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ECTOMYCORRHIZAL COLONIZATION AND GROWTH OF *Anthoshorea javanica* UNDER SOIL AMELIORANT TREATMENTS

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ABSTRACT

Anthoshorea javanica (sin. Shorea javanica) is an ectomycorrhiza-dependent Dipterocarpaceae species whose successful nursery establishment relies on the rapid formation of functional ectomycorrhizal symbiosis. Although the Scleroderma spore inoculum is practical for large-scale nursery application, delayed spore germination may limit early seedling growth. Previous studies have mainly evaluated ectomycorrhizal species or inoculum dosage, whereas the interaction between spore-based ectomycorrhizal inoculation and soil ameliorants remains poorly understood. This study aimed to evaluate the effects of soil ameliorants on ectomycorrhizal colonization and growth of A. javanica seedlings inoculated with Scleroderma dictyosporum and Scleroderma columnare. A factorial completely randomized design was employed using two ectomycorrhizal species and three soil ameliorant treatments (control, humic acid, and Bio-Nature 50), with six replications per treatment. Growth variables and ectomycorrhizal colonization were analyzed using two-way ANOVA followed by Fisher's LSD test ($P < 0.05$). The interaction between ectomycorrhizal species and soil ameliorants significantly affected leaf area and shoot dry weight. The combination of S. dictyosporum and Bio-Nature 50 produced the highest leaf area (33.24 cm²) and shoot dry weight (1.80 g). Ectomycorrhizal colonization remained consistently high (79.23–91.59%) across all treatments, with no significant differences, while anatomical observations confirmed successful symbiosis through the formation of mantle and Hartig net structures. These findings demonstrate that Bio-Nature 50 enhances seedling growth responses when combined with compatible Scleroderma species without altering colonization levels. This information provides a practical basis for selecting combinations of soil ameliorants and ectomycorrhizal inoculum to improve the production of high-quality A. javanica seedlings in forest nurseries.

Keywords: *Soil Conditioner; Scleroderma; Shorea sp.; Mycorrhizal*

INTRODUCTION

Anthoshorea javanica (Sin. Shorea javanica) is an important tree species in the Dipterocarpaceae family that has high ecological and economic value in Indonesia (Nuraini *et al*., 2020). This species is known as a producer of natural resin that has long been used by the community, especially in the Lampung region, as a raw material for varnish, paint, cosmetics, pharmaceuticals, and various derivative product industries (Widiyono, 2021). In addition to serving as a source of non-timber forest products, *A. javanica* plays an important ecological role in maintaining forest cover, increasing carbon storage, and supporting biodiversity conservation in tropical rainforest ecosystems (Supriana, 2018). Therefore, the success of developing resin plantation forests and rehabilitating areas involving these species depends heavily on the availability of healthy, high-quality seedlings (Silva *et al*., 2019; Brower *et al*., 2024).

The production of *A. javanica* seedlings in the nursery faces various obstacles, one of which concerns the biological characteristics of Dipterocarpaceae species, which are highly dependent on mycorrhizal symbiosis (Riniarti & Wahyuni, 2017). Studies have shown that most Dipterocarpaceae species are highly responsive to ectomycorrhizal associations, and some are highly dependent on these associations to support their growth and survival (Sato *et al*., 2024). Ectomycorrhizal symbiosis increases the area of root absorption by forming external hyphal networks that reach soil pores inaccessible to plant roots. Through this mechanism, plants achieve greater nutrient absorption, especially of phosphorus and nitrogen, and increased tolerance to environmental stresses (Li *et al*., 2025; Henriksson *et al*., 2023).

In *A. javanica*, the presence of ectomycorrhizae has been reported as an important factor determining seed growth success. According to Rangkuti *et al*. (2024), ectomycorrhiza-colonized seedlings generally exhibit increased growth, biomass, nutritional status, and adaptability after field planting. The high dependence of *A. javanica* on ectomycorrhizae has made mycorrhizal inoculation an important part of this plant's nursery system. Therefore, various efforts have been made to obtain ectomycorrhizal fungi that are highly compatible with *A. javanica* roots (Kaewgrajang *et al*., 2023).

