PROJECT REPORT

On

IDENTIFICATION, CLONAL MULTIPLICATION AND MICROBIAL ASSOCIATION OF *LEUCAENA* HYBRIDS (LOW SEED YIELDERS) COMPARED TO *EUCALYPTUS* AND *ACACIA* HYBRIDS FOR OPTIMIZING LAND PRODUCTIVITY

SUBMITTED TO

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Submitted by

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Project Title

"Identification, clonal multiplication and microbial association of *Leucaena* hybrids (low seed yielders) compared to *Eucalyptus* and *Acacia* hybrids for optimizing land productivity".

Objectives

I. Production of large scale planting material of economically important multipurpose trees *viz.* Subabul, *Eucalyptus* and *Acacia* hybrid

The increasing demand for pulpwood is being met from the agro-forestry plantations on farmer's fields. There is a great need to increase the production efficiency by using superior quality clones. A viable and concerted effort in the tree improvement is required to ensure continuous supply of quality clones. The tree improvement programme requires the introduction of new species, seed sources for testing, seed orchards, controlled crossing, progeny testing and evaluation *etc.*

With a view to increase biomass production per unit area, we have developed disease resistant fast growing superior clones. These high yielding disease resistant genetically superior clones have been tested in the clonal testing area. The tested good clones are multiplied on mass scale, and distributed to farmers.

We have collected seeds from different provenances of *Eucalyptus*, Subabul and *Acacia* hybrid. Plus trees having following characters were selected from plantations of these species and were marked as CPTs.

- HEIGHT
- CLEAR BOLE
- LESS BRANCH WITH SMALL CROWN
- DIAMETER AT GROUND LEVEL
- DIAMETER AT BREAST HEIGHT
- DISEASE RESISTANCE
- MECHANICAL STABILITY OF TREE
- LIGNIN CONTENT - 20 TO 28%
- DENSITY - 550 TO 700 Kgs/M^3
- FIBER LENGTH - 0.8 MM TO 1.2 MM

To mass propagate these CPTs, we have adopted the clonal technology/macro propagation by cuttings.

For mass production of rooted cuttings, we have established the following facilities.

I. Low Cost Mist Chambers

Low Cost Mist Chambers are the pits dug in compact soil, generally of standard size of 12 m length, 1.3 m breadth and 27 cm depth. The pit is lined on all the sides using a single layer of bricks in vertical position. A layer of sand and pebbles is put at the bottom up to 7 cm thickness. Water is filled in the pit up to 2-inch height or in channels of 6-inch width and 9-inch depth on all four sides. The hydropit is covered with polythene sheet, kept like a tunnel using semicircular bamboo or cast iron frames. The fog collected on the inner surface of the polythene sheet will reduce the temperature and the drops formed due to condensation will fall on the leaf laminae and continue to keep the surface wet (Plate 1).

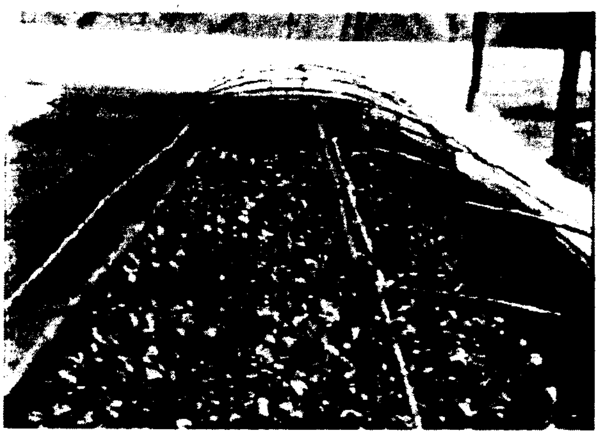

Plate 1. Locally developed mist chamber

For successive rooting of cuttings, the following conditions required are controlled in mist chambers.

a) Temperature

An optimum temperature of 24-30°C required for high photosynthetic activity could be maintained in mist chambers.

b) Humidity

Generally a high humidity of 85-90 per cent is maintained inside a mist chamber for maximum rooting. It reduces transpiration preventing the loss of turgidity in the cuttings. To prevent cuttings from dehydration, a thin film of moisture will always remain on the leaf lamina and no loss of moisture from cuttings is allowed.

c) Light

Both the intensity and longer duration of light influence rooting. Light is also essential for photosynthesis. A light intensity of 1000-1500 flux for 10-12 hrs is always maintained inside a mist chamber for getting maximum rooting.

d) Media

For maximum rooting, media should be well-drained, porous, light in weight and should not be wet to avoid build up of pathogens.

The Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad has developed a microbial technology for converting wood waste generated in chipper house of paper mills into a value added product, 'bio compost' (Plate 2).

Plate 2. Wood waste generated in chipper house of paper mills

We have adopted this technology. The composting technology involves blending of wood dust with nitrogen rich leguminous *Glyricidia* chippings. The material in layers is inoculated with a fungal consortium, specially developed for this solid waste. The material is allowed for composting in windrows for a period of 3 months with fortnightly turning (Plate 3).

Plate 3. Wood waste converted into a biocompost in a composting yard

The bio compost produced is stable, nutrient rich, light in weight and promotes the growth of *Eucalyptus* seedlings (Plate 4).

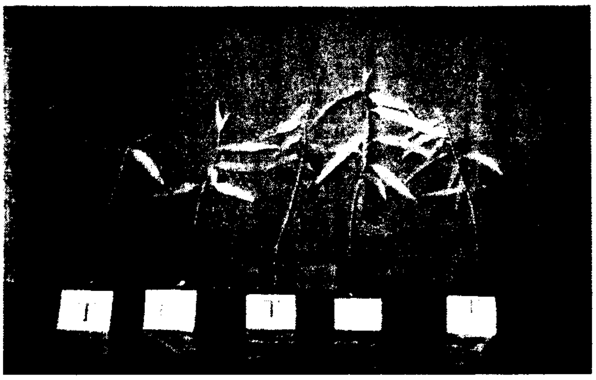

Plate 4. Biocomposted wood waste used as a nursery medium to grow *Eucalyptus* seedlings

We are using such an eco-friendly method of converting an agro industrial waste into value added bio compost.

This bio compost is used as a potting medium for rooting of cuttings as well as for growing seedlings (through seeds) in root trainers.

II. Hardening chamber

We have constructed hardening chambers where the rooted cuttings are hardened for few days before planting in farmers' fields (Plate 5).

Plate 5. Hardening chamber

Currently every year, we produce 30 lakhs of *Eucalyptus* rooted cuttings, 10 lakhs of *Acacia* cuttings, 78 lakhs of *Eucalyptus* seedlings, 30 lakhs *Acacia* seedlings, 2 lakhs of Subabul seedlings and 1 crore 25 lakhs of *Casuarina* seedlings for distribution to farmers.

2. Large scale planting of tree species

At Kulwalli in Karnataka, we have taken up large scale planting of multipurpose tree species. We have prepared trenches all along the contours of hillocks and planted these seedlings. We have taken up soil conservation measures like ripping and bunding, which has resulted in uniformly grown, green plantations (Plate 6).

Plate 6. Soil and water conservation techniques adopted

Now, we have 5 years old, 2100 acres of *Eucalyptus* plantations, 5 years old, 250 acres of *Acacia* plantations and 4 years old, 100 acres of *Casuarina* plantations and 4 years old, 50 acres of Subabul (Plate 7a and b).

