



# Quality Stock Production of *Aquilaria malaccensis* Lamk. Using Arbuscular Mycorrhizal Inoculation: Restoration of Agarwood Source

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

*Aquilaria malaccensis* Lamk. is a critically endangered and economically important forest tree species of North-east India. In the current 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 *Acaulospora* spp. ( $EM_2$ ) 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  $EM_2$  treatment followed by the *Glomus* spp. + *Acaulospora* spp. ( $EM_1+EM_2$ ) treatment ( $1.10 \pm 0.031$ ) and *Glomus* spp. ( $EM_1$ ) ( $0.83 \pm 0.014$ ) treatment. Control seedlings had a lower value

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( $0.14 \pm 0.021$ ) of  $Q_i$  than the rest of the treatments. The plastochrone 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; plastochrone interval index; quality index.

## LIST OF ABBREVIATIONS

$EM_1$	: <i>Glomus spp.</i>
$EM_2$	: <i>Acaulospora spp.</i>
$EM_1+EM_2$	: <i>Glomus spp.</i> + <i>Acaulospora spp.</i>
$B_i$	: Biovolume Index
$Q_i$	: Quality Index
$M_e$	: Mycorrhizal Efficiency
$P_i$	: Plastochrone Interval Index
DAI	: Days After Inoculation
AMF	: Arbuscular Mycorrhizal Fungi
CV	: Coefficient of Variance

## 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 [1]. Although *Aquilaria malaccensis* Lamk., locally known as “Sanchi or Agaru”, is known as the best species of agarwood, its large-scale harvesting has caused rapid depletion of the stock in India’s natural forests [2]. According to the IUCN Red List, the species is globally vulnerable A1cd [3] and ‘Critically Endangered’ in India [4] due to the continued unsustainable overexploitation, leads to a decline of population by over 80%. However, the government takes action to bring international trade within sustainable limits. In addition, 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 [5]. 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 [6].

*Aquilaria 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 [7]. 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 researchers [8]. 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 [9]. Application of mycorrhizal fungi can enhance the growth of agarwood-producing plants even in greenhouse conditions and open land [10,11,12,13]. However, there are no reports of bio-inoculation effect on morpho-metrics of *A. malaccensis* in India. Although, AM inoculation affected damping off disease in the said plant [14], growth effect on *A. malaccensis* and *A. crasna* has been reported [15]. The objective of the present study was to screen the dominant and efficient strains from the rhizosphere of *A. malaccensis* Lamk.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Rhizospheric Soil and Root 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.

### 2.2 Isolation, Quantification, Root Colonization, and Mass Multiplication of AM Spores

Isolation of AM spores was performed using a “wet sieving and decanting technique” [16]. The quantitative estimation of the AM spores was carried out by the modified method of Gaur & Adholeya [17]. The root colonization was assessed by the ‘Rapid clearing and staining

technique' [18]. The mycorrhizal inoculum production was done by using the 'soil funnel technique' [19].

### 2.3 Cultivation and Growth Studies

The plantlets of *Aquilaria 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* spp. consortium containing *Glomus invermaium* Hall, *G. mosseae* Gerd.&Trappe, and *G. maculosum* Miller & Walker) and EM<sub>2</sub> (*Acaulospora* spp. consortium containing *Acaulospora trappei* Ames & Linderman, *A. elegans* Trappe & Gerdemann and *A. lacunosa* Morton) 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 [20].

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

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

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 [21,22].

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

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

### 2.5 Quality Index

The quality index to assess the quality of seedlings was calculated using the following formula [21,22].

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

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

### 2.6 Plastochrone Index

The plastochrone index is generally calculated by the formula derived by Erickson & Michelini [23].

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

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, L<sub>n</sub>= Length of an organ that is equal to or slightly longer than R, L<sub>n+1</sub>= Length of an organ that is just slightly shorter than R.

### 2.7 Mycorrhizal Efficiency

Mycorrhizal Efficiency of the AM strains was calculated using the following formula [24].

