

# SCREENING OF SELECTED NATIVE ARBUSCULAR MYCORRHIZAL FUNGI AT DIFFERENT LEVELS FOR THEIR SYMBIOTIC EFFICIENCY WITH *TECTONA GRANDIS* SEEDLINGS

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A study was conducted to find efficacy of three native species of arbuscular mycorrhizal fungi (AMF) on growth and seedling quality of *Tectona grandis*. The native AMF species (*Funelliformis mosseae*, *Glomus intradices* and *Glomus proliferum*) at different levels (10, 20 and 50 g inoculum per seedling) were applied to the seedlings raised in polythene bags. Inoculated seedlings showed a significant difference in plant growth and quality when compared to the uninoculated seedlings. Though, the growth and quality enhancement differed among AMF species and levels of inoculation, generally, mycorrhizal symbiosis significantly improved seedling growth. Mycorrhizal colonisation of seedlings roots ranged from 15.0 to 36.0%. The physiological parameters also showed improvement with AMF inoculation. The mycorrhizal efficiency index (MEI) of seedlings inoculated with *G. proliferum* (50 g inoculum) was 89.23%. Root colonisation was found to range from 17.33 to 33.33% and from 22.67 to 56.33% when 50 g and 10 g of inoculums were used. The result showed that *G. proliferum* (50 g of inoculums) at the time of transplanting showed maximum growth and seedling quality benefits in nursery as compared to all other fungi used for *T. grandis*. The technology has potential to reduce the nursery period and increase quality of seedlings produced, resulting in considerable economic gains.

Keywords: Inoculation, root colonisation, nursery, economy, mycorrhizal efficiency index (MEI)

## INTRODUCTION

Arbuscular mycorrhizal fungi (AMF), a group of obligate biotrophic fungi belonging to the phylum *Glomeromycota*, are among the oldest fungi in terrestrial systems on earth. The symbiotic relationship of these fungi with plants is assumed to have played an essential role in the establishment of (pre) vascular plants on land. Around 230 morphospecies of these fungi have been identified and described (Schubler 2013). Symbiotic associations of AMF and plant roots are widespread in the natural environment and can provide a range of benefits to the host plant. These include improved nutrition, enhanced resistance to soil-borne pests and disease, improved resistance to drought, tolerance to heavy metals and better soil structure (Gosling et al. 2006). Presence of AMF can significantly increase root surface area by production of extensive hyphae, increase transpiration, reduce leaf temperature and restrain the decomposition of chlorophyll (Abbaspour et al. 2012). Phosphorus is often most important in symbiosis. The AMF host

obtains maximum benefit when the mineral nutrient regime is least favourable for growth (Ezawa et al. 2002). In turn, plants direct 4 to 20% of photoassimilate to mycorrhizas (Ruissen 2013). Hyphae work as conduits that transport carbon from plant roots to other soil organisms involved in nutrient cycling processes. Though the AMF association can offer multiple benefits to the host plant, it may not be obviously mutualistic at all points in time, and under some conditions it is possible for the host plant to lose C with no apparent benefit. In some cases, it can even cause a decline in growth (Lerat et al. 2003). In many tree seedlings, the inoculation of AMF was found beneficial (Dutt et al. 2013, Binu et al. 2015) resulting in seedlings of higher quality. The high percentage of root colonisation in AMF treated seedlings is found to be directly correlated with an improved growth and physiology (Dutt et al. 2013). Arbuscular mycorrhizal fungi is an unexploited potential biofertiliser in forest nurseries which can be utilised for quality tree seedling production.

Teak (*Tectona grandis*) belongs to the family Lamiaceae. It has centuries old reputation as the 'king of timbers' (White 1991). In India, it covers an area of around 9.77 million hectares and this is around 13% of the total broad-leaved forests of India (Berbata 1999). Teak requires a relatively long nursery period lasting about one year. The goal of forest tree nursery practices is to produce high quality seedlings with useful characters. Evolving appropriate nursery management strategies to enhance productivity and thereby reducing the long nursery period has been the basic challenge. Teak seedlings are poorly studied in relation to its physiological responses to AMF applications. Studies on screening of AMF level of inoculation regimes on most tropical trees in nursery are meagre. Screening and standardisation of AMF species is necessary for any afforestation programme, as expenditure on nursery itself takes a major portion of plantation cost. The present study has been formulated to assess the impact of inoculation potential of selected AMF on growth and quality of *Tectona grandis* seedlings.

