Original Research Article

Quality stock production of *Aquilaria malaccensis* Lamk. using Arbuscular Mycorrhizal inoculation: Restoration of agarwood source

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±0.024) than the rest of the inoculation treatments. The Quality index (Q_i) value was also high (1.22±0.024) in EM_2 treatment followed by the *Glomus* spp. + *Acaulospora* spp. (EM_1+EM_2) treatment (1.10±0.031) and *Glomus* spp. (EM_1) (0.83±0.014) treatment. Control seedlings had a lower value (0.14±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 2nd leaf was 1 day than rest of the treatments in which the time interval was 4 days for initiation of the 2nd 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 8th leaf primordia appeared on the 36th 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

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 & Khan 2012). Although *A. 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 (Saikia & Khan 2014). According to the IUCN Red List, the species is globally vulnerable A1cd (Ver 2.3; IUCN 2017) and 'Critically Endangered' in India (Harvey-Brown 2018) 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 (Saikia &

UNDER PEER REVIEW

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 & 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 researchers (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; Satria *et al.* 2023). 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 (Tabin *et al.* 2009), growth effect on *A. malaccencsis* 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* Lamk.

2. Material 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" (Singh & Tiwari 2001). The quantitative estimation of the AM spores was carried out by the modified method of Gaur & Adholeya (1994). The root colonization was assessed by the 'Rapid clearing and staining technique' (Phillips and Hayman 1970). The mycorrhizal inoculum production was done by using the 'soil funnel technique' (Menge & 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_1 (*Glomus* spp. consortium containing *Glomus invermaium* Hall*, G. mosseae* Gerd.&Trappe*,* and *G. maculosum* Miller & Walker) and EM_2 (*Acaulospora* spp. consortium containing

UNDER PEER REVIEW

Acaulospra trappei Ames & Linderman*, A. elegans* Trappe & Gerdemann and *A. lacunosa* Morton) and their mixed consortium ($EM_1 + EM_2$). 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 (Debi & Parkash 2016).

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

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

$$\text{Total biomass} = \text{Shoot biomass} + \text{Root biomass}$$

Where, $F_{w(s)}$ = Fresh weight of shoot, $F_{w(r)}$ = Fresh weight of root, $D_{w(s)}$ = Dry weight of shoot, $D_{w(r)}$ = 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, Bi = 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, $D_{w(S)}$ = Dry weight of seedling, H= Height of seedlings in cm, D= Diameter of stem in mm/cm; $D_{w(s)}$ = Dry weight of shoot, $D_{w(r)}$ = Dry weight of root

2.7 Plastochrone Index: The plastochrone index is generally calculated by the formula derived by Erickson & 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.

UNDER PEER REVIEW

2.7 Mycorrhizal Efficiency: Mycorrhizal Efficiency of the AM strains was calculated using the following formula (Bagyaraj 1994).

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

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

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

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±0.72) in the case of control treatment whereas low (5.60±0.22) in EM_2 treatment. The soil temperature was more or less stable (26° C) in all the treatments. The maximum increase in height (19.7± 0.29cm) was observed in the case of EM_1 inoculated plants while the minimum (12.3±0.16cm) was reported in control/non-inoculated plants. The maximum AM spore count (11±1.41 per 50gm soil), was in EM_1 + EM_2 treatment while the minimum (1.0±0.47) was in control/non-inoculated plants. The total root colonization percentage (100±0%) was high in the case of EM_1 + EM_2 treatment while low (40±0.82) in control/non-inoculated plants (see Table 1).

The soil temperature (25 ^{o}C) in all treatments was stable after 60 days. The control treatment showed a higher pH (6.22±0.10) than the EM_2 treatment in which the pH was low (5.76±0.24). The maximum increase in height (26±0.44cm) was observed in EM_1 inoculated plantlets. The minimum increase in height (14.3±0.42cm) was observed in control/non-inoculated plants. The maximum AM spore count (26±1.69 per 50gm soil) was observed in the EM_1 + EM_2 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).

UNDER PEER REVIEW

After 90 days of inoculation, all treatments had constant soil temperature (24 ºC) 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_1 treatment. The maximum increase in height (32.7±0.86 cm) was observed in the EM_1 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_1 + EM_2 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 ºC) was invariable in all the treatments than the control in which it was high (22 ºC). The maximum pH (6.29±0.09) was measured in EM_1 + EM_2 treatment and the minimum (5.87±0.25) was measured in EM_2. The maximum increase in height (40±0.84cm) was observed in EM_1 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_1 + EM_2 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 ºC) in all treatments including control treatment. All the treatments including the control had acidic pH values but EM_1 + EM_2 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 EM1-inoculated plants. The maximum AM spore count (237±0.24 per 50g soil) was observed in the EM_1 + EM_2 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_2 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_1 treatment followed by EM_2 treatment and EM_1 + EM_2 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).