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

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

Mycorrhizal Efficiency of strains (ME strains)  
= MEs + MEr

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



**Fig. 1. *Glomus* spp. consortium (left to right; A. *G. invermaium* B. *G. mosseae* C. *G. maculosum*), *Acaulospora* spp. consortium (left to right; D. *A. trappei*, 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 seedlings)**

## 2.9 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 *Aquilaria 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 ( $19.7 \pm 0.29$  cm) was observed in the case of EM<sub>1</sub> inoculated plants while the minimum ( $12.3 \pm 0.16$  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 ( $26 \pm 0.44$  cm) was observed in EM<sub>1</sub> inoculated plantlets. The minimum increase in height ( $14.3 \pm 0.42$  cm) was observed in control/non-inoculated plants. The maximum AM spore count ( $26 \pm 1.69$  per 50 gm 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 ( $12 \pm 2.16$ ). The maximum total root colonization percentage ( $100 \pm 0$  %) was seen in all inoculated plants than in control/non-inoculated plants in which the total root colonization percentage was low ( $60 \pm 0.40$ ) (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 50 g 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±0.84 cm) was observed in EM<sub>1</sub> inoculated plants/treatment while a minimum (18±0.37 cm) increase in height was recorded in control/non-inoculated plants. The maximum AM spore count (129±0.41 per 50 g 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±0.28 cm) was observed in EM<sub>1</sub>-inoculated plants. The maximum AM spore count (237±0.24 per 50 g 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 Fig. 2. The increase in circumference and diameter in control/non-inoculated seedlings were minimal (0.03±0.02 cm, 0.06±0.01 cm) respectively in comparison to all treatments/inoculated seedlings. The increase in circumference (1.01±0.021 cm) and diameter (2.02±0.022 cm) 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±0.24 g) 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±0.12 g) 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 Fig. 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 P<sub>i</sub> of *A. malaccensis* after 60 days of inoculation/first-stage inoculation has been shown in Fig. 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 primordial 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 primordial 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).

**Table 1. Effect of Arbuscular Mycorrhizal inoculation on *Aquilaria malaccensis* after 30 days\***

Treatment	Temperature (°C)	pH	Spore count (50 g 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 Arbuscular Mycorrhizal inoculation on *Aquilaria malaccensis* after 60 days\***

Treatment	Temperature (°C)	pH	Spore count (50 g 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 Arbuscular Mycorrhizal inoculation on *Aquilaria malaccensis* after 90 days\***

Treatment	Temperature (°C)	pH	Spore count (50 g 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

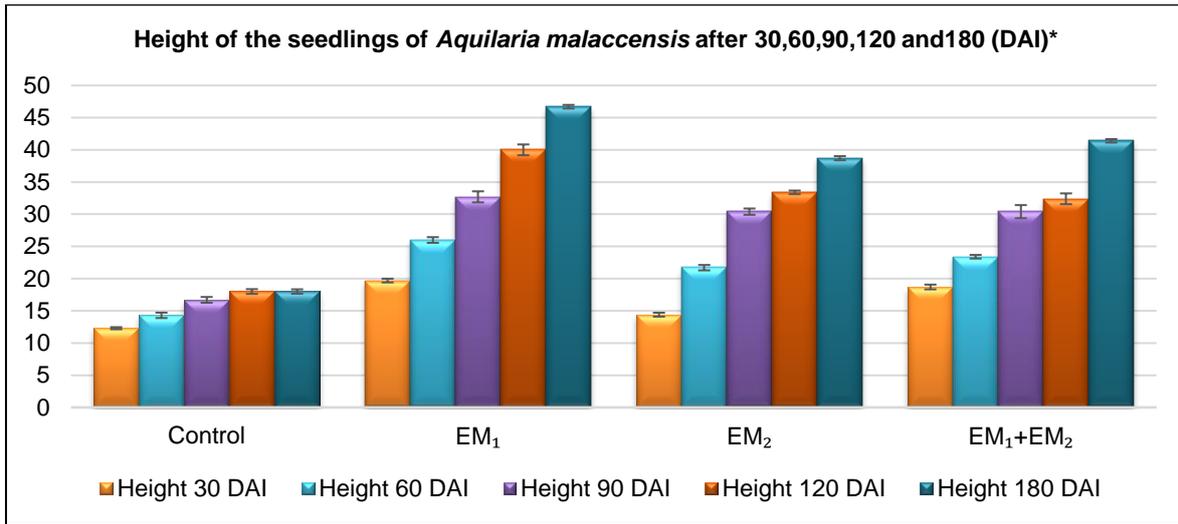
**Table 4. Effect of Arbuscular Mycorrhizal inoculation on *Aquilaria malaccensis* after 120 days\***