## MATERIALS AND METHODS

The investigation was conducted at the College of Forestry, Kerala Agricultural University, Thrissur district, Kerala, India (10° 32' N latitude and 76° 26' E longitude). Three isolates of AMF, commonly seen in the rhizosphere region of teak, were selected for the study. Growth of teak seedlings were compared by subjecting the seedlings to different inoculum concentrations (10, 25 and 50 g). A 3<sup>2</sup> factorial experiment was laid in a completely randomised design with five replication. The different treatments are shown in Table 1. The experiment was conducted for nine months.

Teak seeds were collected from three plantations of Nilambur Forest Division (10° 15' & 10° 26' N latitudes, and 75° 46' & 76° 33' E longitudes) of Kerala Forest Department. The large sized fruit of teak (above 9 mm) were used for the production of seedlings (Jijeesh and Sudhakara 2013). Seeds were pretreated by alternate wetting (night) and drying (day in shade) for seven days (Bedell 1989). The potting media used in this experiment were soil (sieved in 0.5 mm mesh) and sand mixture (1:1), which was used after fumigation with 0.5% formaldehyde for 20 days. The mixture was kept

**Table 1** Different treatment combinations given to teak seedlings with three mycorrhizae at different concentrations

Treatment combinations
Teak + <i>Funneliformis mosseae</i> + 10 g inoculum
Teak + <i>Funneliformis mosseae</i> + 25 g inoculum
Teak + <i>Funneliformis mosseae</i> + 50 g inoculum
Teak + <i>Glomus intraradices</i> + 10 g inoculum
Teak + <i>Glomus intraradices</i> + 25 g inoculum
Teak + <i>Glomus intraradices</i> + 50 g inoculum
Teak + <i>Glomus proliferum</i> + 10 g inoculum
Teak + <i>Glomus proliferum</i> + 25 g inoculum
Teak + <i>Glomus proliferum</i> + 50 g inoculum
Teak as control

in open and then mixed repeatedly to remove the formaldehyde residues.

Pure cultures of native species of *Funneliformis mosseae*, *Glomus intraradices* and *Glomus proliferum*, vermi-paste based (1000 spores in 100 g), were obtained from The Energy Research Institute (TERI), New Delhi, and stored in refrigerated condition. All the three species of AMF were multiplied in fumigated soil in polythene bags (55 cm × 25 cm). Ten gram of AMF was mixed in the poly bag and five sterilised (0.01% sodium hypochloride for 10 min and washed in sterile water) maize seeds were sown. The plants were irrigated daily using sterile water. At every 10 days interval, the plants were applied with Hoagland's solution (Hoagland & Arnon 1950), 50 ml per plant. The maize roots were checked for colonisation frequently by clearing and staining method (Philips & Hayman 1970). The shoot portion of the maize was removed when root colonisation was more than 80%, and the soil containing the roots were thoroughly mixed to obtain inoculum. The spore count obtained was 10 per g of soil.

Fumigated soil was immediately transferred to the greenhouse, and polythene bags (11.43 × 15.24 cm, gauge 75 micron) were filled with potting media, leaving 4 cm space at the top. The inoculums were placed in polybags, as per the treatments, and covered with 1:1 mixture of sterilised sand and soil, up to 2 cm above the inoculum. Pre-germinated teak seeds were placed in these polybags to a depth of 2 cm.

The pretreated seeds of teak were surface sterilised (0.01% sodium hypochloride for 10 min), washed in sterile water and sown in sterilised sand beds in trays. Thirty days old

seedlings were transplanted to polybags. These seedlings were arranged in three blocks of 30 seedlings each, grown in open condition throughout the experimental period and irrigated well. The 45 days old established seedlings of uniform size were inoculated with selected species of AMF at different levels, at the base of plant of 2 cm depth.

