Effect of arbuscular mycorrhizal fungi and planting media on seedlings growth of *Helicteres isora* L. in the nursery

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Abstract. A screw tree (*Helicteres isora* L.) is a small/large shrub species that grows and spreads in many Asian countries, including Indonesia (NTT and Maluku). It is a medicinal plant commonly used to treat many diseases, such as bleeding and constipation. This study aimed to determine the effect of Arbuscular Mycorrhizal (AM) fungi and planting media on the growth of screw tree seedlings in a nursery. This research was conducted at Bogor Forest Research and Development Center's nursery, Indonesia. This study consisted of two factors: AM fungi with three levels, namely control, *Glomus aggregatum* and *Glomus clarum* and growth media with two levels, namely mixed media of soil: rice husk charcoal (2:1) and mixed soil of media: rice husk charcoal: cocopeat (2:1:1). The results showed that treatment of *G. aggregatum* and soil mixed of media: rice husk charcoal: cocopeat (2:1:1) was significantly different from other treatments except for *G. clarum* and soil mixture of media: rice husk charcoal: cocopeat (2:1:1) that significantly increased height, diameter and dry weight of seedlings and the values were 97, 56, 126 and 46, 37, 127% compared to the control. Mycorrhizal dependency of screw tree was very high (126 and 127%). Generally, interaction treatment of *G. clarum* and mixed media of soil: rice husk charcoal: cocopeat (2:1:1) increased the growth of 11-month-old screw tree in the nursery.

1. Introduction

A screw tree (*Helicteres isora* L.) from the family Malvaceae is a small tree or large shrub and a potential medicinal plant in Southeast Asia. In Indonesia, this species naturally occurs in the province of East Nusa Tenggara. The review results showed that *H. isora* has chemical compounds or secondary metabolites that can be used for therapeutic, phytochemical and pharmacological purposes [1, 2]. Traditionally, the fruit, bark and roots can be used for diabetes and lowering blood sugar levels. In addition, screw tree fruit is also able to help overcome sleep disorders [3].

*H. isora* has the potential to be developed as a leading medicinal plant in Indonesia. It is necessary to master the cultivation techniques to support these efforts. This species can be reproduced both generatively [4, 5, 6] and vegetatively [3, 7]. Production of quality seedlings is very supportive of the *H. isora* plantations' development. Therefore, it is necessary to use beneficial soil microbes such as arbuscular mycorrhizal fungi (AM) and improve the quality of the growing media. Medicinal plants are reported to have a symbiosis with AM fungi. The review results showed that AM fungi could increase growth, productivity and secondary metabolite compounds [8-10]. In addition,
AM fungi can absorb nutrients (especially P) and water and increase plant resistance to biotic and abiotic stress [11-19]. However, studies regarding the effectiveness of AM fungi on the growth of *H. isora* plants were still limited. Therefore, the determination of plant growth media needs attention. The growth media commonly used is soil plus rice husk, soil + cocopeat + rice husk charcoal. One of the materials that are expected to add nutrients and improve soil structure is organic matter such as compost for rice husks and cocopeat. The benefits of rice husk charcoal are to act as a soil manager to absorb nutrients [20].

This study aimed to know the effect of AM fungi: *Glomus aggregatum* and *G. clarum* and growth media on the growth of screw tree seedlings in the nursery.

2. Materials and Methods

2.1. Time and place of research

This research was conducted from April 2020 to March 2021 at the Silviculture Greenhouse, Forest Research and Development Center, Bogor. Location coordinate is 06º35'46" S and 106º46'52" E (GPS point), with an altitude of ± 263 meters above sea level (asl).

2.2. Research design

The research design was a completely randomized design in factorial of two factors: the AM fungi and the growth media. AM fungal factors consisted of three levels, namely control (A1), *Glomus aggregatum* (A2) (Seameo Biotrop Collection), and *G. clarum* (A3) (Forest Microbiology Collection, Forest Research and Development Centre). The growth media factors consisted of two levels, namely: T1 = soil (Topsoil of Red Yellow Podzolic Soil, Dramaga Forest Areas with Special Purposes); rice husk charcoal (2:1), and T2 = soil: rice husk charcoal: cocopeat (2:1:1). The number of repetitions per treatment was fifty (50). All media were sterilized in autoclave with 121°C for 60 minutes. For the record, there was a shortage of seeds at the time of the research due to the low percentage of seed germination.