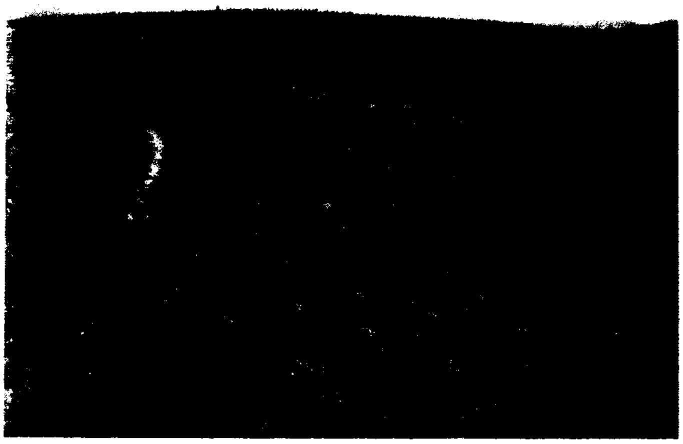

Plate 7a. A view of the *Eucalyptus* plantation

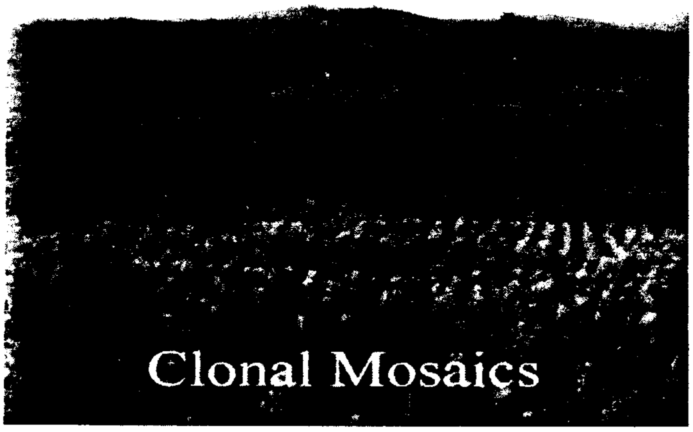

Plate 7b. Eucalyptus clonal orchard

3. Use of biofertilizers for sustaining production of trees

Biofertilizers such as Nitrogen fixers (*Azotobacter* and *Azospirillum*) and P-solubilizers are known to support sustainable agriculture stimulating plant growth and improving soil fertility.

These inoculants were purchased from the Department of Agricultural Microbiology, University of Agricultural Sciences, and Dharwad and used. All the tree species, before planting, were dipped in biofertilizers, mycorrhiza, cowdung slurry and planted.

The results of the effects of inoculation of different biofertilizers are furnished in Table 1, 2, 3 and 4.

4. Multiplication and inoculation of suitable mycorrhizae to MPTS

Mycorrhizae are mutually beneficial interactions between plant roots and certain fungi.

Mycorrhizae are known to mobilize various plant nutrients and supply them to the plants. Plants with good nourishment grow vigorously and healthy.

The fruiting bodies of *Pisolithus tinctorius* were collected from the *Eucalyptus* plantations and a spore suspension in cowdung slurry was prepared. The *Eucalyptus* seedlings were dipped in this suspension before planting. And, in case of experiments using VA mycorrhizae, *Glomus fasciculatum*, *G. mossae*, *Gigasposa margarita*, and *G. aggregatum* were obtained from the Department of Agricultural Microbiology, University of Agricultural Sciences, Bangalore and inoculated to nursery beds and seeds of *Acacia* and *Eucalyptus* were sown. The VAM-pre-colonized seedlings were transplanted to polybags and maintained for 6 months and later planted in the main field.

The effect of these inoculants on the height and girth of trees are furnished in Table 1, 2, 3 and 4.

5. Leaves as fodder for dairy animals

Leguminous plants, by virtue of association with *Rhizobium,* fix atmospheric nitrogen and are rich in protein. The leafy biomass of leguminous trees like *Leucaena* are harvested and used as fodder for dairy animals. Secondly, in the initial stages of plantations of MPTS, several leguminous forage species are grown as an inter crop between rows (Plate 8) and the biomass harvested and used as fodder for animals (Plate 9).

Plate 8. Forage grown as an intercrop in *Eucalyptus* plantation

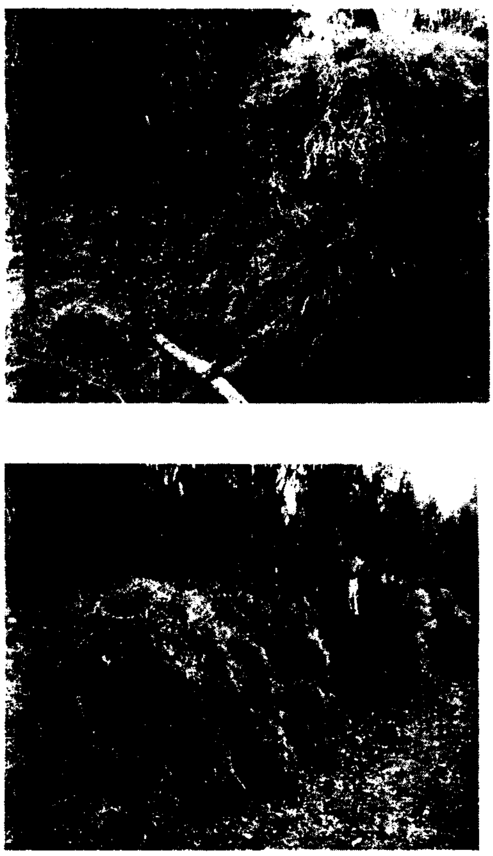

Plate 9. Harvested fodder from *Eucalyptus* plantation

This would not only increase the nutrition of animals but also increase the milk yield, thus, increasing profits of farmers.