Observations were taken after 150 days of inoculation of AMF. Observations were taken (12 plants per replication) on seedling height, collar diameter and number of functional leaves at 150 days after inoculation. Another set of 12 seedlings were destructively sampled simultaneously to determine biometric observations and growth observations. The vigour index I of the seedlings was calculated by multiplying germination percentage to seedling length and expressed in percentage (Kharb et al. 1994). Similarly, multiplying germination percentage to seedling biomass, and expressed in percentage, gives vigour index II. Chlorophyll content, photosynthetic rates, transpiration rate, water potential and relative water content (RWC) of seedlings were also measured (Scholander et al. 1965).

The percentage AMF colonisation in the root samples of different treatments at the end of study was determined following the procedure of Philips and Hayman (1970). The extrametrical chlamydo spores produced by the AMF were estimated following the wet sieving and decanting technique (Gerdemann and Nicolson, 1963). Quality index, which is a measure to assess the quality of seedling based on height, stem diameter and dry biomass, was calculated using the formula by Hatchell (1985). Biovolume index, which is a non-destructive quick method to calculate the above-ground portion of the tree seedlings, was calculated using the formula by Hatchell (1985). Mycorrhizal use efficiency index (MEI) or mycorrhizal dependency allows assessment of growth improvement produced by inoculation of plants with a mycorrhizal fungus. MEI was estimated according to Secilia and Bagyaraj (1994).

### Statistical analysis

Data was subjected to one-way analysis of variance (ANOVA). Based on the outcome of ANOVA, post-hoc analysis (Duncan 1955) was performed to separate the means.

## RESULTS

There were variations in the growth, physiology and quality of teak seedlings produced due to AMF inoculations (Table 2–5). For all the biometric characters, except number of lateral roots, the seedlings inoculated with *G. proliferum* showed a higher value, when compared to other treatments (Table 2). The lowest value obtained was for the uninoculated control. Among the various doses of *G. proliferum*, the performances of seedlings inoculated with 50 g inoculum were the best. The number of lateral roots was more for treatment T1 (*F. mosseae* + 10 g inoculum), followed by the treatment T4 (*G. intraradices* + 10 g inoculum) and T8 (*G. proliferum* + 25 g inoculum). Inoculating the seedlings with AMF also influenced growth parameters. Leaf area ratio, leaf weight ratio and specific leaf area were higher for seedlings inoculated with *G. proliferum*, although the values did not vary significantly with different doses of *G. proliferum* inoculum. Specific leaf weight was highest for seedlings in treatment T3 (*F. mosseae* + 50 g inoculum) and lowest for all the doses of inoculation with *G. proliferum*. The observation on absolute growth rate ( $\text{cm day}^{-1}$ ) was more for treatment T9 (*G. proliferum* + 50 g inoculum) followed by T8 (*G. proliferum* + 25 g inoculum) and T7 (*G. proliferum* + 10 g inoculum). It was observed that the relative growth rate was highest for treatment T4 (*G. intraradices* + 10 g inoculum). Not many variations were observed for the other treatments. The physiology of teak seedlings, influenced by different treatments, were also studied (Table 3). The values showed that photosynthetic rate, transpiration rate, stomatal conductance and plant water potential were not significantly different on seedlings among different mycorrhizal treatments. The chlorophyll content, leaf temperature and relative water content showed significant difference with respect to the mycorrhizal treatments. The seedlings treated with *G. proliferum* (50 g inoculum) showed the highest value. The root colonisation percent of teak seedlings, as influenced by different mycorrhizal treatments (Table 4) revealed that colonisation percentage of roots and number of spores per 10 g of soil, was greater for seedlings inoculated with *G. proliferum*. The dose (50 g inoculum) was found to be better than the other two dosages. The difference in quality indices of teak seedling, subjected to different mycorrhizal treatment,