Treatment	Temperature (°C)	pH	Spore count (50 g 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

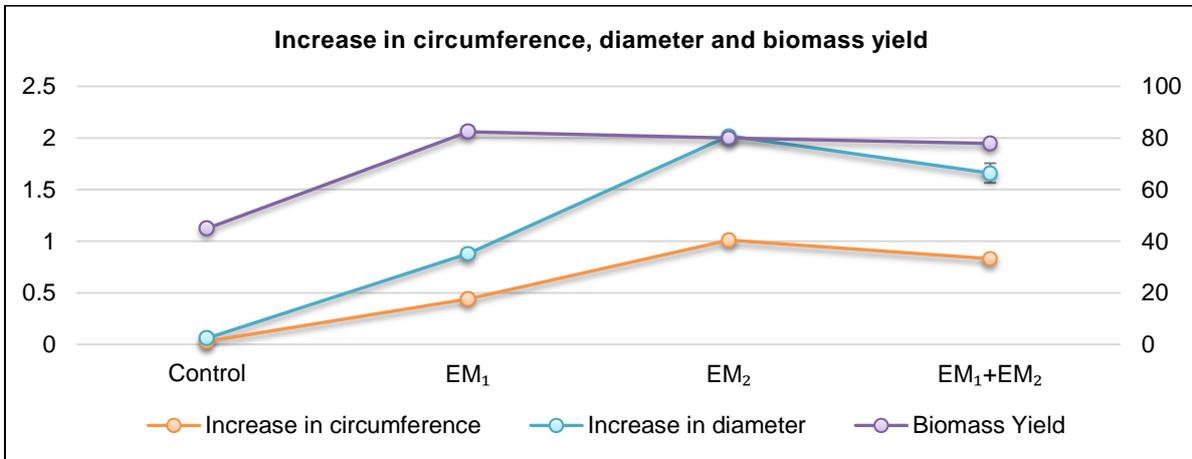
**Table 5. Effect of arbuscular mycorrhizal inoculation on *Aquilaria malaccensis* after 180 days\***

Treatment	Temperature (°C)	pH	Spore count (50 g 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

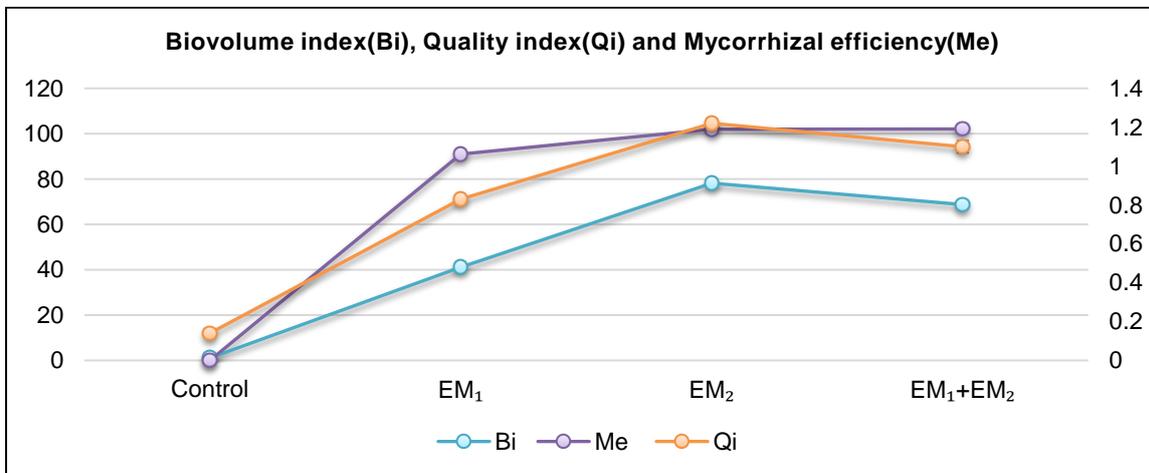
± Standard Error of mean; \* Average of five replications



**Fig. 2. Effect of Arbuscular Mycorrhizal inoculation on height of *Aquilaria malaccensis* seedlings after 30, 60, 90,120, and 180 (DAI) \***