UNDER PEER REVIEW

B_i, Qi, and M_e of AM strains are represented in Figure 3. The B_i was higher in all inoculated plants than in non-inoculated control plants but EM_2 treatment had a maximum B_i (78.17±0.024) than the rest of the inoculation treatments. The Q_i value was also higher (1.22±0.024) in EM_2 treatment than EM_1+EM_2 (1.10±0.031) treatment and EM_1 (0.83±0.014) treatment. Control seedlings had a lower value (0.14±0.021) of Q_i than the rest of the treatments. The maximum M_e of AM strains was found approximately equal (102.06± 0.076 & 102.01±0.076) in EM_2 and EM_1+ EM_2 treatments followed by EM_1 treatment having low M_e (90.93±0.084) of AM strains. The control/non-inoculated plants had zero (0±0) M_e of AM strains because no inoculum/AM strain was added in this treatment (see Fig. 4).

The P_i of *A. malaccensis* after 60 days of inoculation/first-stage inoculation has been shown in Figure 4. The time interval for initiation of 2nd leaf primordia in treatment/inoculation of EM_1+EM_2 was 1 day than the rest of the treatments (EM_1, EM_2, and Control) in which the time interval was 4 days for initiation of the 2nd leaf primordia. The appearance of 10th leaf primordia in treatment/inoculation of EM_1 was observed on the 41st day, thereafter no leaf primordial observance was reported. Similarly, the appearance of 13th leaf primordia in treatment/inoculation of EM_2 was observed on the 41st day, thereafter no leaf primordial observance was reported in this treatment also. Substantially, the leaf primordia appearance in the treatment/inoculation of EM_1+EM_2 was spontaneous and it was the 17th 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 8th leaf primordia appeared on the 36th day and after that, there was no appearance of leaf primordia. The time interval was maximum (16 days) between the 7th to 8th leaf primordia appearance in non-inoculated control plants (see Fig. 5).

4. Discussion

The current study highlighted that the EM_2 treatment was the best single/alone treatment of bio-inoculation followed by EM_1+EM_2 synergistic treatment than non-inoculated control seedlings, for quality stock production of *A. malaccensis* seedlings. In the case of plastochrone interval index, it was observed that *A. malaccensis* seedlings inoculated with EM_1+EM_2 synergistic treatment showed the emergence of leaf primordia. In contrast, EM_1 in a single treatment resulted in the appearance of leaf primordial, till the 60th day of inoculation.

Mycorrhizal infection may improve the growth of plants through nutrient uptake by increasing the absorbing surface area of roots (Wang *et al*. 2017). 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

UNDER PEER REVIEW

plants with the highest root colonization showed numerous 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 crucial.

Nelson *et al.* (2000) 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 (Youpensuk *et al.* 2005). AM fungi also play an important role in the conservation of endangered tree species. Turjaman *et al.* (2006) 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 (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 *Gigaspore* 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). 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_2 treatment was the best single treatment of bio-inoculation followed by the EM_1+EM_2 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 programes.

References

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), 14-21.

Bagyaraj, D.J. (1994). Vesicular–arbuscular mycorrhiza: application in agriculture. In: Norris, J.R., Read, D.J., & Varma, A.K. (Eds.), *Techniques for the Study of Mycorrhiza*. Academic Press, London, UK, pp. 819–833.

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

Debi, C., & Parkash, V. (2016). Rhizospheric Inoculation Influence on Seedling Growth, Development and Biomass Yield in Oroxylum indicum (L.) Benth. Ex Kurz. *International Journal Science and Research*, 424-429.

Erickson, R. O., & Michelini, F. J. (1957). The plastochrone index. *American Journal of Botany*, 297-305.

Gaur, A., & Adholeya, A. (1994). Estimation of VAMF spores in soil: a modified method. *Mycorrhiza news*, *6*(1), 10-11.

Harvey-Brown, Y. (2018). *Aquilaria malaccensis*. The IUCN Red List of Threatened Species. e. T32056A2810130. DOI: https://dx.doi.org/10.2305/IUCN.UK.2018-1.RLTS.T32056A2810130.en

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). DOI: 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.

