

**Arbuscular mycorrhization promotes quality stock production of *Aquilaria malaccensis*  
Lamak.: A critically endangered source of agarwood**

**Abstract**

*Aquilaria malaccensis* Lamk., a critically endangered, slow-growing but economically important forest tree species of North-east India. In this study, a biotization experiment was performed to show the effect of arbuscular mycorrhizae on obtaining high-quality agarwood-producing plants. One-month-old seedlings were inoculated in a designed experiment with single and combined endomycorrhizal treatments. All inoculated seedlings showed significant biomass production than control seedlings. The Biovolume index ( $B_i$ ) was higher in all inoculated plant seedlings than in non-inoculated control seedlings but  $EM_2$  (*Acaulospora* species) treatment had a higher  $B_i$  ( $78.17 \pm 0.024$ ) than the rest of the inoculation treatments. The Quality index ( $Q_i$ ) value was also high ( $1.22 \pm 0.024$ ) in *Acaulospora* species ( $EM_2$ ) treatment followed by the *Glomus* species + *Acaulospora* species ( $EM_1+EM_2$ ) treatment ( $1.10 \pm 0.031$ ) and *Glomus* species ( $EM_1$ ) ( $0.83 \pm 0.014$ ) treatment. Control seedlings had a lower value ( $0.14 \pm 0.021$ ) of  $Q_i$  than the rest of the treatments. The plastochron interval index ( $P_i$ ) of *A. malaccensis* after 60 days of inoculation was low in  $EM_1+EM_2$  treatment as the time interval for initiation of 2<sup>nd</sup> leaf was 1 day than rest of the treatments in which the time interval was 4 days for initiation of the 2<sup>nd</sup> leaf primordia. Substantially, the leaf primordia appearance in the  $EM_1+EM_2$  treatment was impetuous than rest of the treatments. In the control treatment, the 8<sup>th</sup> leaf primordia appeared on the 36<sup>th</sup> day and after that, there was no appearance of leaf primordia. Therefore, the  $EM_2$  treatment was the best single/alone treatment of mycorrhizal inoculation followed by  $EM_1+EM_2$  synergistic treatment for quality stock production of *A. malaccensis* seedlings.

**Keywords:** AM fungi, Biotization, Biovolume index, Growth promotion, Plastochron interval index, Quality index

**1. Introduction**

Agarwood (Aloeswood, Eaglewood, Lign-aloes), traded in several forms ranging from round-wood to processed products e.g., medicine, sedatives, incense, and perfume, is one of the most promising commercial products of the world and it has considerable economic importance (Saikia and Khan 2012). Although *A. malaccensis* Lamk., locally known as “Sanchi or Agaru”, is the best-known species of agarwood, its large-scale harvesting has caused rapid depletion of the stock in the natural forests of India (Saikia and Khan 2014). According to the IUCN Red List, the species is globally Vulnerable A1cd (Ver 2.3; IUCN 2017), ‘Critically Endangered’ in India (IUCN 2018) due to the continued unsustainable exploitation, leading to decline of the population by over 80%. Although the government takes action to bring international trade within sustainable limits, various studies have also revealed the population dynamics of this important tree species in home gardens of north-east India which have contributed towards the

conservation, artificial regeneration, and management of this species (Saikia and Khan 2013). The species is commonly cultivated in the home gardens of Upper Assam in association with other useful plants for its high commercial value and conservation (Saikia and Khan 2016).

*A. malaccensis* Lamk. is a semi-tolerant tree species and difficult to plant this species directly in open land so requires a variety of treatments like mycorrhization (Muin 2019). The occurrence of mycorrhizal fungi in soils, its association with forest trees and crops, its influence on plant growth, nutrition uptake, and disease resistance are well documented by various workers (Parkash *et al.* 2005). The role of Arbuscular Mycorrhizal (AM) fungi in improving the quality and survival of plant seedlings and their growth after plantation has been well recognized (Tanwar *et al.* 2014). Application of mycorrhizal fungi can enhance the growth of agarwood-producing plants even in greenhouse conditions and open land. (Rini *et al.* 2020; Husna *et al.* 2021; Yuwono *et al.* 2021; Benni *et al.* 2023). However, there are no reports of bio-inoculation effect on morpho-metrics on *A. malaccensis* in India. Although, AM inoculation affected damping off disease in the said plant (Tabin *et al.* 2009), growth effect on *A. malaccensis* and *A. crasna* has been reported (Turjaman *et al.* 2006). The objective of the present study was to screen the dominant and efficient strains from the rhizosphere of *A. malaccensis* and their utilization to get a good yield of biomass through bio-inoculation after mass production of bio-inoculants as this tree species is slow-growing. So, trials were carried out in pots for inoculating the plant seedlings with efficient and dominant strains of AM fungi alone and in combination.

## **2. Material and methods**

### **2.1 Sampling of root and rhizospheric samples**

Root and soil samples of the target plant were collected at the flowering and fruiting stage. These samples were taken by digging out a small amount of soil close to plant roots up to the depth of 15–30 cm and kept in sterilized polythene bags at 5–10°C for further processing in the laboratory for mycorrhizal quantification and root colonization.

### **2.2 Isolation, quantification, root colonization, and mass multiplication of AM spores**

Isolation of AM spores was done by using a “wet sieving and decanting technique” (Singh and Tiwari 2001). The quantitative estimation of the AM spores will be done by modified method of Adholeya and Gaur (1994). The root colonization will be studied by the ‘Rapid clearing and staining technique’ (Phillips and Hayman 1970). The mycorrhizal inoculum production will be done by using the ‘soil funnel technique’ (Menge and Timmer 1982).

### **2.3 Cultivation and Growth Studies**

The plantlets of *A. malaccensis* Lamk. were raised with the help of AM inoculation in root trainers in laboratory conditions. These inoculated seedlings were transplanted in bigger pots and then in field conditions again with mycorrhizal inoculation for primary establishment and better growth of quality seedlings. The experiment, set up in Randomized Block Design (RBD) was conducted in the nursery of

Rain Forest Research Institute, located at a distance of 10 km East of Jorhat City, Assam, India (26°46'53"N 94°17'29"E, 107 msl). The annual average precipitation is 500 mm and the annual average temperature is 26°C. The bio-inocula taken were of two different genera with isolates/strains of endomycorrhizae *i.e.*, EM<sub>1</sub> (*Glomus* species consortium containing *Glomus invermaium*, *G. mosseae*, and *G. maculosum*) and EM<sub>2</sub> (*Acaulospora* species consortium containing *Acaulospora trappae*, *A. Elegans* and *A. lacunosa*) and their mixed consortium (EM<sub>1</sub> + EM<sub>2</sub>). In control sets, no AM inoculum was given. Five replications of each treatment were taken (see Fig. 1)

Observations were recorded to see the AM inoculation effect on plant seedlings for parameters such as soil Temperature, pH, increase in height, AM spore count, and total colonization in root (%) after specific time intervals (up to 180 days after inoculation /DAI).