Effect of biofertilizers on growth and development of MPTS

The principal aim of agro-forestry is to create positive interactions between woody perennials, herbaceous plants and their biotic and abiotic environments in order to increase the overall productivity of the system. It is important to understand these interactions in order to predict the behaviour of agro-forestry systems following management practices such as tillage, pruning, fertilization or microbial inoculation. Microbial inoculants are not only cost-effective but also eco-friendly, leading to sustainability.

Effect of microbial inoculants on Subabul

Biofertilizers tested

a) *Azospirillum*

b) *Azotobacter* and

c) P-solubilizer

Observations were recorded after 2 years of planting in field.

Effect of inoculation of VAM fungi on height and girth of *Acacia* hybrid

Mycorrhizal association is known to increase growth and yield of crops by enhanced nutrient uptake, resistance to drought and increased to tolerance to pathogens (Hayman, 1993) and production of phytohormones.

The ability of mycorrhizal fungi to release auxins has been reported by several workers. Ek *et al.* (1993) identified IAA in the culture media of 17 of 19 isolates of ectomycorrhizal fungi. Addition of precursors like indole or tryptophan resulted in increased production of auxins by mycorrhizal fungi.

Several studies have demonstrated increased auxin content in response to mycorrhizal infection, which may indicate a role of auxin in symbiosis. Mitchell *et al.* (1996) reported that the level of IAA in *Pinus echinata* roots was increased three fold by inoculation with *Pisolithus tinctorius*, indicating that hyper auxiny is associated with mycorrhizal symbiosis.

The mycorrhizae are vital for uptake and accumulation of nutrient ions from soil and translocation to hosts because of their high metabolic rate and strategically diffused distribution in the upper soil layers. The fungus serves as an additional root system. It is estimated that VAM fungi may increase the effective absorbing surface of roots by as much as 10 times. VAM fungi are also known to produce auxins, cytokinins and vitamins that increase root let size and longevity. They protect roots from pathogens by producing some fungi-static compounds. They absorb and translocate water to the host also.

Different VAM fungi were inoculated to *Acacia* hybrid and their effects on height and girth were evaluated.

VAM fungi inoculated

1. *Glomus fasciculatum*
2. *G. mossae*
3. *Gigaspora margarita*
4. *G. aggregatum*

Observations were recorded after 2½ years. It can be seen from Table 5 that tremendous increase in growth and girth of trees were obtained in the mycorrhizal treatment. The maximum height and girth observed were 7.8 m and 32 cm respectively in case of *G. margarita* treatment. The increase in the height was by 80 per cent over the uninoculated control. And the interment in girth was by over 100 per cent. Thus, *G. margarita* was found to be the preferred fungus for *Acacia* treatment was followed by *Glomus fasciculatum*.

Similarly, Selvaraj *et al.* (1994) also found that in *Acacia nicotica* and *A. mangium,* plant height was enhanced by *G. margarita* and *G. fasciculatum*.

Effect of inoculation of VAM fungi on plant growth biomass, per cent root colonization and P content in *Acacia* hybrid seedlings in nurseries

VA Mycorrhizae tested include

Glomus fasciculatum

G. mossae

Gigaspora margarita and

G. aggregatum

These cultures were inoculated to soil in polybags, and the seeds of *Acacia* hybrid inoculated.

It can be seen from Table 6 that all the VAM cultures substantially increased plant height, biomass and P content in seedlings. However, the highest plant height of 23.9 cm, shoot and root dry weight of 478 mg and 72 mg, P content of 0.41% and mycorrhizal root colonization of 85 per cent were obtained by inoculation with *Gigaspora margarita*. This was followed by *Glomus fasciculatum*.

Selvaraj *et al.* (1994) also reported *G. margarita* to be the best VAM fungus for *Acacia margarita*.

Allen *et al.* (1990) reported higher cytokinin activity in mycorrhizal plants when compared with non-infected plants. Several other workers also have confirmed that inoculation with mycorrhizal fungi increase the endogenous cytokinin contents of host plants (Danneberg *et al.*, 1992). Dixon (1989) reported elevated cytokinin activity of citrus seedlings when inoculated with *Glomus fasciculatum* with a concomitant increase in total dry weight, P nutrition and VA mycorrhizal colonization. He suggested that a minimum level of P is required to sustain cytokinin activity and cytokinins may facilitate P utilization. It is well established that mycorrhizal infection can improve mineral uptake particularly P by host plants. Cytokinins are known to mobilize plant nutrients in plant tissues. Recent studies have also revealed that lower cytokinin levels may lead to restricted mineral uptake in host plants.