The six treatments mentioned above were: 1. A1T1 = No AM fungi + soil: rice husk charcoal (2:1); 2. A1T2 = No AM fungi + soil: rice husk charcoal: cocopeat (2:1:1); 3. A2T1 = *G. aggregatum* + soil: rice husk charcoal (2:1); 4. A2T2 = *G. aggregatum* + soil: rice husk charcoal: cocopeat (2:1:1); 5. A3T1 = *G. clarum* + soil: rice husk charcoal (2:1); and 6. A3T2 = *G. clarum* + soil: rice husk charcoal: cocopeat (2:1:1).

2.3. Research procedure

Screw tree seeds were obtained from some mother trees in the Bosen village, Mollo Utara subdistrict, Timor Tengah Selatan district. Location coordinate were 09º42'34"1" S and 124º18'03"5" E (GPS point), altitude ± 691m asl, and rainfall 2733.4 mm year⁻¹. The seeds were germinated on sand medium + cocopeat + rice husk charcoal (6: 3: 1) and watered every day, and four weeks old seedlings or two leaves were weaned according to treatment. The AM fungi inoculum was reproduced on zeolite media and the host *Pueraria javanica*. Before inoculation of AM fungi (10 g), polybags (15 x 20 cm) were filled with media according to treatment. Plants that were not inoculated with AM fungi were appointed as control. Seedlings were maintained and watered daily and observed for 11 months. The average temperature, relative humidity and light intensity were 30°C, 85% and 8500 lux, respectively.

2.4. Observation parameters

Seedling height measurement (cm) was carried out every month with a height gauge (cm), starting from the base of the stem to the highest growing point on the stem line. Seedling diameter measurement (mm) was done every month on the stem 1 cm above the media using calipers. At the end of the experiment, biomass parts of the seedlings were put in an oven at 70°C for 3 x 24 hours until they reached a constant weight and then weighed. Roots were cleared in 10% (w/v) KOH solution for 60 minutes to remove plant phenol, then washed with running water. Then the roots were soaked in 2% (w/v) HCl solution for 10 minutes, then washed with running water. Finally, the roots were stained with try pan-blue overnight, then washed with running water. Root samples were observed using Nikon H550L camera to assess
structural colonization of AMF associated with roots. Twenty segment roots were mounted on each slide and examined under the microscope. The presence of mycelia, vesicles and arbuscules were recorded and analyzed to assess structural colonization. Colonization of AM Fungi, was $\left[ \frac{\Sigma \text{mycorrhizal viewpoint}}{\Sigma \text{field of view observed}} \right] \times 100\%$ [21].

2.5. Data analysis
Data were analyzed with the John's Macintosh Project (JMP) Start Statistics 14 statistical program, and data showing significant differences were further tested by Duncan.

3. Results and Discussion
The results of variable analysis of height, diameter and AM fungi colonization are presented in Table 1. Table 1 shows that the interaction between G. clarum and mixture media soil: rice husk charcoal: cocopeat (2:1:1) was significantly different from other treatments on the height variable of screw tree seedlings aged 11 months. In the diameter variable, the treatment of G. clarum and mixture media of soil: rice husk charcoal: cocopeat (2:1:1) was significantly different from other treatments except for the treatment of G. aggregatum and mixed media of soil: rice husk charcoal: cocopeat (2:1:1). The effect of AM fungi and media interactions of 11 month s old H. isora seedlings in the greenhouse can be seen in Figure 1. AMF colonization ranges from 30-65%. AMF structure in root seedlings can be seen in Figure 2.

**Table 1.** The effect of AM Fungi and growth media on height, diameter and root colonization of screw tree seedlings aged 11 months old in the nursery.

| Treatment | Height (cm) | Diameter (mm) | Root colonization (%) |
|-----------|-------------|---------------|-----------------------|
| A3T2      | 28.00 a     | 3.10 a        | 65                    |
| A2T2      | 21.07 b     | 2.73 ab       | 60                    |
| A2T1      | 20.53 b     | 2.43 bc       | 53                    |
| A1T2      | 20.17 b     | 2.37 bc       | 44                    |
| A3T1      | 17.57 b     | 2.37 bc       | 53                    |
| A1T1      | 14.43 b     | 2.00 c        | 30                    |
| Remarks:  |             |               |                       |
1. The numbers followed by the same letter show no significant difference at the level of p = 0.05 based on the Duncan test.
2. The numbers in parentheses are the increase in height/diameter compared to the control.
3. A1T1 = No AM Fungi + soil : rice husk charcoal (2:1)
4. A1T2 = No AM Fungi + soil : rice husk charcoal : cocopeat (2:1:1)
5. A2T1 = G. aggregatum + soil : rice husk charcoal (2:1)
6. A2T2 = G. aggregatum + soil : rice husk charcoal : cocopeat (2:1:1)
7. A3T1 = G. clarum + soil : rice husk charcoal (2:1)
8. A3T2 = G. clarum + soil : rice husk charcoal : cocopeat (2:1:1)
Figure 1. Effect of AM Fungi and media on the performance of 11 months old *H. isora* seedlings (A = A3T2; B = A2T2; C = A2T1; D = A1T2; E = A3T1; F= A1T1).