One of the ectomycorrhizal fungi that is widely used in Dipterocarpaceae nurseries is the genus *Scleroderma* (Lin *et al*., 2021). Some species in this genus are known to have high colonization potential, be adaptive to various environmental conditions, reproduce easily, and form effective associations with host plants in the Dipterocarpaceae family (Lakhanpal *et al*., 2021). The high level of compatibility between *Scleroderma* and Dipterocapaceae has been reported in various studies, so this fungus is often used as an inoculant to improve seedling quality at the seedbed stage (Handayani *et al*., 2018). The successful colonization of *Scleroderma* not only increases plant nutrient uptake but also increases the biomass and quality of the seedlings produced.

The success of ectomycorrhizal inoculation is greatly influenced by the form of inoculum used. Various forms of inoculum have been developed, including mycelium, colonized roots, fruit bodies, and spores (Repáč, 2010). Among these various forms, spores have several advantages, including ease of obtaining in large quantities, applicability at specific doses, relative ease of storage, and the ability to standardize inoculum quality. The use of spores also provides an opportunity to produce more practical and economical inoculation techniques at the seedbed scale.

However, the use of spores as an inoculum source has limitations. Ectomycorrhizal spores generally undergo a dormant phase before germinating and forming hyphae capable of colonizing plant roots (Agathokleous *et al*., 2023). The process of spore germination, hyphal growth, and the formation of ectomycorrhizal structures takes longer than using inoculum in the form of active mycelium. As a result, there is an early period of seed growth when plants have not yet received optimal benefits from ectomycorrhizal symbiosis. Based on the research by Grove *et al*. (2019), this condition can inhibit the formation of rapid, effective mycorrhizal colonies, especially in seedbed media with limited availability of nutrients and organic matter.

One approach to improving the effectiveness of spore inoculation is to use soil ameliorants (Zhao *et al*., 2023). Amelioran is a substance added to the growing medium to improve the physical, chemical, and biological properties of the soil, thereby supporting plant growth and the activity of soil microorganisms (Fitriatin *et al*., 2021; Purnamayani *et al*., 2019). Ameliorant materials such as humate and organic fertilizer-based soil improvement products are known to increase cation exchange capacity, improve nutrient availability, stimulate microbial activity, and create a rhizosphere environment more conducive to the development of mycorrhizal fungi (Kusuma & Riniarti, 2018). Improving the condition of the growing medium is suspected to accelerate spore germination, hyphal growth, and root colonization by ectomycorrhizal fungi.

Although the benefits of ectomycorrhizae for the growth of Dipterocarpaceae have been widely reported, information on the role of amelioran in supporting the colonization of ectomycorrhizae derived from spore inoculum remains relatively limited. Most previous studies have focused on the effectiveness of fungal types or inoculum doses, whereas the interaction between ectomycorrhizal spore inoculum and soil ameliorants has been studied only to a limited extent, particularly in *A. javanica*. In fact, understanding these relationships is very important for developing more efficient breeding technology and producing high-quality seeds in less time.

Based on this description, this study was conducted to analyze the effect of soil ameliorant on ectomycorrhizal colonization and growth of *A. javanica* seedlings inoculated with ectomycorrhizal fungi of the genus *Scleroderma*. The results of the study are expected to provide scientific information on strategies to enhance the effectiveness of ectomycorrhizal inoculation during the seedbed phase and to support the development of higher-quality, sustainable Dipterocarpaceae seed production technology.

METHOD

The research was conducted over 6 months at the Greenhouse and Laboratory of Silviculture and Forest Protection, Faculty of Agriculture, University of Lampung.

The tools used in this study include a 15x20 cm polybag, a net with an intensity of 75%, a magnetic stirrer, an Erlenmeyer flask, a measuring cup, a ruler, a hand counter, a stereo microscope, a petri dish, a leaf area meter, an oven, an analytical scale, a digital camera, and stationery. The material used consisted of 3-month-old *A. javanica* seeds with an average height of 40 cm, topsoil from the Integrated Field Laboratory, Faculty of Agriculture, Universitas Lampung. inoculant of ectomycorrhizal spores *Scleroderma columnare* and *Scleroderma dictyosporum*, humic acid, and Bio-Nature 50 (BN50).

The study used a factorial Complete Randomized Design with two factors. The first factor is the type of ectomycorrhizae, which consists of:

- M1 = *Scleroderma dictyosporum*
- M2 = *Scleroderma columnare*

The second factor is the type of soil ameliorant, which consists of:

- A0 = without amelioran
- A1 = humic acid
- A2 = Bio-Nature 50 (BN50)

The combination of the two factors resulted in six treatment combinations: M1A0, M1A1, M1A2, M2A0, M2A1, and M2A2. Each treatment combination was replicated six times, resulting in 36 experimental units. Each experimental unit consisted of three seedlings; therefore, the total number of seedlings used in this study was 108. The experimental unit, not the individual seedling, was defined as the statistical replicate. Accordingly, data from the three seedlings within each experimental unit were averaged before statistical analysis.