**Fig. 3. Effect of Arbuscular Mycorrhizal inoculation on circumference, diameter, and biomass of *Aquilaria malaccensis* after 180 (DAI)\***



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

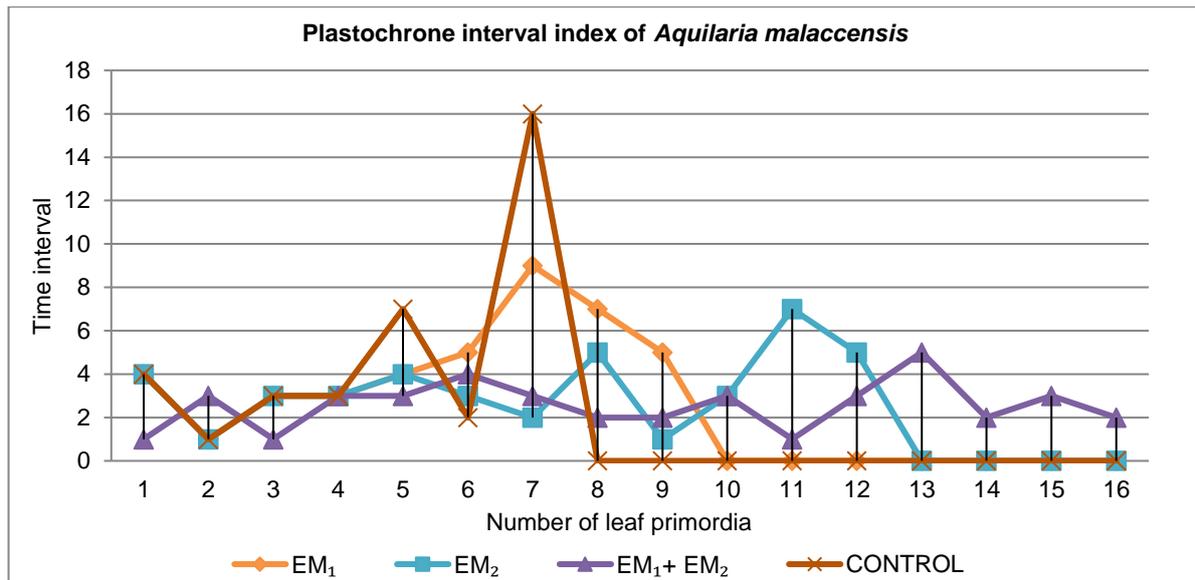


Fig. 5. Plastochrone interval index of *Aquilaria malaccensis* on 180 (DAI)\*

#### 4. DISCUSSION

The current study highlighted 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 *Aquilaria malaccensis* seedlings. In the case of plastochrone 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 may improve the growth of plants through nutrient uptake by increasing the absorbing surface area of roots [25]. In this work, 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. Additionally, the number of mycorrhizal spores was higher in mycorrhizal inoculated soil and directly related to mycorrhizal root colonization. The plants with the highest root colonization showed numerous mycorrhizal

spores in the soil [26]. 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 [21]. 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 [27]. Inoculation with suitable AM fungal strains to improve the growth and survival of plant seedlings in forestation is crucial.

Nelson et al. [28] reported 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 [29]. AM fungi also play an important role in the conservation of endangered tree species. Turjaman et al. [15] revealed that, 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 [14]. Recently, Husna et al. [11] 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 [10]. Mycorrhizal fungi act as the best ameliorant in promoting *A. malaccensis* tree growth for revegetation of post-mining limestone land [12] and former gold mining soil [13]. Accordingly, the previous studies support the present study which also revealed the positive effect of AM inoculation in enhancing various morphometric parameters of *A. malaccensis* Lamk.

Results of this study revealed that the consortium of *Glomus* spp. and *Acaulospora* spp. 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. In this study, the inoculated seedlings had a more pronounced root length than non-inoculated control plants. High root colonization of inoculated plants can increase the absorption of nutrients that promote the production of leaf area, i.e., increases the surface area for photosynthesis, and also minimizes the time interval between the appearance of two leaf primordia i.e. Plastochrone index.

## 5. CONCLUSION

Endomycorrhizae 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 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. This study provides valuable information for 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 programmes.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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