**2.4 Biomass estimation:** Shoot biomass and Root biomass of plants were calculated using the following formulae.

$$\text{Shoot biomass} = \frac{F_{w(s)} - D_{w(s)}}{F_{w(s)}} \times 100$$

$$\text{Root biomass} = \frac{F_{w(r)} - D_{w(r)}}{F_{w(r)}} \times 100$$

**Total biomass = Shoot biomass + Root biomass**

Where, F<sub>w(s)</sub> = Fresh weight of shoot, F<sub>w(r)</sub> = Fresh weight of root, D<sub>w(s)</sub> = Dry weight of shoot, D<sub>w(r)</sub> = Dry weight of root

**2.5 Biovolume index:** The biovolume index of the seedlings was calculated using the following formula (Parkash *et al.* 2011; Basumatary *et al.* 2014).

$$B_i = D^2 \times H$$

Where, B<sub>i</sub> = Biovolume index, H= Height of seedlings in cm, D= Diameter of stem in mm/cm

**2.6 Quality index:** The quality index to assess the quality of seedlings was calculated using the following formula (Parkash *et al.* 2011; Basumatary *et al.* 2014).

$$Q_i = D_{w(s)} / [H/D + D_{w(s)} / D_{w(r)}]$$

Where, D<sub>w(s)</sub> = Dry weight of seedling, H= Height of seedlings in cm, D= Diameter of stem in mm/cm; D<sub>w(s)</sub> = Dry weight of shoot, D<sub>w(r)</sub> = Dry weight of root

**2.7 Plastochron Index:** The plastochron index is generally calculated by the formula derived by Erickson and Michelini (1957).

$$P_i = n + (\ln L_n - \ln R) / (\ln L_n - \ln L_{n+1})$$

Where, n= the sequential index number of the organ for which the PI is being calculated with n increasing in an acropetal order, n = 0 when leaves on seedlings are being studied, R= Reference length of an organ, Ln= Length of an organ that is equal to or slightly longer than R, Ln+1= Length of an organ that is just slightly shorter than R.

**2.8 Mycorrhizal Efficiency:** Mycorrhizal Efficiency of the AM strains was calculated using the following formula.

$$\text{Shoot Mycorrhizal Efficiency (MEs)} = \frac{F_{w(s)} - D_{w(cs)}}{F_{w(s)}} \times 100$$

$$\text{Root Mycorrhizal Efficiency (MEr)} = \frac{F_{w(r)} - D_{w(cr)}}{F_{w(r)}} \times 100$$

$$\text{Mycorrhizal Efficiency of strains (ME strains)} = \text{MEs} + \text{MEr}$$

Where,  $F_{w(s)}$  = Fresh weight of shoot,  $F_{w(r)}$  = Fresh weight of root,  $D_{w(cs)}$  = Dry weight of shoot of control,  $D_{w(cr)}$  = Dry weight of root of control

### Statistical analyses

The standard error of mean and coefficient of variance were calculated for all parameters studied. MS Excel software 2021 was used for the data analysis.

### 3 Results

The effects of AM inoculation on *A. malaccensis* after 30, 60, 90, 120, and 180 days are shown in Tables 1 to 5. The analysis was carried out on different parameters such as soil Temperature, pH, increase in height, AM spore count, and total colonization in root (%).

After 30 days of inoculation, pH was high ( $6.1 \pm 0.72$ ) in the case of control treatment whereas low ( $5.60 \pm 0.22$ ) in EM<sub>2</sub> treatment. The soil temperature was more or less stable ( $26^\circ\text{C}$ ) in all the treatments. The maximum increase in height ( $19.7 \pm 0.29\text{cm}$ ) was observed in the case of EM<sub>1</sub> inoculated plants while the minimum ( $12.3 \pm 0.16\text{cm}$ ) was reported in control/non-inoculated plants. The maximum AM spore count ( $11 \pm 1.41$  per 50gm soil), was in EM<sub>1</sub> + EM<sub>2</sub> treatment while the minimum ( $1.0 \pm 0.47$ ) was in control/non-inoculated plants. The total root colonization percentage ( $100 \pm 0\%$ ) was high in the case of EM<sub>1</sub> + EM<sub>2</sub> treatment while low ( $40 \pm 0.82$ ) in control/non-inoculated plants (see Table 1).

The soil temperature ( $25^\circ\text{C}$ ) in all treatments was stable after 60 days. The control treatment showed a higher pH ( $6.22 \pm 0.10$ ) than the EM<sub>2</sub> treatment in which the pH was low ( $5.76 \pm 0.24$ ). The maximum increase in height ( $26 \pm 0.44\text{cm}$ ) was observed in EM<sub>1</sub> inoculated plantlets. The minimum increase in height ( $14.3 \pm 0.42\text{cm}$ ) was observed in control/non-inoculated plants. The maximum AM spore count ( $26 \pm 1.69$ )

per 50gm soil) was observed in the EM<sub>1</sub> + EM<sub>2</sub> treated seedlings while control/non-inoculated seedlings had minimum AM spore count (12±2.16). The maximum total root colonization percentage (100±0%) was seen in all inoculated plants than in control/non-inoculated plants in which the total root colonization percentage was low (60±0.40) (see Table 2).

After 90 days of inoculation, all treatments had constant soil temperature (24 °C) including control/non-inoculated treatment. The pH (6.19±0.069) was observed high in the case of control plantlets whereas low pH (5.78±0.094) was observed in EM<sub>1</sub> treatment. The maximum increase in height (32.7±0.86 cm) was observed in the EM<sub>1</sub> treatment while the low increase in height (16.7±0.45 cm) was observed in control/non-inoculated plants. The maximum AM spore count (27±1.24 per 50g soil) was seen in EM<sub>1</sub> + EM<sub>2</sub> treatment whereas AM spore number was low in control/non-inoculated plants. The maximum total root colonization percentage (100±0%) was seen in all the inoculated plantlets than in control/non-inoculated plants in which it was low (70±0.82%) (see Table 3).