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

Nelson, R., Krishnamurthy, K. V., & Senthilkumar, S. (2000). Growth and stimulation of *Santlum album* seedlings by vesicular-arbuscular mycorrhizal fungi. *Mycorrhiza News*, *12*(2), 14-15.

UNDER PEER REVIEW

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

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. In *IOP Conference Series: Earth and Environmental Science* (Vol. 449, No. 1, p. 012004). IOP Publishing. DOI: 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

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), 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

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

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 malacensis* 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. italica Plenck). *New Zealand Journal of Crop and Horticultural Science*, *42*(4), 288-302. DOI: 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 malaccencsis* and *A. crasna* Under Greenhouse Conditions. *Indonesian Journal of Forestry Research*, *3*(2), 139-148. DOI: 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. (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

UNDER REVIEW

Figures

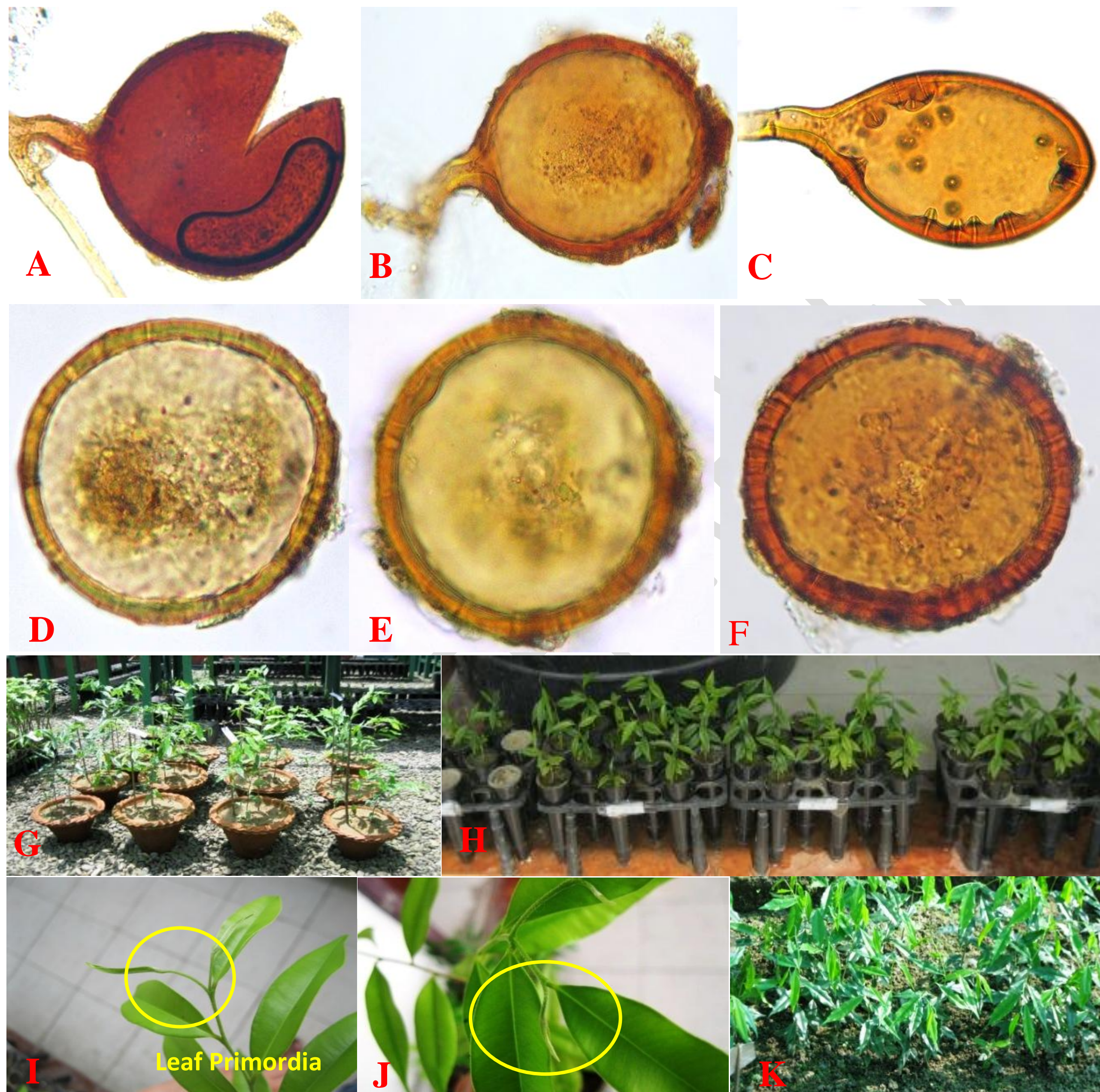

Fig. 1: ***Glomus*** **spp. consortium** (left to right; **A**. *G. invermaium* **B.** *G. mosseae* **C.** *G. maculosum*), ***Acaulospora*** **spp. consortium** (left to right; **D.** *A. trappae,* **E.** *A. elegans* **F.** *A. lacunosa*), **G.** Bioinoculation experiment in pot condition on *A. malaccensis*, **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 seedlings)