In the shoot dry weight variable, the interaction treatment of *G. aggregatum* and mixture media of soil: rice husk charcoal: cocopeat (2:1:1) was significantly different from other treatments except for *G. clarum* and mixture media of soil: rice husk charcoal: cocopeat (2:1:1) (Table 2). There were no differences between treatments on root dry weight variables. In the total dry weight variable of screw tree seedlings aged 11 months, *G. aggregatum* treatment and mixture media of soil: rice husk charcoal: cocopeat (2:1:1) were not significantly different in the treatment of *G. aggregatum* and mixture media of soil: rice husk charcoal (2:1) and treatment without AM fungi and mixed media of soil: rice husk (2:1).

Table 2. Biomass performance of screw tree seedlings due to AMF and growth media treatments.

| Treatment | Leaf (g) | Root (g) | Total (g) |
|-----------|----------|----------|-----------|
| A2T2      | 15.57 a  | 6.36 a   | 21.92 a   |
|           | (313)    | (25)     | (127)     |
| A3T2      | 13.23 ab | 8.63 a   | 21.87 a   |
|           | (251)    | (70)     | (126)     |
| A1T2      | 7.67 bc  | 6.73 a   | 14.40 ab  |
|           | (104)    | (33)     | (49)      |
| A3T1      | 6.63 bc  | 5.78 a   | 12.41 ab  |
|           | (76)     | (14)     | (28)      |
| A2T1      | 5.48 c   | 5.08 a   | 10.56 b   |
|           | (45)     | (0)      | (9)       |
| A1T1      | 3.77 c   | 5.90 a   | 9.67 b    |
|           | (0)      | (16)     | (0)       |

Remarks:
1. The numbers followed by the same letter show no significant difference at the level of p = 0.05 based on the Duncan test
2. The numbers in parentheses are the increase in height / diameter compared to the control
3. A1T1 = No AM Fungi + soil : rice husk charcoal (2:1)
4. A1T2 = No AM Fungi + soil : rice husk charcoal : cocopeat (2:1:1)
5. A2T1 = *G. aggregatum* + soil : rice husk charcoal (2:1)
6. A2T2 = *G. aggregatum* + soil : rice husk charcoal : cocopeat (2:1:1)
7. A3T1 = *G. clarum* + soil : rice husk charcoal (2:1)
8. A3T2 = *G. clarum* + soil : rice husk charcoal : cocopeat (2:1:1)
Figure 2 shows an incision in the root of *H. isora* aged 11 months that is not infected with mycorrhizae (A /control) and infected with mycorrhizae (B, C). These conditions are where vesicle and hypha intervals are visible.

![Figure 2](image)

**Figure 2.** AM Fungi structure in fine root seedlings (A. control, B. A2T2 and C. A3T2, V = vesicle, IH = internal hypha).

In general, the interaction treatment of *G. clarum* and mixed media soil: rice husk charcoal: cocopeat (2:1:1) increased the growth of the 11-months-old screw tree in the nursery. Increased growth is strongly associated with the role of AMF, especially *G. clarum*, in absorbing water and nutrients. Many research results showed that the application of AMF could increase plant growth. [8] reported the application of *G. fasciculatum*, which increased the dry weight of four medicinal plants, namely *Ocimum sanctum*, *Catharanthus roseus*, *Coleus forskholii* and *Cymbopogon flexuosus*. AM symbiosis will benefit the cultivation of medicinal plants and improve the total yield and quality of herbal materials [9]. The potential use of AM fungi for promoting growth and disease resistance in medicinal plants was found in the southern part of Assam [10].

AM Fungi effectively increased plant growth and dry weight, presumably because AM fungi quickly spread to plant roots, forming high root colonization [22]. The effectiveness of AM fungi in increasing plant growth is that AM fungi can supply P and N nutrients by forming external hyphae so that they can be available to plants [23]. It can help plants to absorb water and protect plants from land contaminated with heavy metals. P is a nutrient needed by plants to store and transfer energy in ADP and ATP [24], carbohydrate and protein metabolism, and carbohydrate transport in leaf cells [25]. Increased P content in plants will increase the rate of photosynthesis and stimulate the formation of new leaves [26]. In addition, N nutrient is needed by plants to help the process of forming amino acids and proteins in plants [27]. It helps stimulate vegetative growth of plants, namely increasing height, diameter, stimulating the formation of leaf seedlings, and can make plants greener because it is a constituent of chlorophyll [28].