After 120 days of inoculation, the soil temperature (21 °C) was invariable in all the treatments than the control in which it was high (22 °C). The maximum pH (6.29±0.09) was measured in EM<sub>1</sub> + EM<sub>2</sub> treatment and the minimum (5.87±0.25) was measured in EM<sub>2</sub>. The maximum increase in height (40±0.84cm) was observed in EM<sub>1</sub> inoculated plants/treatment while a minimum (18±0.37cm) increase in height was recorded in control/non-inoculated plants. The maximum AM spore count (129±0.41per 50g soil) was reported in the case of EM<sub>1</sub> + EM<sub>2</sub> treatment while the minimum (60±0.27) AM spore count was observed in control/non-inoculated plants. The maximum total root colonization percentage (100±0%) was seen in all inoculated plants than in control/non-inoculated plants in which the total root colonization percentage was (90±0.94%) (see Table 4).

After 180 days, the soil temperature remained the same (20 °C) in all treatments including control treatment. All the treatments including the control had acidic pH values but EM<sub>1</sub> + EM<sub>2</sub> inoculated plants had slightly higher pH (6.3±0.14) than the rest of the treatments. The maximum increase in height (46.7±0.28cm) was observed in EM<sub>1</sub>-inoculated plants. The maximum AM spore count (237±0.24 per 50g soil) was observed in the EM<sub>1</sub> + EM<sub>2</sub> treatment. The maximum total root colonization percentage (100±0%) was observed in all inoculated seedlings whereas total root colonization percentage (80±0%) was low in control/non-inoculated plants (see Table 5). Fig. 2 graphically represents the trends of increase in height, in all inoculated and non-inoculated seedlings.

The increase in circumference, diameter, and biomass yield of *A. malaccensis* after 180 DAI is shown in Figure 2. The increase in circumference and diameter in control/non-inoculated seedlings were minimal (0.03±0.02cm, 0.06±0.01cm) respectively in comparison to all treatments/inoculated seedlings. The increase in circumference (1.01±0.021cm) and diameter (2.02±0.022cm) were reported in EM<sub>2</sub> treatment. The biomass yield of seedlings was considerably higher in inoculated seedlings than in control/non-inoculated seedlings. A higher biomass yield (82.38±0.24g) was reported in EM<sub>1</sub> treatment followed by

EM<sub>2</sub> treatment and EM<sub>1</sub> +EM<sub>2</sub> treatment which had a moderate biomass yield (80.01±0.28g and 77.91±0.26 g) respectively. The lowest biomass yield (44.94±0.12g) was reported in control/non-inoculated plants (see Fig. 3).

B<sub>i</sub>, Q<sub>i</sub>, and M<sub>e</sub> of AM strains are represented in Figure 3. The B<sub>i</sub> was higher in all inoculated plants than in non-inoculated control plants but EM<sub>2</sub> treatment had a maximum B<sub>i</sub> (78.17±0.024) than the rest of the inoculation treatments. The Q<sub>i</sub> value was also higher (1.22±0.024) in EM<sub>2</sub> treatment than EM<sub>1</sub>+EM<sub>2</sub> (1.10±0.031) treatment and EM<sub>1</sub> (0.83±0.014) treatment. Control seedlings had a lower value (0.14±0.021) of Q<sub>i</sub> than the rest of the treatments. The maximum M<sub>e</sub> of AM strains was found approximately equal (102.06± 0.076 & 102.01±0.076) in EM<sub>2</sub> and EM<sub>1</sub>+ EM<sub>2</sub> treatments followed by EM<sub>1</sub> treatment having low M<sub>e</sub> (90.93±0.084) of AM strains. The control/non-inoculated plants had zero (0±0) M<sub>e</sub> of AM strains because no inoculum/AM strain was added in this treatment (see Fig. 4).

The Pi of *A. malaccensis* after 60 days of inoculation/first-stage inoculation has been shown in Figure 4. The time interval for initiation of 2<sup>nd</sup> leaf primordia in treatment/inoculation of EM<sub>1</sub>+EM<sub>2</sub> was 1 day than the rest of the treatments (EM<sub>1</sub>, EM<sub>2</sub>, and Control) in which the time interval was 4 days for initiation of the 2<sup>nd</sup> leaf primordia. The appearance of 10<sup>th</sup> leaf primordia in treatment/inoculation of EM<sub>1</sub> was observed on the 41<sup>st</sup> day, thereafter no leaf primordia observance was reported. Similarly, the appearance of 13<sup>th</sup> leaf primordia in treatment/inoculation of EM<sub>2</sub> was observed on the 41<sup>st</sup> day, thereafter no leaf primordia observance was reported in this treatment also. Substantially, the leaf primordia appearance in the treatment/inoculation of EM<sub>1</sub>+EM<sub>2</sub> was spontaneous and it was the 17<sup>th</sup> leaf primordia that appeared after 41 days interval, and still, the leaf primordia continued their appearance afterward. But in the Control treatment (without any bio-agent), the 8<sup>th</sup> leaf primordia appeared on the 36<sup>th</sup> day and after that, there was no appearance of leaf primordia. The time interval was maximum (16 days) between the 7<sup>th</sup> to 8<sup>th</sup> leaf primordia appearance in non-inoculated control plants (see Fig. 5).

#### 4 Discussion

The present study observed that the EM<sub>2</sub> treatment was the best single/alone treatment of bio-inoculation followed by EM<sub>1</sub>+EM<sub>2</sub> synergistic treatment than non-inoculated control seedlings, for quality stock production of *A. malaccensis* seedlings. In the case of plastochron interval index, it was observed that *A. malaccensis* seedlings inoculated with EM<sub>1</sub>+EM<sub>2</sub> synergistic treatment showed the emergence of leaf primordia. In contrast, EM<sub>1</sub> in a single treatment resulted in the appearance of leaf primordia, till the 60<sup>th</sup> day of inoculation.

Mycorrhizal infection improving the growth of plants through nutrient uptake by increasing the absorbing surface area of roots (Wang *et al.* 2017). In the present study, soil-based inoculum was used for all the experiments. Hence, the better growth responses were seen. This might be due to the higher reproduction of VAM fungi present in the soil-based inoculum, which sprouted rapidly from extracellular and intracellular hyphae present in the soil and root inoculum. The root colonization by mycorrhiza was directly related to

nutrient uptake by plants. The lowest nutrient uptake was observed in non-mycorrhizal plants and the highest nutrient in mycorrhizal plants grown in sterilized soil. The number of mycorrhizal spores was also higher in mycorrhizal inoculated soil and directly related to mycorrhizal root colonization. The plants with the highest root colonization showed a greater number of mycorrhizal spores in the soil (Salim *et al.* 2020). In the present investigation, the same trend was observed in both the plant seedlings as far as mycorrhizal spore number and root colonization were concerned after 120-180 days.