Research Procedure

A. Weaning Seedlings

A. javanica seedlings are weaned into polybags that have been filled with topsoil media. Before use, the planting medium is dried for 48 hours and sifted using a 2 mm sieve to obtain a homogeneous medium.

B. Ectomycorhiza Preparation and Inoculation

The ectomycorrhizal inoculum used was a suspension of spores derived from the fruit bodies of Scleroderma spp., collected under the stand of *Shorea seminis*. The ripe fruit body is cleaned of sticky dirt and dried at room temperature. After drying, the fruit body is split, and the glebal portion containing the spores is used as the inoculum. Spore suspension is prepared by mixing 5 g of spores with 1000 mL of aqueous solution, to which 8 drops of Tween 80 solution are added as a wetting agent. The mixture is then homogenized with a magnetic stirrer for 2 hours to obtain a uniform spore suspension.

Inoculation is carried out a week after weaning the seedlings. Each seedling is inoculated with 20 mL of spore suspension, applied to the root area using a measuring cup, and then re-covered with planting medium to maintain contact between the spores and the plant's roots.

C. Amelioran App

Humic acid and Bio-Nature 50 were applied at a rate of 20 mL per seedling. Bio-Nature 50 is a liquid organic soil ameliorant formulated with natural plant growth regulators, essential macro- and micronutrients, organic acids, and beneficial microorganisms to improve soil physical, chemical, and biological properties while promoting root development and nutrient availability. Both soil ameliorants were first applied two weeks after ectomycorrhizal inoculation and subsequently applied twice weekly throughout the experimental period.

D. Seed Maintenance

Seed maintenance includes watering, weeding, and pest and disease control as needed until the end of the research period.

E. Observations

Observations were made on the growth and colonization parameters of ectomycorrhiza, which include: increase in plant Height (cm); Leaf area (cm^2); Shoot dry weight (g); root dry weight (g); Total biomass (g); Root length (cm); and Percentage of ectomycorrhizal colonization (%). The increase in plant Height was calculated as the difference between the seedlings' final and initial heights during the research period. Leaf area is measured using a leaf area meter. The shoot dry weight and roots are determined after the plant sample is dried in an oven at 80°C until a constant weight is reached. Total biomass is calculated as the sum of the shoot dry weight and the root dry weight.

Ectomycorrhizal colonization was observed at the end of the study using the gridline intersection method, as described by Brundrett *et al*. (1996). Root samples were observed under stereo microscopy to identify colonized roots by ectomycorrhiza. The percentage of colonization is calculated using the formula:

$$\text{Colonization (\%)} = \left(\frac{\text{number of colonized root points}}{\text{total number of observation points}}\right) \times 100$$

Data Analysis

Statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Data were first tested for normality using the Shapiro–Wilk test and homogeneity of variance using Levene's test before conducting a two-way analysis of variance (ANOVA). When the two-way ANOVA indicated significant treatment effects, mean comparisons were performed using Fisher's Least Significant Difference (LSD) test at the 5% and 1% significance levels, as appropriate.

RESULTS AND DISCUSSION

The ANOVA results recapitulate the research data and are presented in Table 1. From the table, it can be seen that the interaction between ectomycorrhiza and the soil conditioner has a significant effect on the dry weight of the shoot and on leaf area, but not on other variables. Thus, the combination of the two treatments primarily affects the growth of the plant's crown section, while other parameters respond to the individual effects of each factor or show no response during the observation period.

A single factor of ectomycorrhizae has a significant effect on leaf area and shoot dry weight at the 5% level, and a very significant effect on total biomass at the 1% level. Moreover, it does not affect height increase, root dry weight, root length, or percentage of colonization. These results indicated that the response of *A. javanica* seedlings to ectomycorrhizal inoculation is more reflected in increased shoot growth and biomass accumulation than changes in height growth, root development, or root colonization percentages. A single factor of soil improvement has a significant effect on leaf area and total biomass, but also does not affect other variables. These results showed that improving the condition of the growing media through the provision of soil ameliorants played a greater role in supporting the formation of photosynthesis fields and plant biomass accumulation than other growth parameters.