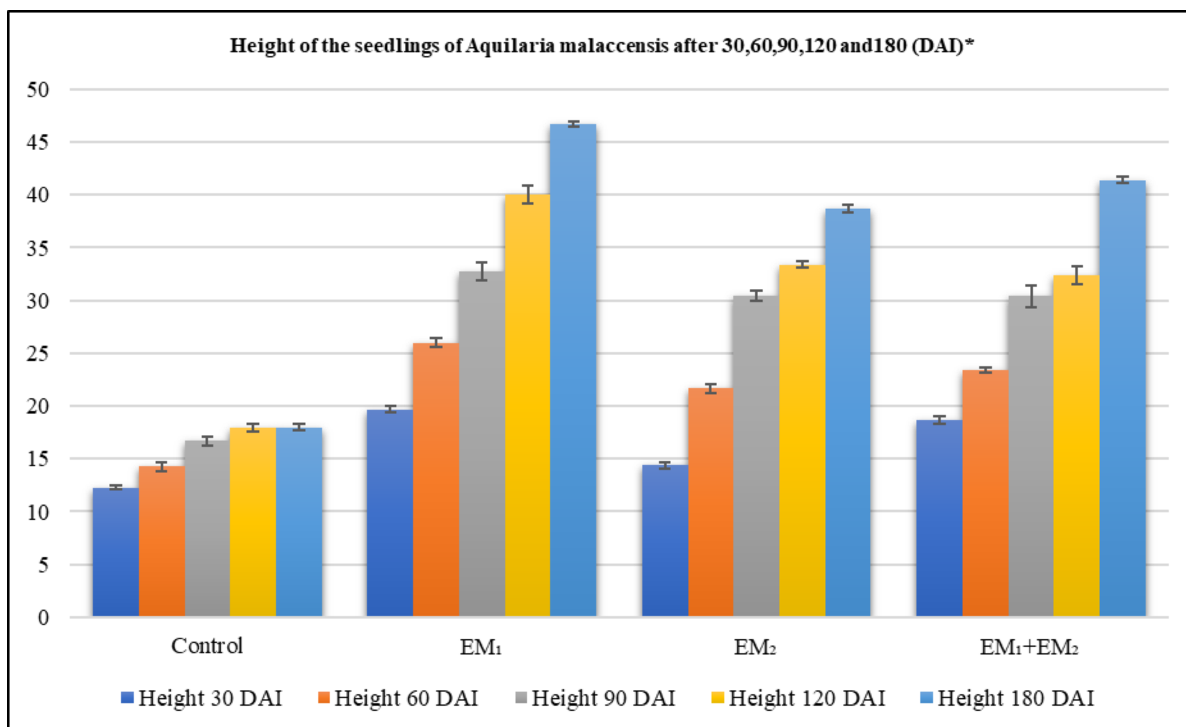

Fig. 2: Effect of Arbuscular Mycorrhizal inoculation on height of *A. malaccensis* seedlings after 30, 60, 90,120, and 180 (DAI) *