The role of AM fungi in improving the quality and survival of plant seedlings and their growth after plantation has been well recognized (Parkash *et al.* 2011). Restoration of lands devastated by resource extraction is an immediate priority and a challenging task for arid land ecologists. During the last two decades, more stress has been given to using mycorrhizal fungi to restore land programs (de Moura *et al.* 2022). Inoculation with suitable AM fungal strains to improve the growth and survival of plant seedlings in forestation is essential.

Nelson *et al.* (2000) observed that AM inoculation improved the growth of the *Santalum album* L. seedlings as indicated by increased shoot and root length, stem thickness, and surface area of leaves when inoculated with *Glomus fasciculatum*. The shoot length increased by 66.2%, fresh weight by 96.4%, and plant biomass by 94.7% over the control plants. The effects of mycorrhizal inoculation on plant growth may also be due to improved rhizogenesis of the otherwise poorly rooted transplants, as has been suggested with other crops (Youpensuk *et al.* 2005). AM fungi also play an important role in the conservation of endangered tree species. Turjaman *et al.* (2006) revealed the positive effect of AM symbiosis in the establishment of seedling stock production of *Aquilaria* sp. on the nursery scale. AM inoculation improves plant-soil interaction by enhancing nutrient status and protecting the host plant against pathogens (Tabin *et al.* 2009). Recently Husna *et al.* (2021) studied the effect of AM fungi on the growth of tropical endangered species *Pterocarpus indicus* and *Pericopsis mooniana* in Southeast Sulawesi, Indonesia. Inoculation of *Glomus* sp. and consortium of *Glomus* sp. with *Gigaspora* sp. improve the agarwood seedling growth by increasing seedling height, total leaf area, and total biomass (Rini *et al.* 2020). Mycorrhizal fungi act as the best ameliorant in promoting *A. malaccensis* tree growth for revegetation of post-mining limestone land (Yuwono *et al.* 2021) and former gold mining soil (Satria *et al.* 2023). These previous studies support the present study which also revealed the positive effect of AM inoculation in enhancing various morphometric parameters of *A. malaccensis* Lamk.

In this study, the consortium of *Glomus* species and *Acaulospora* species increased the spore production and root colonization with the passage of experiment time. The greater root length and number of root branches probably indicate that the mycorrhizal plant has a higher potential for uptake and absorption of relatively mobile nutrients through exploration of a greater soil volume. This subsequently, results in higher nutrient concentrations in the shoots of these plants. The inoculated seedlings in this study also had a more pronounced root length than non-inoculated control plants. High root colonization of inoculated plants, subsequently, greater absorption of nutrients could also result in greater production of

leaf area, *i.e.*, increases the area for photosynthesis and also minimizes the time interval between the appearance of two leaf primordia *i.e.* plastochron index

## 5 Conclusion

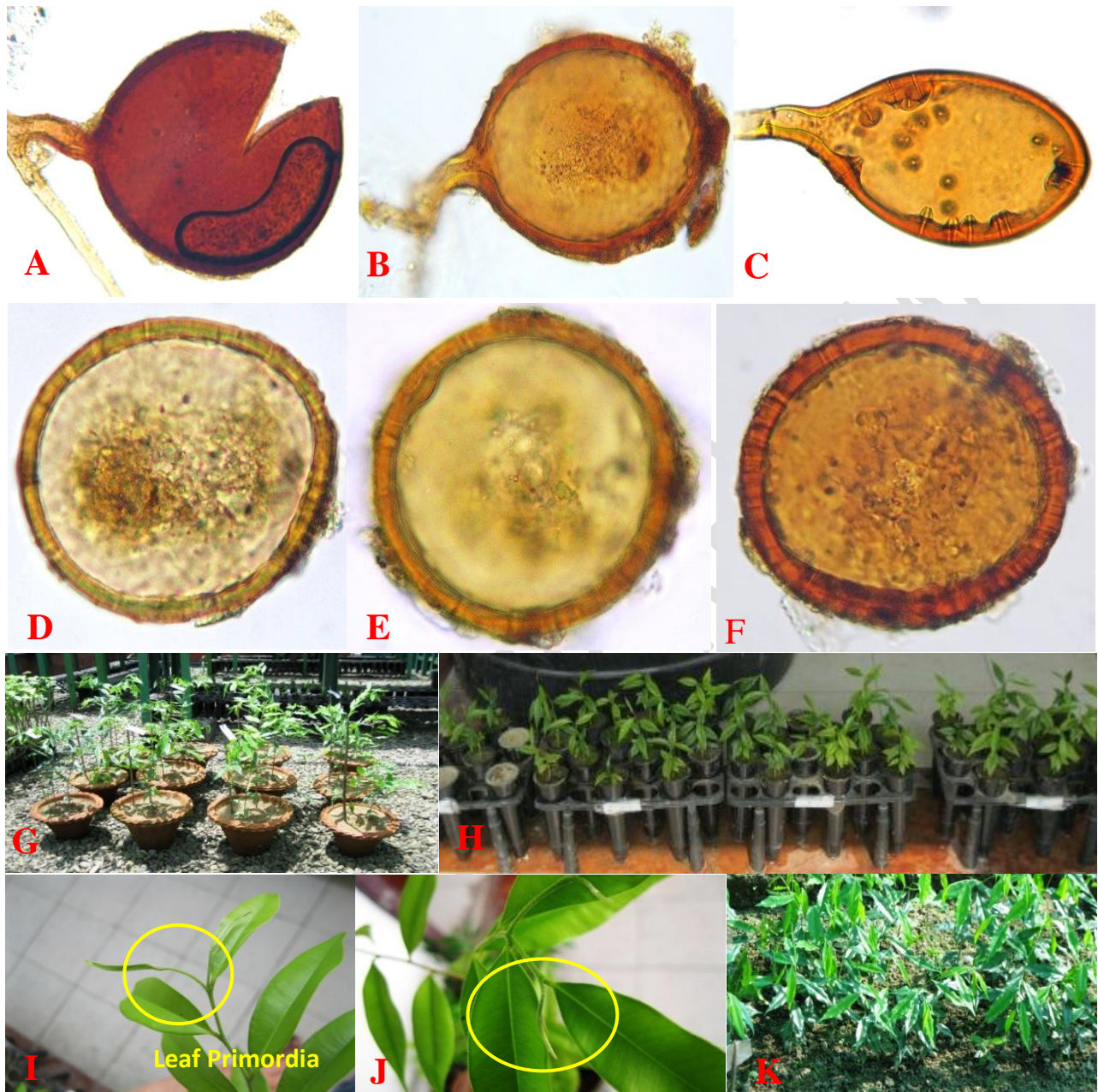
Mycorrhizae are a large component of the soil ecosystem, either due to their ubiquitous nature or the benefits that can be derived from them. Mycorrhizae can contribute to and serve as indicators of plant and soil health. They are essential in the establishment and survival of plants. Therefore, based on the results of different parameters undertaken, the bio-inoculation improves the quality stock production of *A. malaccensis* seedlings than non-inoculated control seedlings. The EM<sub>2</sub> treatment was the best single treatment of bio-inoculation followed by the EM<sub>1</sub>+EM<sub>2</sub> synergistic treatment for quality stock production of *A. malaccensis* seedlings. Although few studies reveal the effect of mycorrhizal inoculation on the growth of *A. malaccensis*, in India there is no confirmed study on the application of mycorrhiza for the quality stock production of this critically endangered tree species. The present study becomes helpful in the regeneration and conservation of this economically important tree species and also increases the survival rate of *A. malaccensis* seedlings for the success of reforestation programs.