**Table 2** Biometric observations of *Tectona grandis* seedling as influenced by different treatments

Treatments	Seedling height (cm)	Collar diameter (mm)	Number of leaves	Leaf area per plant (cm <sup>2</sup> )	Dry weight of leaves (g)	Tap root length	Number of lateral roots	Dry weight of roots (g)	Total dry weight (g)	Shoot-root length ratio	Shoot-root biomass ratio	Vigour Index I	Vigour Index II
T1	19.9 <sup>c</sup>	6.99 <sup>cd</sup>	10.7 <sup>b</sup>	353.80 <sup>c</sup>	2.72 <sup>cd</sup>	29.53 <sup>d</sup>	45.00 <sup>a</sup>	2.47 <sup>c</sup>	6.25 <sup>c</sup>	0.69 <sup>b</sup>	1.52 <sup>b</sup>	25.83 <sup>de</sup>	3.26 <sup>c</sup>
T2	14.2 <sup>d</sup>	4.87 <sup>c</sup>	9.7 <sup>b</sup>	83.80 <sup>d</sup>	1.31 <sup>d</sup>	27.73 <sup>d</sup>	37.00 <sup>ab</sup>	2.13 <sup>c</sup>	4.00 <sup>c</sup>	0.53 <sup>bc</sup>	0.89 <sup>b</sup>	21.91 <sup>c</sup>	2.09 <sup>c</sup>
T3	16.4 <sup>cd</sup>	4.78 <sup>c</sup>	8.7 <sup>bc</sup>	61.96 <sup>d</sup>	1.17 <sup>d</sup>	30.30 <sup>d</sup>	36.00 <sup>ab</sup>	2.18 <sup>c</sup>	3.91 <sup>c</sup>	0.54 <sup>bc</sup>	0.80 <sup>b</sup>	24.36 <sup>de</sup>	2.04 <sup>c</sup>
T4	14.9 <sup>d</sup>	5.01 <sup>c</sup>	8.7 <sup>bc</sup>	80.07 <sup>d</sup>	1.21 <sup>d</sup>	28.33 <sup>d</sup>	32.33 <sup>b</sup>	1.79 <sup>c</sup>	3.56 <sup>c</sup>	0.53 <sup>bc</sup>	1.01 <sup>b</sup>	22.59 <sup>de</sup>	1.86 <sup>c</sup>
T5	17.8 <sup>cd</sup>	5.74 <sup>de</sup>	8.0 <sup>bc</sup>	233.79 <sup>cd</sup>	2.01 <sup>cd</sup>	34.93 <sup>cd</sup>	37.00 <sup>ab</sup>	2.21 <sup>c</sup>	5.00 <sup>c</sup>	0.51 <sup>bc</sup>	1.30 <sup>b</sup>	27.55 <sup>d</sup>	2.61 <sup>c</sup>
T6	16.1 <sup>cd</sup>	4.95 <sup>c</sup>	7.7 <sup>bc</sup>	97.47 <sup>d</sup>	1.50 <sup>d</sup>	34.40 <sup>cd</sup>	39.67 <sup>ab</sup>	2.25 <sup>c</sup>	4.31 <sup>c</sup>	0.48 <sup>c</sup>	1.00 <sup>b</sup>	26.38 <sup>de</sup>	2.25 <sup>c</sup>
T7	20.5 <sup>c</sup>	7.25 <sup>c</sup>	6.7 <sup>c</sup>	430.39 <sup>c</sup>	3.26 <sup>c</sup>	42.00 <sup>bc</sup>	38.67 <sup>ab</sup>	4.00 <sup>bc</sup>	8.64 <sup>c</sup>	0.49 <sup>c</sup>	1.14 <sup>b</sup>	32.63 <sup>c</sup>	4.51 <sup>c</sup>
T8	45.9 <sup>b</sup>	10.48 <sup>b</sup>	10.0 <sup>ab</sup>	1880.42 <sup>b</sup>	12.34 <sup>b</sup>	45.13 <sup>ab</sup>	32.33 <sup>b</sup>	5.18 <sup>b</sup>	22.85 <sup>b</sup>	1.02 <sup>b</sup>	3.69 <sup>a</sup>	47.51 <sup>b</sup>	11.93 <sup>b</sup>
T9	60.8 <sup>a</sup>	13.42 <sup>a</sup>	13.3 <sup>a</sup>	3167.48 <sup>a</sup>	22.50 <sup>a</sup>	52.00 <sup>a</sup>	40.00 <sup>ab</sup>	12.05 <sup>a</sup>	47.53 <sup>a</sup>	1.17 <sup>a</sup>	2.97 <sup>a</sup>	58.87 <sup>a</sup>	24.82 <sup>a</sup>
T10	14.5 <sup>d</sup>	5.18 <sup>c</sup>	6.3 <sup>c</sup>	90.77 <sup>d</sup>	1.15 <sup>d</sup>	34.53 <sup>cd</sup>	36.67 <sup>ab</sup>	2.36 <sup>c</sup>	5.06 <sup>c</sup>	0.42 <sup>c</sup>	1.11 <sup>b</sup>	25.58 <sup>de</sup>	2.64 <sup>c</sup>
SEM ±	2.83*	0.53*	0.44*	184.39*	1.62*	1.68*	1.07*	0.61*	2.76*	0.05*	0.19*	2.19*	1.31*