The heavier the dry weight of the plant, the better the plant growth indicators and the more nutrients absorbed by the plant [29, 30], also added that plants with a greater total dry weight mean that the productivity and development of tissue cells are high and fast. This study is in line with [23] that AMF inoculation can increase the dry weight of *L. leucocephala* plants.

The results of the chemical analysis of soil growing media (T1) and soil mixed with cocopeat and husk charcoal (T2) are presented in Appendix 1. Overall, soil pH was very low (4.30-4.90). Most of the soil in Indonesia is in the acid category with a pH range of 4.0-5.5 [27]. For T1 and T2 growth media, the clay texture was about 59-61%. All media are categorized as heavy textures because the clay content is greater than sand or dust contents in all media.

Organic matter (C-organic 3.55-3.77% low to high) in the soil plays an important role in forming the soil structure. In addition, it can increase the CEC (cation exchange capacity) of the soil (20.49-26.20), which is moderate to high, so that the soil fertility conditions are better.

Based on soil nutrient content, the essential macronutrient N in soil media is in moderate condition, namely 0.17-0.24%. Phosphorus (P) nutrient content in soil medium (T1) and mixed soil cocopeat and husk charcoal (T2) is high, namely 54.00 mg per 100 g (T1). Potassium (K) nutrients in soil media and
soil mixture with cocopeat and husk charcoal are moderate to low. The Ca element is low to moderate (2.92-3.87).

The combination of AM fungi species and the media significantly affected growth in height, diameter, root colonization, and total dry weight of screw. The media nutrient condition is quite good, causing the role of mycorrhizae not to be seen in helping plant growth, but a significant role can be seen in each media composition. The use of charcoal is a non-nutritional material that is not easily damaged. Therefore, it can increase the absorption of water, nutrients and air so soil microbes and mycorrhizal fungi can develop adequately [31].

The success of plant adaptation can be measured by plant biomass which is the result of the interaction between the environment and physiological processes in plants. Stand biomass is the total amount of plant tissue living material at one time [32]. The total dry weight is an important parameter because it provides an overview of the interaction of the environment, genetics and physiological processes of plants.

4. Conclusion
The interaction treatment of G. clarum + soil: rice husk charcoal: cocopeat (2:1:1) gave the best plant growth on the height and diameter parameters of H. isora seedlings. Meanwhile, in a single factor, G. clarum and media of soil: rice husk charcoal: cocopeat (2:1:1) gave better plant growth of H. isora.

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Appendix 1. Soil chemical analysis of *Helicteres isora* growth media in greenhouses

| Parameter                      | Soil+ rice husk (2:1) | Soil+Cocopeat+Rice husk charcoal (2:1:1) |
|-------------------------------|-----------------------|------------------------------------------|
| pH H2O                        | 4.30                  | 4.9                                      |
| pH KCL                        | 3.7                   | 3.7                                      |
| C organik                     | 3.55                  | 3.77                                     |
| N Total                       | 0.17                  | 0.24                                     |
| C/N ratio                     | 21.00                 | 16                                       |
| P2O5 (av) Bray I (ppm)        | 5.00                  | 5.30                                     |
| P2O5                          | 54.00                 | 67.00                                    |
| K2O                           | 30.00                 | 33                                       |
| Ca (meq 100gr⁻¹)              | 2.92                  | 3.87                                     |
| Mg (meq 100gr⁻¹)              | 2.43                  | 1.81                                     |
| K (meq 100gr⁻¹)               | 0.54                  | 1.34                                     |
| Na (meq 100gr⁻¹)              | 0.21                  | 0.80                                     |
| Total                         | 6.10                  | 7.82                                     |
| CEC (meq 100gr⁻¹)             | 20.49                 | 26.20                                    |
| Base saturation (%)           | 30.00                 | 30.00                                    |
| Al3 exchangeable              | 1.15                  | 1.09                                     |
| H+ exchangeable               | 0.10                  | 0.68                                     |
| Texture:                      |                       |                                          |
| Sand                          | 7.00                  | 10.00                                    |
| Silt                          | 32.00                 | 31                                       |
| Clay                          | 61.00                 | 59                                       |