Improved plant growth due to inoculation with VAM fungi has been demonstrated under P deficient conditions also (Hayman, 1993). The investigation of Hattingh *et al.* (1994) and several other studies conducted later have conclusively indicated that this growth response is largely explainable by improved P uptake facilitated by VAM fungi. Further, the direct involvement of VAM fungi in uptake and translocation of zinc and copper (Cooper and Tinker, 1981), Potassium (Powell, 1975) Sulphur (Gray and Gerdendan, 1973) and ammonium (Ames *et al.*, 1983) has been demonstrated.

The VAM fungi are also thought to be involved in protecting plants against root invading nematodes and pathogenic fungi (Bagyaraj, 1984), improved water uptake (Safir *et al.*, 1983) and production of phytohormones. These fungi are also known to play an important role in nodulation and nitrogen fixation in legumes (Hayman, 1986), which is mainly attributed to, improved mineral nutrition particularly that of Phosphorus.

Several investigators have attempted to study the mechanisms of improved nutrient uptake by these fungi. VAM fungi have been classified based on physiological, chemical and physiological mechanisms by Hayman (1993).

Physiological mechanisms

Thompson *et al.* (1983) observed that roots colonized with VAM fungi were more active and remained functional for longer time than those of non-mycorrhizal roots. Harrison (1997) reported that a high activity phosphate transporter for VAM fungus has been isolated and cloned.

This phosphate transporter is active and probably operates by proton-coupled sympost. Further, the transcripts of this transporter have been detected in the external hyphae of *Glomus versiformae*. Tarafdar (1994) has observed higher levels of phosphate activities within 10 min from root in the rhizosphere of wheat plants colonized with VAM fungus. Kothari *et al.* (1990) observed selective stimulation of microorganisms in the rhizosphere of maize colonized with VAM fungus, which they consider may play a role in enhancing nutrient uptake.

Chemical mechanisms

Plants colonized with VAM fungi were able to absorb more P while growing in soils amended with insoluble P such as rock phosphate, bone meal, TCP and apatite. This led to a conclusion that VAM fungi may solubilize P from insoluble sources. Later studies, however, involving isotypically exchangeable P suggest that VAM fungi do not solubilize P (Raj *et al.*, 1981). The results of the investigation conducted by Bolan *et al.* (1984) suggest that VAM fungi were able to utilize P, which was not accessible to non-mycorrhizal plants in soils amended with graded levels of iron hydroxide. This observation appears to have renewed interest in this area of research. Further, VAM fungi are also implicated in modifying rhizosphere pH through the exudations and by differential uptake of ions, which may influence P availability.

Physical mechanisms

Hattingh *et al.* (1983) conclusively demonstrated that VAM fungi can absorb and translocate P from a distance of 1.9 to 7.0 cm from roots, which is beyond the nutrient depletion zone around plant roots. Thus, the extra-matrical hyphae ramifying in soil, significantly increase the surface area for absorption of diffusion-limited nutrients. Conferring the kinetics of P movement in soils and distribution of VAM hyphae in soil, it appears that physical exploration of soil by ramifying hyphae is the main mechanism by which VAM fungi improve nutrient uptake of host plants.

The mycorrhizal association is considered crucial for survival and growth of majority of plant species in natural ecosystems.

In many tropical soils, lack of phsophate is the most important constraint on plant growth. The mycorrhizal roots with a network of mycelia explore large soil volume than non-mycorrhizal roots and enhance uptake of P into the plant.

It has been demonstrated that inoculation of seedlings in nurseries with selected mycorrhizal fungi can enhance the productivity of trees in plantations.

The physico-chemical characters of soil in *Acacia* sp. plantations were compared with those of adjacent fallows to ascertain the effect of *Acacia* sp. growth on soil properties. Soil analysis revealed that there was no significant difference between *Acacia* sp. plantations and adjacent fallow land in physical and chemical properties of soil as well as soil nutrient contents.

Acacia sp. plantations have been reported to contribute to higher litter production than from major plantation species like teak, *Eucalyptus* sp. *etc.* (Sankaran *et al.*, 1993). In general, the rate of decomposition of *Acacia* sp. leaf litter was slower compared to that of *Tectona* (Teak), *Xylia, Albizia, Terminalia etc.* in Kerala. The observation that the litter partially buried in soil decomposed much faster than the litter laid on the surface of soil indicates that the periodic raking of soil in *Acacia* sp. plantations would accelerate litter decay. The major reasons for litter accumulation in *Acacia* sp.

plantations are the high litter production and apparent low rate of litter degradation.