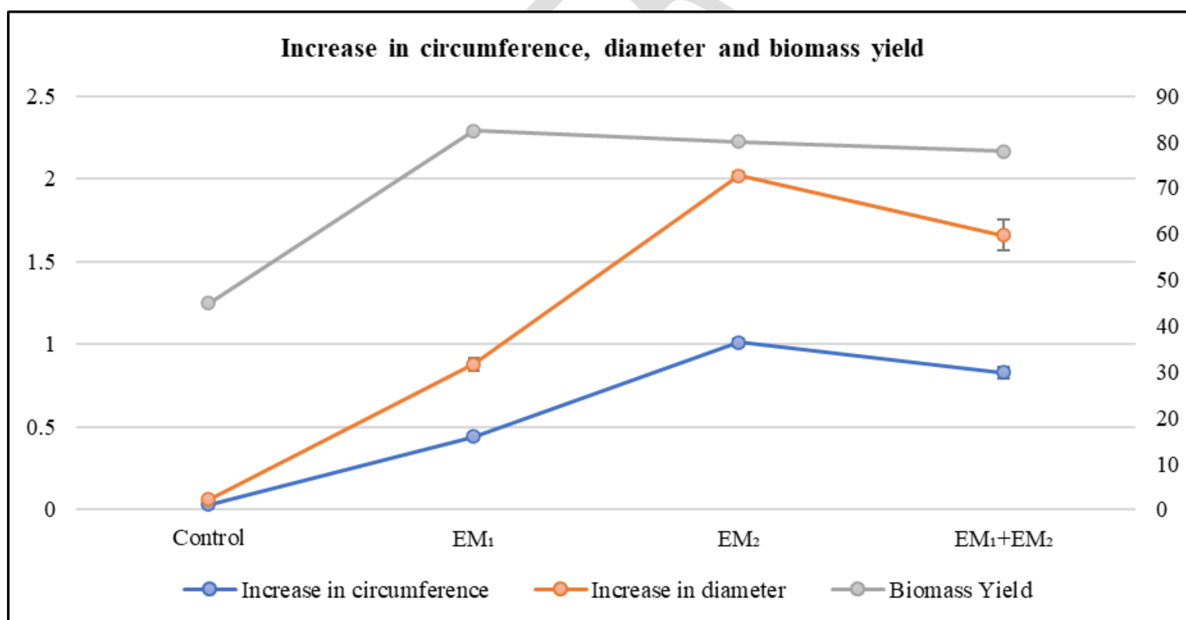

Fig. 3: Effect of Arbuscular Mycorrhizal inoculation on circumference, diameter, and biomass of *A. malaccensis* after 180 days*.

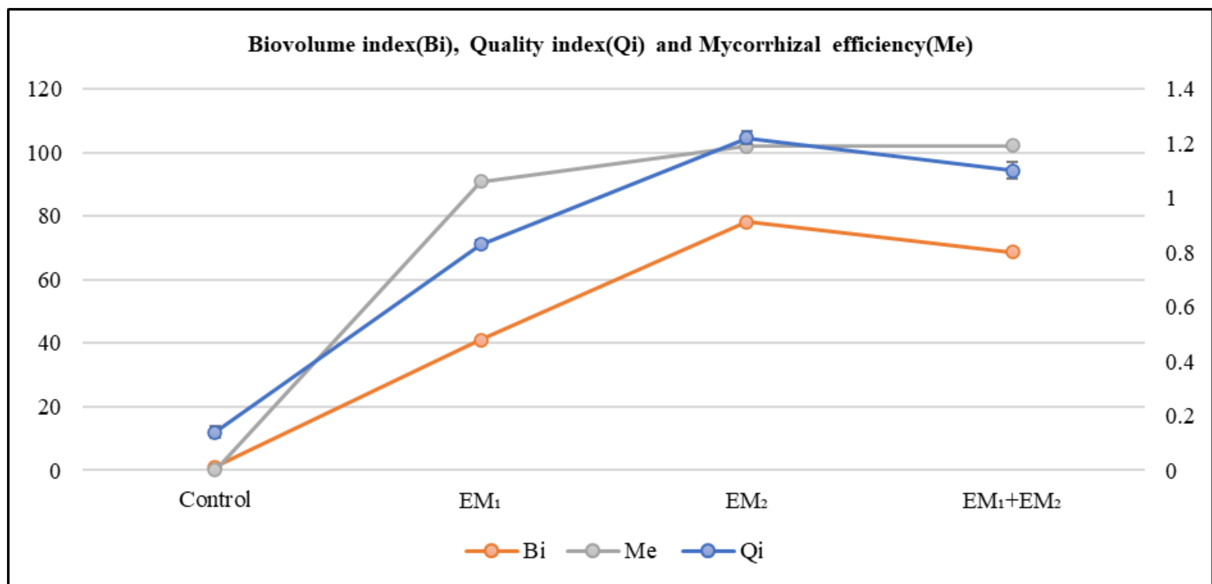

Fig. 4: Effect of Arbuscular Mycorrhizal inoculation on (Q_i) Quality Index, (B_i) Biovolume index, and (M_e) Mycorrhizal efficiency of strains of *A. malaccensis* after 180 days of inoculation (DAI) *