## References

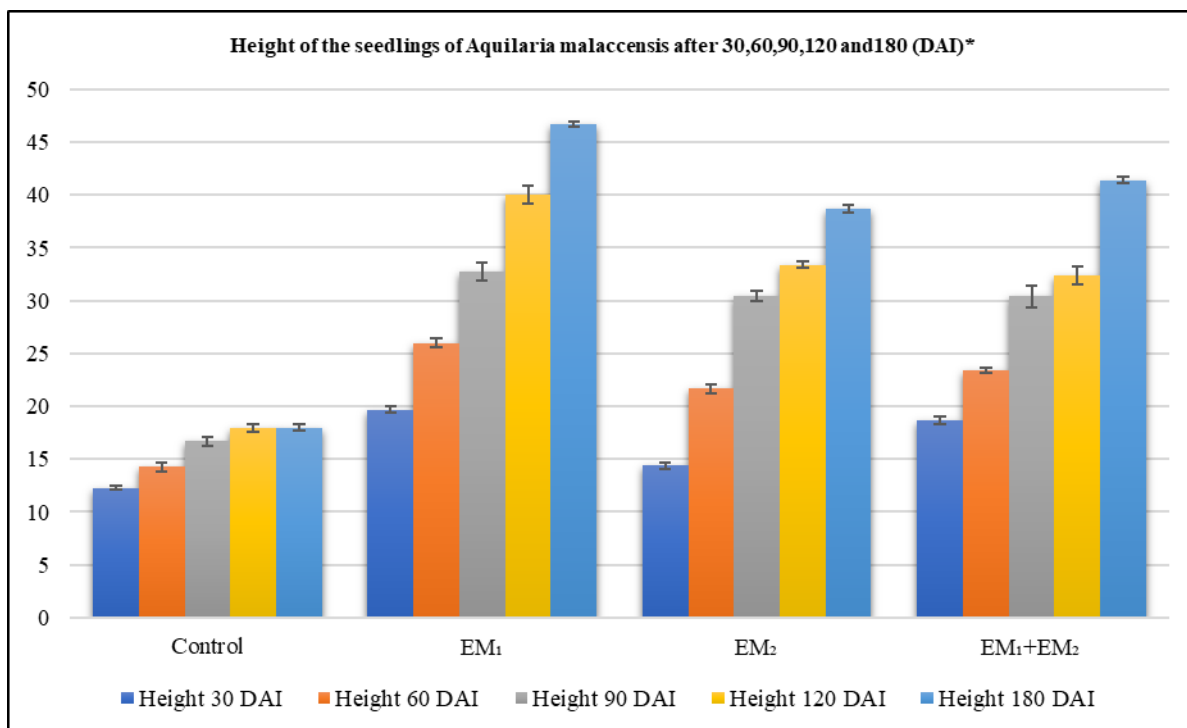
- Adholeya A., Gaur A. 1994. Estimation of VAM fungal spores in soil. *Mycorrhiza News* 61: 10-11.
- Basumatary N., Parkash V., Tamuli A.K., Saikia A.J., Teron R. 2014. Arbuscular mycorrhizal inoculation affects growth and rhizospheric nutrient availability in *Hevea brasiliensis* (Willd. Ex. A. Juss.) Müll. Arg. Clones. *International Journal of Current Biotechnology* 2(7): 14-23.
- de Moura M.A., Oki Y., Arantes-Garcia L., Cornelissen T., Nunes Y.R.F., Fernandes G.W. 2022. Mycorrhiza fungi application as a successful tool for worldwide mine land restoration: Current state of knowledge and the way forward. *Ecological Engineering* 178: 106580. DOI: [10.1016/j.ecoleng.2022.106580](https://doi.org/10.1016/j.ecoleng.2022.106580)
- Erickson R.O., Michelini F.J. 1957. The plastochron index. *American Journal of Botany* 44(4): 297-305.
- Husna H., Tuheteru F.D., Arif A. 2021. Arbuscular mycorrhizal fungi to enhance the growth of tropical endangered species *Pterocarpus indicus* and *Pericopsis mooniana* in post gold mine field in Southeast Sulawesi, Indonesia. *Biodiversitas Journal of Biological Diversity* 22(9): 3844-3853. DOI: [10.13057/biodiv/d220930](https://doi.org/10.13057/biodiv/d220930)
- IUCN 2017. The IUCN Red List of Threatened Species. Version 2017-3. International Union for Conservation of Nature. Accessed at <http://www.iucnredlist.org>. Downloaded on 27 December 2017.
- IUCN. 2009. Asian Regional Workshop (Conservation & Sustainable Management of Trees, Viet Nam) 1998. *Aquilaria malaccensis*. In: IUCN 2009. IUCN Red List of Threatened Species. Version 2009.2.
- Menge J.A., Timmer L.W. 1982. Procedure for Inoculation of Plants with Vesicular-Arbuscular Mycorrhizae in Laboratory, Greenhouse and Field. In: Schenck, N.C., Ed., *Methods and Principles of Mycorrhizal Research*, American Phytopathological Society, St. Paul.
- Muin A. 2019. The Utilization of Arbuscular Mycorrhizal Fungi for Planting Agarwood (*Aquilaria* spp) Seedling in Open Land. *Microbiology Indonesia* 13(3): 83-89. DOI: [10.5454/mi.13.3.2](https://doi.org/10.5454/mi.13.3.2)
- Nelson R., Krishnamurthy K.V., Senthilkumar S. 2000. Growth and stimulation of *Santalum album* seedlings by vesicular–arbuscular mycorrhizal fungi. *Mycorrhiza News* 12(2): 14-15.
- Ng L.T., Chang Y.S., Kadir A.A. 1997. A review on agar (gaharu) producing *Aquilaria* species. *Journal of Tropical Forest Products* 2(2): 272-285.