\*Significant at 0.05 levels; values with similar superscript alphabets within a column do not vary significantly

**Table 3** Growth observations of *Tectona grandis* seedlings as influenced by different treatments

Treatments	Leaf area ratio (cm <sup>2</sup> g <sup>-1</sup> )	Leaf weight ratio (cm <sup>2</sup> g <sup>-1</sup> )	Specific leaf area (cm <sup>2</sup> g <sup>-1</sup> )	Specific leaf weight (g cm <sup>-2</sup> )	Absolute growth rate (cm day <sup>-1</sup> )	Relative growth rate (g g <sup>-1</sup> day <sup>-1</sup> )	Net assimilation rate (g g <sup>-1</sup> day <sup>-1</sup> )
T1	56.47 <sup>bc</sup>	0.43 <sup>bc</sup>	130.47 <sup>ab</sup>	0.008 <sup>d</sup>	19.38 <sup>c</sup>	0.00 <sup>bc</sup>	0.00 <sup>ab</sup>
T2	20.57 <sup>e</sup>	0.33 <sup>def</sup>	62.75 <sup>c</sup>	0.017 <sup>ab</sup>	13.88 <sup>d</sup>	0.01 <sup>ab</sup>	0.00 <sup>ab</sup>
T3	15.81 <sup>e</sup>	0.30 <sup>ef</sup>	52.99 <sup>c</sup>	0.019 <sup>a</sup>	15.94 <sup>cd</sup>	0.01 <sup>ab</sup>	0.00 <sup>ab</sup>
T4	21.77 <sup>e</sup>	0.35 <sup>cde</sup>	63.91 <sup>c</sup>	0.016 <sup>ab</sup>	14.56 <sup>d</sup>	0.01 <sup>a</sup>	0.00 <sup>b</sup>
T5	42.58 <sup>d</sup>	0.40 <sup>bcd</sup>	105.83 <sup>b</sup>	0.010 <sup>cd</sup>	17.36 <sup>cd</sup>	0.01 <sup>ab</sup>	0.00 <sup>ab</sup>
T6	22.63 <sup>e</sup>	0.35 <sup>cde</sup>	65.03 <sup>c</sup>	0.016 <sup>ab</sup>	15.72 <sup>cd</sup>	0.01 <sup>ab</sup>	0.00 <sup>ab</sup>
T7	48.94 <sup>cd</sup>	0.37 <sup>cde</sup>	133.75 <sup>ab</sup>	0.008 <sup>d</sup>	19.94 <sup>c</sup>	0.00 <sup>bc</sup>	0.00 <sup>ab</sup>
T8	82.34 <sup>a</sup>	0.54 <sup>a</sup>	152.33 <sup>a</sup>	0.007 <sup>d</sup>	44.45 <sup>b</sup>	0.00 <sup>c</sup>	0.00 <sup>ab</sup>
T9	67.48 <sup>b</sup>	0.48 <sup>ab</sup>	141.39 <sup>a</sup>	0.007 <sup>d</sup>	58.86 <sup>a</sup>	0.00 <sup>c</sup>	0.00 <sup>a</sup>
T10	19.06 <sup>e</sup>	0.25 <sup>f</sup>	78.20 <sup>c</sup>	0.013 <sup>bc</sup>	14.10 <sup>d</sup>	0.01 <sup>bc</sup>	0.00 <sup>ab</sup>
SEM ±	4.27*	0.02*	7.15*	0.001*	2.74*	0.00	0.00*

\*Significant at 0.05 level; values with similar superscript alphabets within a row do not vary significantly