Table 1. Recapitulation of ANOVA Analysis for all Variables Observed in the Research on Using Soil Ameliorant to Accelerate Ectomycorhiza Colonization and Growth of A. javanica

	ΔH	LSA	SW	RW	TB	RL	C
Ectomycorhiza	ns	*	*	ns	**	ns	ns
Soil Ameliorant	ns	*	ns	ns	*	ns	ns
Ectomycorhiza * Soil Ameliorant	tn	**	*	ns	ns	ns	ns

*Description: ΔH: increase in plant height; LA: leaf area; SW: shoot dry weight; RW: root dry weight; TB: total biomass; RL: root length; C: percent colonization; ns: no significant difference; *: differs in the significant level by 5%; **: differs in the significant level by 1%.*

Leaf area, shoot dry weight, and total biomass showed a more noticeable reaction than other growth metrics, indicating that ectomycorrhizal inoculation and soil amendment first impact plant physiological activity and nutrient absorption efficiency before leading to increased growth or root system development. Growing leaf area increased the capacity for photosynthesis, thereby raising photosynthetic accumulation and boosting the production of both total and crown biomass. In Dipterocarpaceae, increased leaf area and biomass are often early indicators of successful ectomycorrhizal symbiosis, occurring before a noticeable change in plant height (Budi *et al*., 2020). Recent studies have also shown that ectomycorrhizal associations can increase the efficiency of phosphorus and nitrogen uptake and improve plant nutrient status, thereby increasing biomass even when colonization rates or root growth do not increase significantly (Ma *et al*., 2021; Teste *et al*., 2020). In addition, according to Rui *et al*. (2024), organic and humate-based ameliorants improve nutrient availability, rhizosphere microbial activity, and the quality of growth media, ultimately enhancing canopy growth and plant biomass production. Therefore, the increase in leaf area and biomass observed in this study is likely due to a synergy between improved growing media conditions and increased nutrient utilization efficiency in A. javanica seedlings inoculated with ectomycorrhizal fungi.

To find out the effect of treatment, LSD follow-up tests were carried out on variables that were influenced by the interaction between ectomycorrhiza application and soil ameliorant. The results of the LSD test for variable leaf area and shoot dry weight are presented in Table 2. The results of the BNT test showed that the interaction between ectomycorrhiza and a soil ameliorant produced different responses in leaf area and shoot dry weight of *A. javanica* seeds. The highest leaf area was observed with the combination of S. dictyosporum and Bio-Nature 50 (33.24 cm²), which differed markedly from most other treatments. In contrast, the lowest leaf area was observed with the combination of *S. dictyosporum* and humat (17.30 cm²). The same pattern was observed for shoot dry weight, with the combination of S. dictyosporum and Bio-Nature 50 yielding the highest value (1.80 g). In comparison, the lowest was observed in the treatment with *S. columnare* without a soil ameliorant (1.15 g). These results show that the growth response of seedling crowns is influenced by the suitability of the ectomycorrhizal type and the soil ameliorant used.

Table 2. Effects of the Interaction between Ectomycorrhizal Species and Soil Ameliorants on Leaf Area (LA) and Shoot Dry Weight (SW) of Anthoshorea javanica Seedlings.

Ectomycoriza * Soil Ameliorant	LA (m2)	SW (g)
***Scleroderma dictyosporum* (Sd)**	27.803 a	1.250 b
Sd+Humat	17.304 c	1.400 b
Sd+BN50	33.237 a	1.800 a
***Scleroderma columnare* (Sc)**	21.528 bc	1.150 b
Sc+Humat	23.483 bc	1.375 b
Sc+BN50	20.618 c	1.200 b
BNT 5%	**6.8**	**0.348**

Description: Values followed by different lowercase letters within each column are significantly different according to Fisher's Least Significant Difference (LSD) test at P < 0.05.

The increase in leaf area in the *S. dictyosporum*-Bio-Nature 50 combination indicates that the interaction between the two treatments improves plants' resource utilization efficiency. Leaf area is an important indicator of photosynthesis capacity because it is directly related to the plant's ability to capture solar radiation and produce photosynthetic pigments (Domaratskyi, 2021). Ectomycorrhizae are known to increase the uptake of nitrogen and phosphorus through external hyphal networks that expand the root exploration area, thereby supporting the formation of better photosynthetic organs (Budi *et al*., 2020). Recent studies (Li *et al*., 2025; Ma *et al*., 2021) have shown that ectomycorrhizal associations improve plant nutrient status, nutrient absorption efficiency, and biomass productivity by transferring nutrients from soil to host plants.