- Parkash V., Aggarwal A., Sharma S., Sharma D. 2005. Effect of endophytic mycorrhizae and fungal bioagent on the development and growth of *Eucalyptus saligna* Sm. seedlings. Bulletin of National Institute of Ecology 15: 127-131. DOI: <http://dx.doi.org/10.17221/120/2008-JFS>
- Parkash V., Aggarwal A., Sharma V. 2011. Rhizospheric effect of vesicular-arbuscular mycorrhizal inoculation on biomass production of *Ruta graveolens* L.: a potential medicinal and aromatic herb. Journal of plant nutrition 34(9): 1386-1396. DOI: <http://dx.doi.org/10.1080/01904167.2011.581044>
- Phillips J. M., Hayman D.S. 1970. Improved produces for clearing roots and staining parasitic and VAM fungi for rapid assessment of infection. Transactions of the British Mycological Society 55(1): 158-161. DOI: [https://doi.org/10.1016/S0007-1536\(70\)80110-3](https://doi.org/10.1016/S0007-1536(70)80110-3)
- Rini M.V., Susilowati E., Riniarti M., Lukman I. 2020. Application of *Glomus* sp. and a mix of *Glomus* sp. with *Gigaspora* sp. in improving the Agarwood (*Aquilaria malaccensis* Lamk.) seedling growth in Ultisol soil. Vol. 449(1), p. 012004. In: IOP Conference Series: Earth and Environmental Science. April, 2020. IOP Publishing. DOI: [10.1088/1755-1315/449/1/012004](https://doi.org/10.1088/1755-1315/449/1/012004)
- Saikia P., Khan M. L. 2014. Homegardens of upper Assam, northeast India: a typical example of on-farm conservation of Agarwood (*Aquilaria malaccensis* Lamk.). International Journal of Biodiversity Science, Ecosystem Services & Management 10(4): 262-269. DOI: [10.1080/21513732.2014.973449](https://doi.org/10.1080/21513732.2014.973449)
- Saikia P., Khan M.L. 2012. Agar (*Aquilaria malaccensis* Lamk.): a promising crop in the homegardens of Upper Assam, northeastern India. Journal of Tropical Agriculture 50 (1-2): 8-14.
- Saikia P., Khan M.L. 2013. Population structure and regeneration status of *Aquilaria malaccensis* Lamk. in homegardens of Upper Assam, northeast India. Tropical Ecology 54(1): 1-13.
- Saikia P., Khan M.L. 2016. Tree species diversity and its population and regeneration status in homegardens of Upper Assam, Northeast India. Journal of Forest and Environmental Science 32(2): 129-139. DOI: [10.7747/JFES.2016.32.2.129](https://doi.org/10.7747/JFES.2016.32.2.129)
- Salim M.A., Setyaningsih L., Iskandar I., Wahyudi I., kirmi H. 2020. Root colonization by arbuscular mycorrhizal fungi (AMF) in various age classes of revegetation post-coal mine. Biodiversitas Journal of Biological Diversity 21(11): 5011-5022 DOI: [10.13057/biodiv/d211105](https://doi.org/10.13057/biodiv/d211105)
- Satria B., Martinsyah R.H., Armansyah A., Erona M., Warnita W. 2023. The Influence of Arbuscular Mycorrhizal Fungi (AMF) Dosage and Yomari Liquid Organic Fertilizer on the Growth of Seedlings of Agarwood-Producing Plants (*Aquilaria malaccensis* Lamk.) on Former Gold Mining Soil. International Journal of Environment, Agriculture and Biotechnology 8(6): 073-084. DOI: <https://dx.doi.org/10.22161/ijeab.86.10>
- Singh S.S., Tiwari S.C. 2001. Modified wet sieving and decanting technique for enhanced recovery of spores of vesicular-arbuscular mycorrhizal (VAM) fungi in forest soils. Mycorrhiza News 12(4): 12-13.
- Tabin T., Arunachalam A., Shrivastava K., Arunachalam K. 2009. Effect of arbuscular mycorrhizal fungi on damping-off disease in *Aquilaria agallocha* Roxb. seedlings. Tropical Ecology 50(2): 243-248.
- Tanwar A, Aggarwal A, Parkash V (2014). Effect of bioinoculants and superphosphate fertilizer on the growth and yield of broccoli (*Brassica oleracea* L. var. italic Plenck). New Zealand Journal of Crop and Horticultural Science 42(4): 288-302. <https://doi.org/10.1080/01140671.2014.924537>
- Turjaman M., Santoso E., Sumarna Y. 2006. Arbuscular Mycorrhizal Fungi Increased Early Growth of Gaharu Wood of *Aquilaria malaccensis* and *A. crasna* Under Greenhouse Conditions. Indonesian Journal of Forestry Research 3(2): 139-148. DOI: [10.20886/ijfr.2006.3.2.139-148](https://doi.org/10.20886/ijfr.2006.3.2.139-148)
- Wang W., Shi J., Xie Q., Jiang Y., Yu N., Wang E. 2017. Nutrient exchange and regulation in arbuscular mycorrhizal symbiosis. Molecular Plant 10(9): 1147-1158. DOI: <http://dx.doi.org/10.1016/j.molp.2017.07.012>
- Youpensuk S., Rerkasem B., Dell B, Lumyong S (2005). Effects of Arbuscular mycorrhizal fungi on a fallow enriching tree (*Macaranga denticulata*). Fungal Diversity 18(1): 189-199.
- Yuwono S.B., Alawiyah A., Riniarti M., Dermiyati D. 2021. Revegetation of Critical Land with Gaharu (*Aquilaria malaccensis*) under Various Ameliorants Application. Journal of Tropical Soils 26(1): 19-28. DOI: [10.5400/jts.2021.v26i1.19-28](https://doi.org/10.5400/jts.2021.v26i1.19-28)