The high rate of litter production in *Acacia* plantations is evidently due to the fast vegetative growth exhibited by the species. The favourable climatic conditions like temperature and rainfall prevailing in the area will contribute to higher primary productivity of the plantations leading to higher amount of litter production. According to Penfold and Willis (1961) that the faster growing tree species will produce higher the litter.

A regular increase in the litter yield with successive growth years in *Acacia* sp. plantations shows continuous development of canopy, which is characteristic of young plantations. Yearly litter yield is known to be a function of annual synthesis of fresh organic matter as foliage and other components in the plantations (Bray and Gorham, 1964). An increasing trend in the production of litter based on stand ages have been reported by O'Connell and Menage, 1982; Das and Ramakrishnan, 1985.

Plant litters with high initial Nitrogen content and low C:N ratios are known to decompose rapidly. Though *Acacia* sp. leaves have a high initial Nitrogen content and low C:N ratio interestingly the decay rate was relatively low. The low decomposability of

Acacia sp. litter can be attributed to the high content of crude fibers in the phyllodes and also the presence of a thick cuticle on the phyllode surface. Moreover, the estimated lignin content to be higher in *Acacia* sp. leaf litter than that of *Tectona*, *Xylia* and other tropical tree species. Kumar and Deepu, 1992 opined that physical and chemical properties of litter might exert a strong influence on decomposition. Pandey and Singh (1992) reported that a negative correlation between the weight loss and fiber content of the litter. The allelopathic effect of *Acacia* sp. leaves on the decomposer microorganisms may be another possible reason for its low degradability. Lignin content of litter is recognized to be one of the most important factors controlling decay rates. Further, the decomposition of lignin of the Nitrogen rich litters is known to be significantly lower than those with poor Nitrogen content. Similar results were also reported for leaf litters of *Albizia falcataria* (Sankaran, 1993) and *Leucaena leucocephala* (Sandhu et al., 1990).

The organic Carbon content of the soil under *Acacia* sp. litter was significantly more after decomposition of the litter. It is possible that a good portion of the organic Carbon content was liberated as CO_2 during the decomposition process and a substantial increase in organic Carbon content levels may occur only after prolonged period of decay process.

Effect of inoculation of Ectomycorrhizal fungi on plant growth, biomass, root colonization, and P content in *Eucalyptus* sp. seedlings in nurseries

The fruiting body of the ectomycorrhizal fungus, *Pisolithus tinctorius* with spores (Plate 10) was collected from *Eucalyptus* plantation and inoculated to soil in polybags after mixing with cowdung slurry. And, the seedlings of *Eucalyptus* transplanted to poly bags.

Plate 10. Fruiting body of *Pisolithus tinctorius*

It can be observed from Table 7 that the ectomycorrhizal inoculation substantially increased plant height, biomass, mycorrhizal root inoculation and P content in seedlings. A height of 26.6 cm was obtained due to inoculation, which was more than 100 per cent compared to UIC. A shoot dry weight of 518 mg, which was three times greater than that of the control, was obtained in the mycorrhiza treated seedlings. A P content of 0.44 per cent was observed which was four times more than that of the control. Similarly, Natarajan (1999) observed *Eucalyptus* sp. forming association with *Pisolithus* sp., which led to increased growth and biomass.

Before

After

Plate 10. Fruiting body of *Pisolithus tinctorius*

Gibberellins also have been known to be produced by mycorrhizal fungi. Ho (1997) found all 8 isolates of *Pisolithus tinctorius* to produce phytohormones including GA. Similarly, significant quantities of GA like compounds were detected in filtrates of certain ectomycorrhizal fungi. Ho and Trappe (1992) detected exctracellular growth regulators including GA, IAA and cytokinins in the culture of two mycorrhizal fungi. GA like substances *in vitro* indicates that excertion of these compounds by the fungus after infection may have a physiological role on the growth and development of the host. Allen *et al.* (1992) observed that infection by *Glomus fasciculatum* significantly increased GA activity in the leaves of the host plant with a tendency for decreased GA activity in the roots.

After detecting significant amounts of GA like compounds in the filtrates of mycorrhizal fungi, Hanky and Greece (1997) attempted to mimic the possible effects of GAs on the host with exogenous application of GA in various concentrations to pine seedlings.