The high shoot dry weight in the *S. dictyosporum*-Bio-Nature 50 combination suggested that increased leaf area is associated with *greater biomass accumulation*. Bio-Nature 50 is thought to improve the condition of the growing medium by increasing nutrient availability and soil biological activity, thereby supporting the effectiveness of ectomycorrhizal symbiosis (Lee *et al*., 2012). In contrast, humate treatments combined with *S. dictyosporum* resulted in relatively low leaf area, indicating that the plant's response to soil amendments may vary with the compatibility of the fungi used. Several recent studies report that humates and organic soil enhancers can increase nutrient availability, cation exchange capacity, rhizosphere microbial activity, and plant biomass production, although the magnitude of the response is strongly influenced by plant type, media conditions, and interactions with the soil microorganisms involved (Purnamayani *et al*., 2019).

Although it has not been proven that the interaction between ectomycorrhizae and the soil ameliorant has a significant effect on the colonization percentage, this parameter remains interesting to discuss further, as all treatments showed relatively high colonization levels (Table 3). The colonization percentage ranged from 79.23% to 91.59%, with the highest in the combination of *S columnare* and Bio-Nature 50 (91.59%) and the lowest in the combination of *S. dictyosporum* and Bio-Nature 50 (79.23%). The LSD test results showed that all treatments were in the same letter group, indicating no meaningful difference among the treatment combinations. These findings indicate that both *S. dictyosporum* and *S. columnare* exhibit similar colonization capabilities in the roots of *A. javanica* seedlings, regardless of the type of soil ameliorant used.

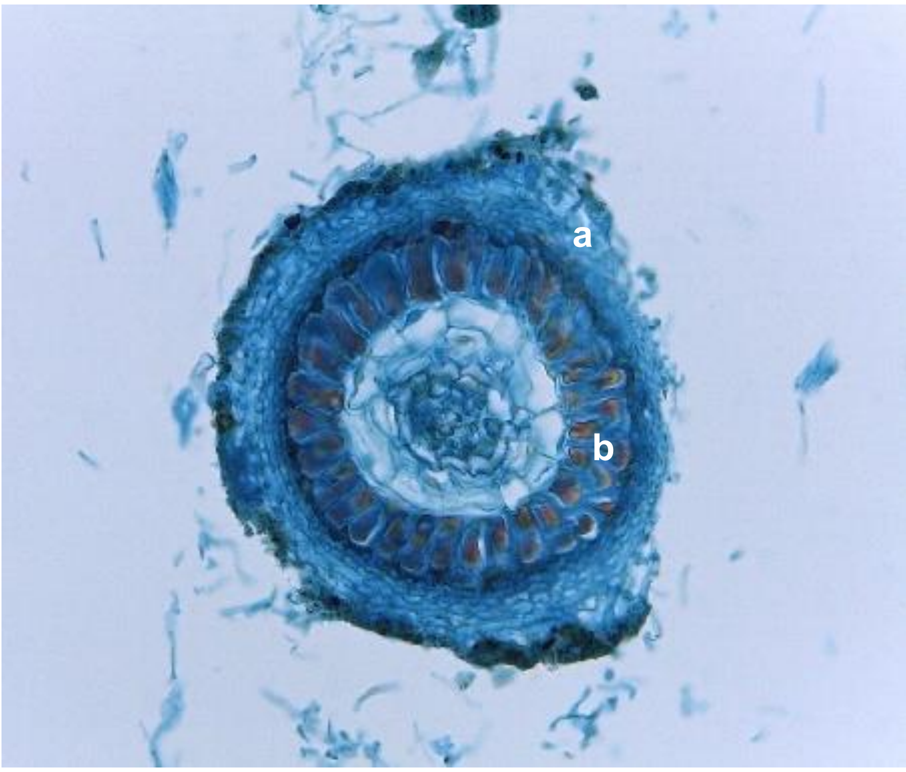
Table 3. Percentage of Ectomycorrhizal Colonization in A. javanica Roots by Treatment with Ectomycorrhizal Inoculum and Soil Ameliorant

Ectomycoriza * Soil Ameliorant	C(%)
Scleroderma dictyosporum (Sd)	81.640 a
Sd+Humat	79.234 a
Sd+BN50	79.236 a
***Scleroderma columnare* (Sc)**	79.710 a
Sc+Humat	84.098 a
Sc+BN50	91.589 a
BNT 5%	**13.29**

Description: Values followed by different lowercase letters within each column are significantly different according to Fisher's Least Significant Difference (LSD) test at $P < 0.05$.