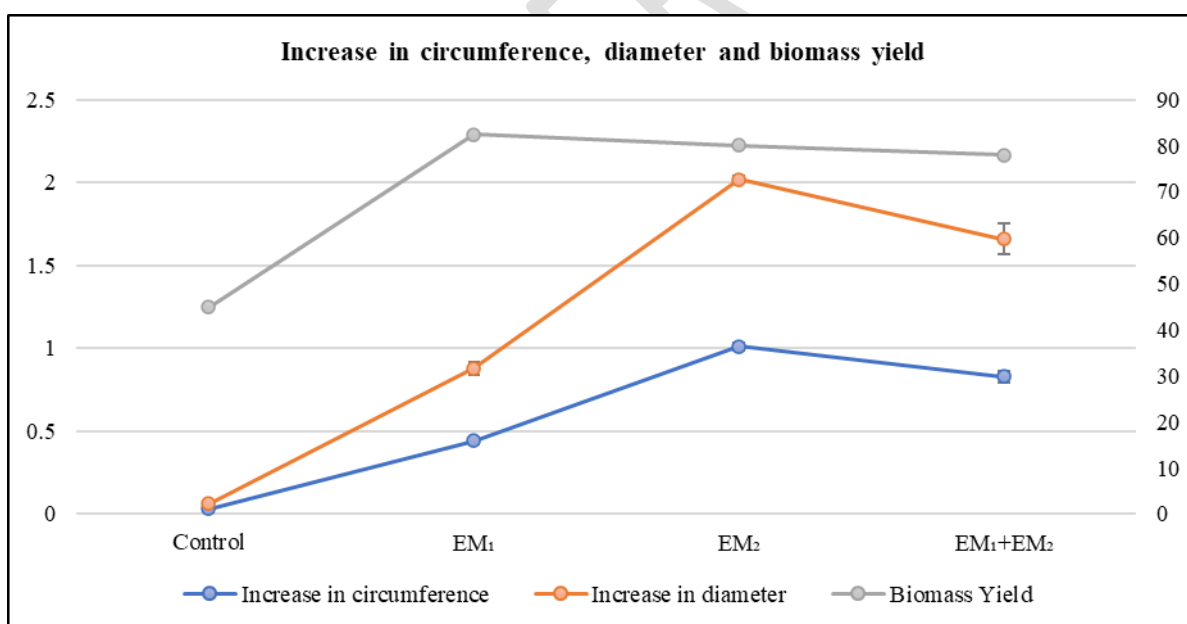
## Figures & Tables



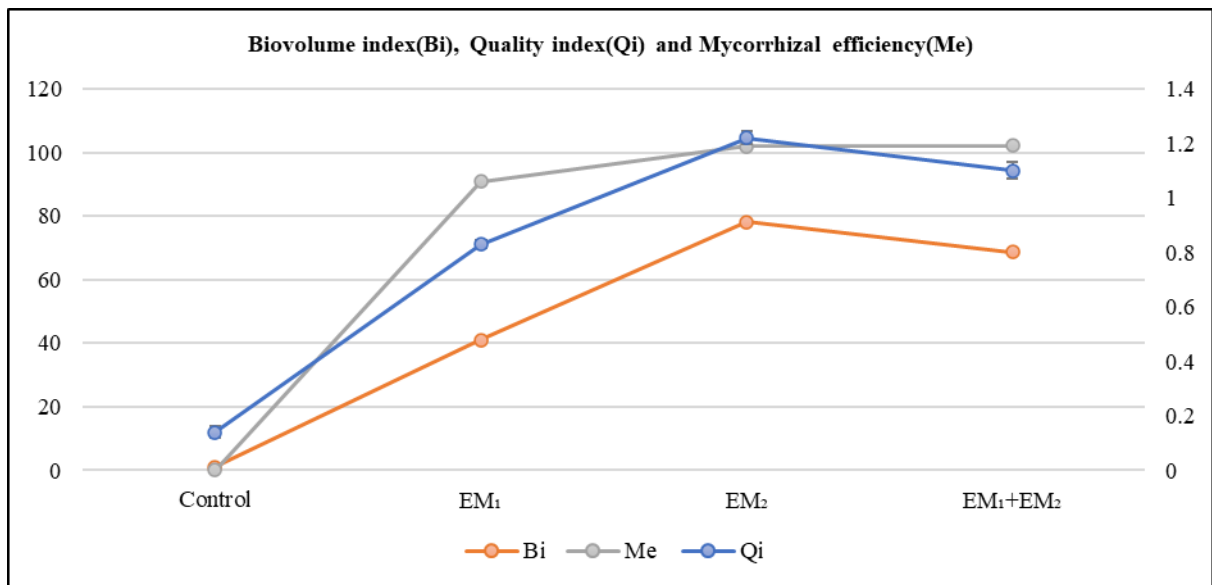
**Fig. 1:** *Glomus* species consortium (left to right; A. *G. invermaium* B. *G. mosseae* C. *G. maculosum*), *Acaulospora* species consortium (left to right; D. *A. trappae*, E. *A. elegans* F. *A. lacunosa*), G. Bioinoculation experiment in pot condition on *A. malaccensis*, H. Raising of *Aquilaria malaccensis* seedlings with AM inoculation, I- J. Leaf primordia initiation due to AM Inoculation, K. Quality stock of *A. malaccensis* in field condition, (Yellow circles show the leaf primordia initiation and appearance in inoculated seedling)