**Table 4** Physiology of *Tectona grandis* seedlings as influenced by different treatments

Treatments	Chlorophyll content	Photosynthesis rate (μmol m <sup>-2</sup> s <sup>-1</sup> )	Transpiration rate (μmol m <sup>-2</sup> s <sup>-1</sup> )	Leaf temperature (°C)	Stomatal conductance (s cm <sup>-1</sup> )	Relative water content (%)	Plant water potential (MPa)
T1	36.07 <sup>abcd</sup>	12.56	2.33	31.90 <sup>d</sup>	0.16	74.31 <sup>ab</sup>	1.40
T2	40.67 <sup>abc</sup>	13.73	2.55	32.40 <sup>bc</sup>	0.17	75.55 <sup>ab</sup>	1.81
T3	35.60 <sup>abcd</sup>	15.84	2.88	32.60 <sup>ab</sup>	0.18	74.76 <sup>ab</sup>	1.58
T4	36.07 <sup>abcd</sup>	11.69	2.61	32.37 <sup>c</sup>	0.16	71.27 <sup>ab</sup>	1.80
T5	34.90 <sup>bcd</sup>	11.47	2.59	32.27 <sup>c</sup>	0.16	70.98 <sup>ab</sup>	1.81
T6	33.90 <sup>d</sup>	14.87	2.93	32.33 <sup>c</sup>	0.20	73.48 <sup>ab</sup>	1.55
T7	34.73 <sup>bcd</sup>	14.48	2.80	32.43 <sup>bc</sup>	0.18	70.15 <sup>ab</sup>	1.84
T8	41.53 <sup>ab</sup>	11.46	2.56	32.60 <sup>ab</sup>	0.16	74.80 <sup>ab</sup>	1.81
T9	42.13 <sup>a</sup>	12.15	2.69	32.80 <sup>a</sup>	0.16	80.08 <sup>a</sup>	1.58
T10	31.57 <sup>d</sup>	14.18	2.96	32.77 <sup>a</sup>	0.19	67.14 <sup>b</sup>	1.42
SEM ±	4.48*	4.09 <sup>ns</sup>	0.50 <sup>ns</sup>	0.27*	0.05 <sup>ns</sup>	6.39*	0.37 <sup>ns</sup>

\*Significant at 0.05 level; values with similar superscript alphabets within a column do not vary significantly

is shown in Table 6. The results showed that *G. proliferum* treated seedlings had higher values for biovolume, seedling quality index and mycorrhizal efficiency index. The best performance was observed for seedlings treated with *G. proliferum* (50 g inoculum).

## DISCUSSION

Host specificity among AMF has been reported by many workers (Rajan et al. 2000, Wu et al. 2011, Binu et al. 2015). The need for selecting efficient native AMF that can be used for inoculating different mycotrophic plants has been further

stressed (Jeffries 1987, Bagyaraj & Varma 1995). The predominant genus of AMF spores occurring in Kerala is *Glomus* due to its adaptability to a wide range of soil and environmental factors (Harikumar & Potty 1999, Gopal et al. 2005). The selection of native AMF species was due to the predominant nature of *Glomus*. Three native species of AMF (*Funelliiformis mosseae*, *Glomus intradices* and *Glomus proliferum*) were selected at different levels (10, 25 and 50 g inoculum per seedling).

AMF inoculation resulted in a significant increase in plant height, stem girth, plant biomass and quality index of teak seedlings. The better



**Table 5** Root colonisation percentage of *Tectona grandis* seedlings as influenced by different treatments

Treatments	Colonisation percentage (%)	Number of spores 10 g <sup>-1</sup> soil
T1	17.33 <sup>f</sup>	24.00 <sup>f</sup>
T2	23.33 <sup>e</sup>	33.00 <sup>e</sup>
T3	28.67 <sup>d</sup>	50.00 <sup>d</sup>
T4	24.00 <sup>de</sup>	37.00 <sup>e</sup>
T5	28.33 <sup>d</sup>	47.67 <sup>d</sup>
T6	22.67 <sup>e</sup>	63.33 <sup>d</sup>
T7	33.33 <sup>c</sup>	90.67 <sup>c</sup>
T8	43.33 <sup>b</sup>	119.00 <sup>b</sup>
T9	56.33 <sup>a</sup>	137.00 <sup>a</sup>
T10	0.00 <sup>g</sup>	15.00 <sup>g</sup>
SEM ±	2.68*	7.27*