The high colonization percentage across all treatments indicates that A. javanica is a highly compatible host for Scleroderma ectomycorrhizae. As a member of Dipterocarpaceae, *A. javanica* is highly dependent on ectomycorrhizae, enabling its roots to form effective symbioses when fungal propagules are available in the rhizosphere. The absence of significant differences between treatments indicates that the growing media conditions used in this study remain within the range that supports spore germination and ectomycorrhizal formation in all media, including ameliorant, humate, and Bio-Nature 50. Some recent studies report that the success of ectomycorrhizal colonization is more influenced by compatibility between fungi and host plants than by modifications to growing media (Hortal *et al*., 2017), especially in Dipterocarpaceae species that are highly mycorrhizal-dependent (Rangkuti et.al., 2024). The colonization rate of above 70% obtained in this study is generally considered excellent and indicates that the Scleroderma spore inoculum used has high viability and infectivity toward the host plant's roots (Policelli *et al*., 2020).

Observation of the root anatomy revealed the formation of a typical ectomycorrhizal structure consisting of a mantle and Hartig net on the root *of A. javanica* (Figure 1). The mantle is a hyphae layer that covers the root surface and functions to protect the roots and expand the absorption field, while the Hartig net is a hyphae network that develops in the intercellular space of the cortex and is the main location for the exchange of water, nutrients, and photosynthetics between fungi and host plants (Smith & Read, 2008). The presence of these two structures indicates that *Scleroderma* fungi successfully form functional ectomycorrhizal associations with the roots of *A. javanica*. According to Jörgensen *et al*. (2023), the formation of mantles and Hartig nets is the primary indicator of successful ectomycorrhizal colonization, as these structures enhance nutrient-absorption efficiency and plant adaptation to unfavorable environmental conditions. These findings reinforce the results of colonization observations showing that the two Scleroderma species used are able to form symbiotic associations on the roots of *A. javanica*.



(a) mantle (b) Hartig net

Figure 1. Cross-sectional Slice of the Root of A. javanica, which shows Evidence of Ectomycorrhiza Colonization

CONCLUSION AND RECOMMENDATION

This study showed that the interaction between ectomycorrhizal application and soil ameliorant affected the growth of *A. javanica* seedlings. The interaction between the two factors had a significant effect on leaf area and shoot dry weight, but did not affect other growth variables. The combination of Scleroderma dictyosporum and Bio-Nature 50 resulted in the highest leaf area and shoot dry weight, suggesting that the compatibility between ectomycorrhizal types and soil enhancers played an important role in supporting seedling growth. Although the colonization percentage did not differ significantly between treatments, anatomical observations of the roots revealed the formation of a mantle and a Hartig net, indicating successful establishment of a functional ectomycorrhizal symbiosis in *A. javanica* roots. This study demonstrates that soil ameliorants primarily influence seedling growth responses rather than ectomycorrhizal colonization, indicating that improvements in seedling performance can occur without significant changes in colonization intensity. This study demonstrates that soil ameliorants primarily influence seedling growth responses rather than ectomycorrhizal colonization, indicating that improvements in seedling performance can occur without significant changes in colonization intensity. Furthermore, the findings provide new evidence that the growth response of *A. javanica* seedlings depends on the compatibility between Scleroderma species and soil ameliorants when spore-based inoculum is used. From a practical perspective, the combination of *S. dictyosporum* and Bio-Nature 50 can be considered a promising nursery treatment to improve the growth of A. javanica seedlings, although it should not be interpreted as an increase in ectomycorrhizal colonization. This information may assist forest nursery managers in selecting compatible inoculum–ameliorant combinations to produce vigorous Dipterocarpaceae seedlings more efficiently. Further research is recommended to evaluate the effectiveness of the combination of ectomycorrhiza and a soil enhancer over a longer observation period and to examine the physiological responses and nutrient uptake of plants to gain a more comprehensive understanding of the mechanisms of symbiosis.

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