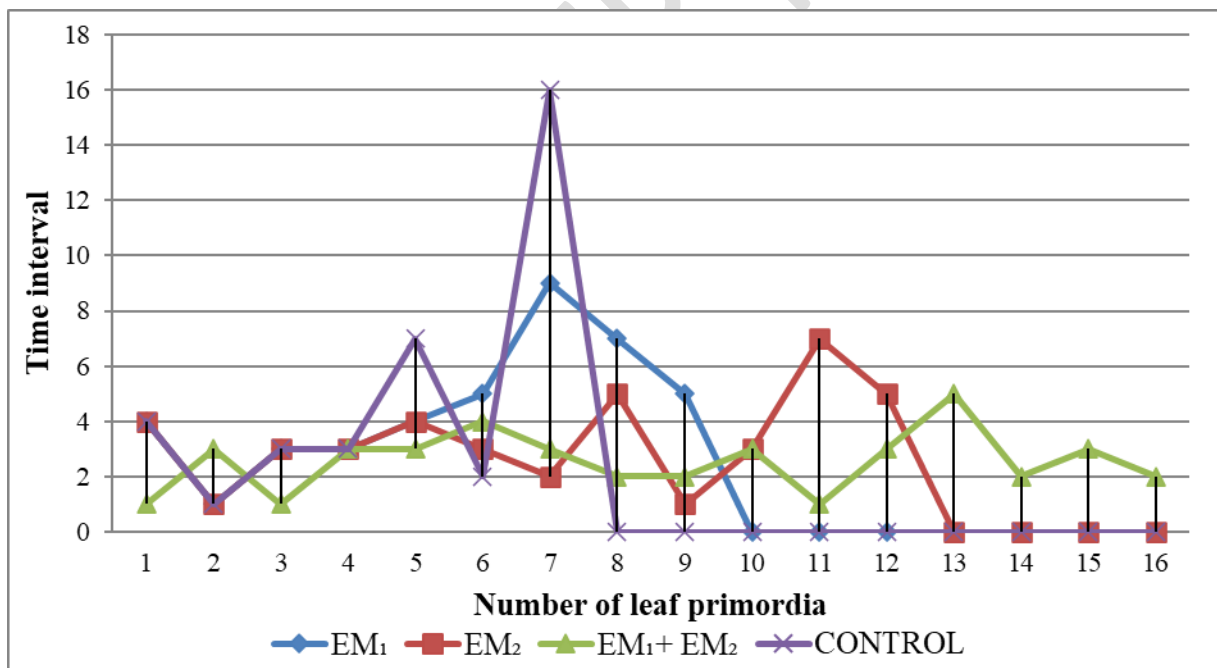
**Fig. 2: Effect of Endomycorrhizal inoculation on height of *A. malaccensis* seedlings after 30,60,90,120 and180 (DAI) \***



**Fig. 3: Effect of Endomycorrhizal inoculation on Circumference, Diameter, and Biomass of *A. malaccensis* after 180 days\*.**



**Fig. 4: Effect of Endomycorrhizal inoculation on (Q<sub>i</sub>) Quality Index, (B<sub>i</sub>) Biovolume index, and (M<sub>e</sub>) Mycorrhizal efficiency of strains of *A. malaccensis* after 180 days of inoculation (DAI) \***



**Fig. 5: Plastochron interval index of *A. malaccensis* on 180 days after inoculation (DAI)\***

**Table 1: Effect of Endomycorrhizal inoculation on *Aquilaria malaccensis* after 30 days\*.**

Treatment	Temperature (°C)	pH	spore count (50gm of soil)	Total Root Colonization (%)
EM <sub>1</sub>	26.0 ±0.47	5.62±0.25	3.0 ±0.82	90.0 ±0.82
EM <sub>2</sub>	26.0 ±0.47	5.60±0.22	9.0 ±1.24	95.0 ±1.24
EM <sub>1</sub> +EM <sub>2</sub>	26.0 ±0	5.8±0.19	11 ±1.41	100 ±0.47
Control	26.0 ±0.82	6.1±0.072	1.0 ±0.47	40.0 ±0.82
CV (%)	0.017	0.032	0.25	0.012

**Table 2: Effect of Endomycorrhizal inoculation on *A. malaccensis* after 60 days\*.**

Treatment	Temperature (°C)	pH	spore count (50gm of soil)	Total Root Colonization (%)
EM <sub>1</sub>	25 ±0	5.84±0.25	18 ±1.24	100 ±0
EM <sub>2</sub>	25 ±0	5.76±0.24	21 ±2.06	100 ±0
EM <sub>1</sub> +EM <sub>2</sub>	25 ±0	6.15±0.024	26 ±1.69	100 ±0
Control	25 ±0	6.22±0.10	12 ±2.16	60 ±0.40
CV (%)	0	0.026	0.103	0.0063

**Table 3: Effect of Endomycorrhizal inoculation on *Aquilaria malaccensis* after 90 days\*.**

Treatment	Temperature (°C)	pH	spore count (50gm of soil)	Total Root Colonization (%)
EM <sub>1</sub>	24 ±0	5.78±0.094	14 ±1.69	100 ±0
EM <sub>2</sub>	24 ±0	5.85±0.14	23 ±0.82	100 ±0
EM <sub>1</sub> +EM <sub>2</sub>	24 ±0	6.1±0.098	27 ±1.24	100 ±0
Control	24 ±0	6.19±0.069	9 ±1.24	70 ±0.82
CV (%)	0	0.017	0.085	0.0029

**Table 4: Effect of Endomycorrhizal inoculation on *A. malaccensis* after 120 days\*.**

Treatment	Temperature (°C)	pH	spore count (50gm of soil)	Total Root Colonization (%)
EM <sub>1</sub>	21 ±0.23	5.9±0.22	121±0.25	100±0
EM <sub>2</sub>	21 ±0	5.87±0.25	127±0.65	100±0
EM <sub>1</sub> +EM <sub>2</sub>	21 ±0	6.29±0.095	129±0.41	100±0
Control	22 ±0.47	6.12±0.047	60±0.27	90±0.94
CV (%)	0.0081	0.026	0.0037	0.0026

**Table 5: Effect of Endomycorrhizal inoculation on *Aquilaria malaccensis* after 180 days\*.**

Treatment	Temperature (°C)	pH	spore count (50gm of soil)	Total Root Colonization (%)
EM <sub>1</sub>	20±0.31	5.89±0.047	228±0.52	100±0
EM <sub>2</sub>	20±0.26	5.85±0.098	233±0.36	100±0
EM <sub>1</sub> +EM <sub>2</sub>	20±0.15	6.3±0.14	237±0.24	100±0
Control	20±0.42	6.15±0.024	76±0.16	70±1.24
CV (%)	0.014	0.013	0.0017	0

± Standard Error of mean; \* Average of five replications

### List of abbreviations

EM<sub>1</sub>: *Glomus* species; EM<sub>2</sub>: *Acaulospora* species; EM<sub>1</sub>+EM<sub>2</sub>: *Glomus* species + *Acaulospora* species; B<sub>i</sub>: Biovolume index; Q<sub>i</sub>: Quality index; M<sub>e</sub>: Mycorrhizal efficiency; P<sub>i</sub>: Plastochron interval index; DAI: Days After Inoculation; AMF: Arbuscular Mycorrhizal Fungi; CV: Coefficient of variance