\*Significant at 0.05 level; values with similar superscript alphabets within a column do not vary significantly

**Table 6** Quality indices of *Tectona grandis* seedlings as influenced by different treatments

Treatments	Biovolume	Seedling Quality Index	Mycorrhizal Efficiency Index
T1	140.11 <sup>c</sup>	0.94 <sup>abc</sup>	14.07 <sup>abc</sup>
T2	69.40 <sup>c</sup>	0.83 <sup>bc</sup>	-24.02 <sup>c</sup>
T3	78.83 <sup>c</sup>	0.76 <sup>bc</sup>	-31.16 <sup>c</sup>
T4	75.55 <sup>c</sup>	0.74 <sup>c</sup>	-53.68 <sup>c</sup>
T5	102.69 <sup>c</sup>	0.82 <sup>bc</sup>	-3.26 <sup>bc</sup>
T6	79.77 <sup>c</sup>	0.82 <sup>bc</sup>	-22.27 <sup>c</sup>
T7	149.11 <sup>c</sup>	1.12 <sup>a</sup>	28.38 <sup>abc</sup>
T8	481.66 <sup>b</sup>	1.04 <sup>ab</sup>	77.67 <sup>ab</sup>
T9	818.49 <sup>a</sup>	1.19 <sup>a</sup>	89.23 <sup>a</sup>
T10	75.00 <sup>c</sup>	0.91 <sup>abc</sup>	0.00 <sup>bc</sup>
SEM ±	44.28*	0.04*	10.70*

\*Significant at 0.05 level; values with similar superscript alphabets within a column do not vary significantly

performance of the seedlings inoculated with *G. proliferum* (50 g inoculum) was the result of the higher photosynthetic area and chlorophyll content of the seedlings that might have resulted in the accumulation of more photosynthates, compared to seedlings in other treatments. Similar results were observed for inoculated teak seedlings, wherein a significant increase in plant height, stem girth, plant biomass

and plant phosphorus content were observed especially for *G. leptotichum* treated seedlings (Rajan et al. 2000). Studies on *Azadirachta indica* (Sumana and Bagyaraj 2003), *Casuarina equisetifolia* (Vasanthakrishna et al. 1995), *Dalbergia sissoo* (Sumana & Bagyaraj 1996), *Prunus persica* (Wu et al. 2011), *Anacardium occidentale* (Ananthakrishnan et al. 2004), *Azadirachta excelsa* (Huat et al. 2002), *Acacia mangium* (Ghosh & Verma 2006), *Acacia holosericea* (Duponnois & Plenchette 2003) and *Santalum album* (Binu et al. 2015) confirmed the results.

There are also contradicting reports, where indigenous AMF were found to be ineffective or less effective (Bagyaraj et al. 1989, Reena & Bagyaraj 1990) compared to exotics. Inoculating with unsuitable AMF did not affect collar girth, root weight and root length of sandal seedlings (Binu et al. 2015). *Glomus proliferum* with higher inoculation (50 g inoculum) resulted in a higher plant growth, physiologically sound and quality seedlings. A positive dose response relationship is generally attributed to a better colonisation of rhizosphere by the introduced microorganism (Raaijmakers et al. 1995). Increase in the amount of inoculum generally increases plant protection (Bull et al. 1991, Raaijmakers et al. 1995). Some detrimental effects on root growth were also observed with high inoculation doses (Kapulnik et al. 1985, Bashan 1986). Mycorrhizal inoculation increased plant height, dry matter yield, root length and root infection percentage in *Prosopis cineraria* seedling. The critical level of spores was 400 germinable spores per polybag (1 kg soil), for *P. cineraria* seedling (Verma et al. 2009). In *Tecomella undulata*, 100 g rhizosphere soil (500 germinable spores) of AMF, was found to be the best dose for better growth (Srivastava et al. 2004).