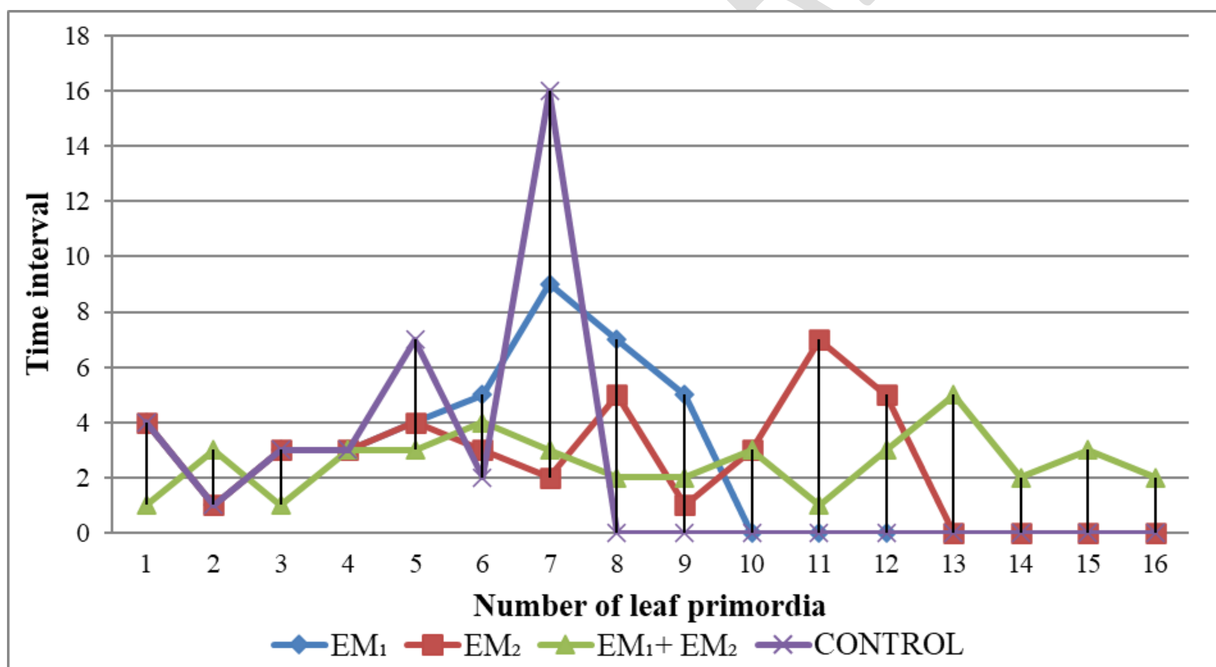

Fig. 5: Plastochrone interval index of *A. malaccensis* on 180 days after inoculation (DAI)*

Tables

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

Treatment	Temperature (°C)	pH	spore count (50g of soil)	Total Root Colonization (%)
EM_1	26.0 ±0.47	5.62±0.25	3.0 ±0.82	90.0 ±0.82
EM_2	26.0 ±0.47	5.60±0.22	9.0 ±1.24	95.0 ±1.24
EM_1+EM_2	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 *A. malaccensis* after 60 days*.

Treatment	Temperature (°C)	pH	spore count (50g of soil)	Total Root Colonization (%)
EM_1	25 ±0	5.84±0.25	18 ±1.24	100 ±0
EM_2	25 ±0	5.76±0.24	21 ±2.06	100 ±0
EM_1+EM_2	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 (50g of soil)	Total Root Colonization (%)
EM_1	24 ±0	5.78±0.094	14 ±1.69	100 ±0
EM_2	24 ±0	5.85±0.14	23 ±0.82	100 ±0
EM_1+EM_2	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

UNDER PEER REVIEW

Table 4: Effect of Arbuscular Mycorrhizal inoculation on *A. malaccensis*after after 120 days*.

Treatment	Temperature (°C)	pH	spore count (50g of soil)	Total Root Colonization (%)
EM_1	21 ±0.23	5.9±0.22	121±0.25	100±0
EM_2	21 ±0	5.87±0.25	127±0.65	100±0
EM_1+EM_2	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 (50g of soil)	Total Root Colonization (%)
EM_1	20±0.31	5.89±0.047	228±0.52	100±0
EM_2	20±0.26	5.85±0.098	233±0.36	100±0
EM_1+EM_2	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_1: *Glomus* spp.; EM_2: *Acaulospora* spp.; EM_1+EM_2: *Glomus* spp. + *Acaulospora* spp.; 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

UNDER PEER REVIEW