Cytokinins regulate cell division, differentiation and senescence in plant tissues. Similar to auxins, cytokinin may also play an important role in the establishment of mycorrhizal as well as evoking a physiological response in mycorrhiza-infected plants. The ability of several mycorrhizal fungi to produce cytokinins is now well characterized. The role of cytokinins can be demonstration from two different viewpoints *viz.*, their role in mycorrhizae formation and in changing the cytokinin balance in the plant as a mycorrhizal response.

Table 5. Growth response of *Acacia* hybrid to inoculation of different VAM fungi (after 2½ years) (Dandeli, Karnataka).

VAM fungi	Plant height (mt)	GBH (cm)
Glomus fasciculatum	7.30	27
Glomus mossae	6.10	24
Gigospora margarita	7.80	32
Gigaspora aggregatum	5.90	18
Uninoculated control	4.35	15

Table 6. Growth response of *Acacia* hybrid to inoculation with different VAM fungi (Dandeli, Karnataka) in nursery bags.

VAM fungi	Plant ht. (cm)	Shoot dry wt. (mg/pl)	Root dry wt. (mg/pl)	Total P (%)	Root colonization (%)
Glomus fasciculatum	23.8	440	69	0.39	80
Glomus mossae	20.6	286	47	0.29	55
Gigaspora margarita	23.9	478	72	0.41	85
Gigaspora aggregatum	17.2	215	42	0.25	43
Uninoculated control	12.6	168	29	0.19	5

Table 7. Growth response of *Eucalyptus* species to inoculation with *Pisolithus tinctorius* in nursery bags

VAM fungi	Plant ht. (cm)	Shoot dry wt. (mg/pl)	Root dry wt. (mg/pl)	Total P (%)	Root colonization (%)
Pisolithus tinctorius	26.6	518	84	0.44	86
Uninouclated control	11.5	171	30	0.10	2

It can be seen from Table 1 that microbial inoculation resulted in increased height as well as girth of Subabul. A maximum height of 6.26 m and maximum girth of 15.4 cm were obtained due to inoculation with *Azospirillum* G3 strain. Whereas uninoculated control yielded only 4.9 m height and 12.0 cm girth. The second best treatment was *Azotobacter*, which produced 6.2 m height and 15.0 cm girth.

Table 1. Effect of different biofertilizers on height and girth of Subabul after 2 years of planting.

Sl. No.	Treatment	Height (m)	Girth (cm)
1.	UIC	4.90	12.0
2.	*Azotobacter* sp.	6.20	15.0
3.	P-solubilizer	5.80	14.4
4.	*Azospirillum* sp.	6.20	13.6
5.	*Azospirillum* G3 strain	6.26	15.4
	CD at 5%	NS	NS
	S.Em±	0.734	1.497

In case of Subabul (low seed yielders) also, height and girth were improved due to inoculation with biofertilizers (Table 2). The highest height of 6.5 m was observed in the trees inoculated with *Azotobacter* and *Azospirillum* accounting to about 44 per cent increase over UIC. This was followed by P-solubilizer and *Azospirillum* treatment with 6.0 m. the higher girth of 13.6 cm was observed in this treatment, again.

Table 2. Effect of different biofertilizers on height and girth of Subabul (low seed yielders) after 2 years of planting.

Sl. No.	Treatment	Height (m)	Girth (cm)
1.	UIC	4.5	12.4
2.	*Azotobacter* + *Azospirillum*	6.5	13.0
3.	P-solubilizer + *Azotobacter* + *Azospirillum* 43	5.4	13.4
4.	P-solubilizer + *Azospirillum*	6.0	13.6
5.	*Azotobacter* + *Azospirillum* G3	5.7	12.6
	CD at 5%	NS	NS
	S.Em±	7.16	1.924

Increased height and girth in *Acacia* hybrid seedlings were noticed due to biofertilizer inoculation. P-solubilizer and G3 strain inoculation was found to be the best combination which yielded 6.36 m height and 26.00 cm girth, which were the highest (Table 3). The second best treatment was P-solubilizer and *Azospirillum* inoculation with 6.28 m height and 24.60 cm girth. The increase in the height was over 32 per cent and increase in the girth was over 50 per cent over the UIC treatment.

Table 3. Effect of biofertilizers on height and girth of *Acacia* hybrid after 2 years of planting.