It is well known that the enhanced nutritional status of a plant manifests in its improved growth (Jeffries 1987). Teak plants grown in the presence of AMF showed a general increase in plant growth parameters, such as plant height, stem girth, leaf area and total dry weight, as against those grown in soils uninoculated with AMF. The *G. proliferum* with 50 g inoculum significantly enhanced plant height and collar diameter, compared to other treatments. The total photosynthetic area, expressed as the leaf area, was significantly greater in plants grown in the presence of *G. proliferum* with 50 g inoculum. This increased leaf area and growth of the seedlings, which probably

resulted in significantly higher biomass compared to other treatments. The enhancement in growth and physiology is also related to root colonisation percentage (Table 5).

The physiological growth of teak seedlings, raised in soil inoculated with AMF, was significantly high for parameters leaf area ratio, leaf weight ratio and specific leaf area (Table 2). Greater than 100% increase in leaf weight ratio was observed in seedlings subjected to *G. proliferum* with 25 g inoculum compared to control (T10). The extent of increase in leaf area ratio, leaf weight ratio, specific leaf area, specific leaf weight, relative growth rate and net assimilation rate in seedlings varied among the treatments studied. Seedlings grown in the presence of *G. proliferum* with 50 g inoculum showed significantly higher growth, except absolute growth rate, compared to those grown in lower level (10 g inoculum) of *G. proliferum*. With regard to leaf area ratio, the highest value recorded was 82.34 cm<sup>2</sup> g<sup>-1</sup> for *G. proliferum* with 25 g inoculum (T8). Greater than 100% increase in leaf weight ratio was observed in seedlings subjected to *G. proliferum* with 25 g inoculum, compared to control. Inoculating with AMF improved the physiology of seedlings (Table 4). The high percentage of root colonisation in AM fungal treated plants is directly correlated with a better nutrient uptake, increased total chlorophyll content, increased rate of photosynthesis and transpiration (Rajasekaran & Nagarajan 2005, Azam & Jalil 2007, Dutt et al. 2013), and thereby improved root and shoot growth (Thaker & Fasrai 2002, Farshian et al. 2007). The presence of AMF on root system of plants is correlated with higher rates of net photosynthesis (Reid et al. 1983, Nylund & Unestam 1987). The difference in photosynthetic rate is probably be due to excessive starch accumulation in leaves of seedlings inoculated with AMF. This observation was further strengthened by the present study, as mycorrhizal fungi used in this study significantly improved chlorophyll content and photosynthesis of soil, compared to uninoculated treatment (Table 4). Physiologically sound seedlings can be obtained from seedlings inoculated with *G. proliferum* with 50 g inoculum, but it did not significantly differ from seedlings inoculated with *G. proliferum* of lower levels (10 g inoculum). The *G. proliferum* with 50 g inoculum showed a significantly higher percentage of root colonisation, compared to other treatments

(Table 5). The spore numbers were also highest in soil samples inoculated with *G. proliferum* with 50 g inoculum, indicating better proliferating ability of fungus with teak as the host. This capacity of *G. proliferum* with 50 g inoculum to sporulate and hence multiply is of great significance, as it will not only increase the colonisation of roots but also improve mycorrhizal potential of the soil to which it would be transplanted. The ability of AMF species to improve growth and physiology of teak seedlings, and to sporulate in higher numbers compared to *F. mosseae* and *G. intradices* with various levels of inoculation, indicates its suitability as a species for teak. Further, seedlings raised in the presence of *G. proliferum* with 50 g inoculum showed a greater biovolume index and quality index compared to other treatments (Table 6). The increase was to an extent of 75.00 and 818.49%, respectively, over those seedlings raised in soil uninoculated with AMF. Such high values of biovolume index and quality index indicate a sturdier stem and proportionate top dry weight compared to seedling dry weight, qualities which are desirable among nursery seedlings (Hatchell 1985).

## CONCLUSIONS

It can be concluded that teak seedlings showed varied responses to different levels of AMF, and *G. proliferum* with 50 g inoculum at the time of transplanting, 10 spores g<sup>-1</sup> in nursery, conferred maximum growth benefits compared to other fungi. Further, it showed the ability to proliferate in greater numbers, and enhance physiological aspects and growth of teak seedlings. Teak seedlings raised in the presence of *G. proliferum* may be more established and perform better when planted in degraded and impoverished lands.

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