Sl. No.	Treatment	Height (m)	Girth (cm)
1.	UIC	4.80	16.80
2.	*Azospirillum* + *Azotobacter*	6.14	23.40
3.	*Azotobacter* + *Azospirillum* G3 strain	6.18	20.60
4.	P-solubilizer + *Azospirillum* G3 strain	6.36	26.00
5.	P-solubilizer + *Azospirillum*	6.28	24.60
	CD at 5%	NS	NS
	S.Em±	0.633	2.324

Eucalyptus sp. seedlings also showed very good response to biofertilizer inoculation. The data revealed that the highest height of 7.46 m was obtained by P-solubilizer and *Azotobacter* inoculation (Table 4) and, the highest girth of 21.8 cm was observed in three combination inoculants of P-solubilizer, *Azospirillum* and *Azotobacter*. The second best treatment was P-solubilizer and G3 strain inoculation with 7.42 m height and 19.8 cm girth after a period of two years of planting.

Table 4. Effect of biofertilizers on height and girth of *Eucalyptus* after 2 years of planting.

Sl. No.	Treatment	Height (m)	Girth (cm)
1.	UIC	5.30	18.8
2.	P-solubilizer + *Azospirillum* G3 strain	7.42	19.8
3.	*Azotobacter* + *Azospirillum* G3 strain	6.72	17.2
4.	*Azotobacter* + P-solubilizer	7.46	16.6
5.	*Azospirillum* + *Azotobacter* + P-solubilizer	7.40	21.8
6.	*Azospirillum* + G3 strain	7.40	15.4
	CD at 5%	NS	NS
	S.Em±	0.954	2.575

Field performance of *Eucalyptus pellita* inoculated with *Pisolithus tinctorius*

Observations on height and girth of *Eucalyptus* were taken after 2 years of planting in field. Tremendous increase in height and girth was noticed due to inoculation with *P. tinctorius*. The average height of 100 trees was found to be 7.55 m, while in case of uninoculated control, it was a mere 5.88 m (Table 8). This increase in height works out to be about 30 per cent over control. There was a substantial increase in the girth also (26.67 cm) when compared to control. Increase in growth, girth and biomass of *Eucalyptus* by *Pisolithus* has also been observed by Natarajan (1999).

Table 8. Effect of inoculation of *Pisolithus* sp. on height and girth of *Eulalyptus pellita* after 2 years of planting at Kulwalli, Karnataka.

Sl. No.	Treatment	Height (m)*	Girth (cm)*
1.	Control	5.88	20.41
2.	*Pisolithus* treated	7.55	26.67

*Average of 100 trees

Influence of *P. tinctorius* on the growth of *Eucalyptus* clones

The inoculation of *Eucalyptus* clones with *P. tinctorius* resulted in about 60 per cent more height than the control treatment (Tables 9 and 10) both after one year and two years of planting in Kulawalli fields. And, there was increment in girth also by about 40 per cent.

Table 9. Effect of inoculation of *Pisolithus* sp. on height and girth of *Eucalyptus* clones after 2 years of planting at Kulawalli, Karnataka.

Sl. No.	Treatment	Height (m)*	Girth (cm)*
1.	Control	6.31	17.54
2.	*Pisolithus* treated	9.43	24.55

*Average of 100 trees

Table 10. Effect of inoculation of *Pisolithus* sp. on height and girth of *Eucalyptus* clones after 1 year of planting at Kulawalli, Karnataka.

Sl. No.	Treatment	Height (m)*	Girth (cm)*
1.	Control	3.84	11.67
2.	*Pisolithus* treated	6.25	16.93

*Average of 100 trees

Jasper *et al.* (1989) reported that several Australian *Acacia* spp. are strongly dependent on VAM fungi for P uptake. Sankaran *et al.* (1993) observed colonization of *Acacia auriculiformis* was VA mycorrhizal and there was no evidence of ectomycorrhizal association in *Acacia* sp. They observed that the seedlings inoculated with VAM fungi had significantly higher height; shoot and root dry mass and mycorrhizal root colonization as compared to uninoculated control. The P content in seedlings also varied significantly between incoulated and control seedlings. Seedlings inoculated with *Glomus fasciculatum* had the maximum increase in weight (183% over control), shoot and root dry mass (278% and 267%, respectively). *Acaulospora* sp. was found the next best efficient fungus.

Sankaran *et al.* (1993) also assessed the status of root nodulation of *Acacia* sp. in Kerala. There was an inverse relationship between the number of nodules and soil fertility attributes. Nursery experiments using *Rhizobium* sp. inoculated seedlings indicated significant variation in efficiency of various *Rhizobium* isolates in forming root nodules. There was a direct correlation between number of nodules and seedling biomass in nursery experiments.

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