Potential of rhizosphere bacteria and arbuscular mycorrhizal fungi (AMF) on the growth of mindi (*Melia azedarach* L.) seedlings

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Abstract. Rhizosphere bacteria and arbuscular mycorrhizal fungi (AMF) support plant development through nutrient absorption and environmental stress-tolerance mechanisms. This study examined how different types of rhizosphere bacteria and AMF influence the growth of Mindi (*Melia azedarach* L.) seedlings. The rhizosphere bacteria included *Bacillus* sp., *Azotobacter* sp., *Pseudomonas* sp., and *Azospirillum* sp. In contrast, the AMF isolate consisted of a mixture of *Gigaspora margarita*, *Acaulospora spinosa* and *Glomus manihotis*. The results showed that inoculation with a bacterial consortium yielded the highest plant height in mindi seedlings (ρ-value = 0.0419). Inoculation with *Bacillus* sp. resulted in the highest stem diameter compared with the other treatments (ρ-value = 0.0006). For biomass and AMF colonization variables, *Azotobacter* sp. exhibited the highest biomass (ρ-value = 0.0501) and AMF colonization percentage (ρ-value = 0.0051) compared with the control and other bacterial inoculation treatments. Furthermore, Indole Acetic Acid (IAA) production was highest in *Azotobacter* sp. (ρ-value = <0.0001). AMF inoculation in mindi seedlings had no significant effect (ρ-value = 0.3623) on the growth indicators assessed. These results highlight the role of rhizosphere bacteria in improving the quality of mindi seedlings, thereby contributing to sustainable forest management.

1 Introduction

The decline in natural forest production is a critical issue caused by various factors such as deforestation, forest fires, and climate change. This decline in natural forest production affects the availability of raw wood materials, which are becoming increasingly limited. To address this issue, the development of community forests is a promising alternative. Community forest development has the potential to enhance wood production, making it a significant contributor to timber resource supply.

The mindi tree (*Melia azedarach* L.) is a tree species used in community forests. Mindi is a fast-growing forestry species with significant potential for forest development, including in Indonesia. Its use in forestry extends to establishing community forests, where community

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forests planted with mindi trees have shown promising and well-developed growth, instilling hope for the future of sustainable forestry. Mindi is a valuable forestry species with a high economic importance, particularly for timber production and medicinal purposes.

The mindi tree is valued as a source of high-quality timber due to its durability, making it widely used as a raw material for furniture, construction, and handicrafts. Additionally, mindi is a medicinal plant used to treat various ailments, including diarrhea, diabetes, rheumatism, and hypertension [1]. Another benefit of mindi trees is their frequent use in agroforestry practices, which helps reduce soil erosion and improve soil quality [2]. However, despite its diverse benefits, the growth of mindi trees often faces challenges, particularly in marginal lands with low fertility. Optimal soil conditions are crucial for supporting the early growth of mindi trees, particularly during the seedling phase. Efforts to enhance the quality of mindi seedlings are necessary to produce high-quality planting stock. One supporting technique for improving seedling growth is the utilization of soil microorganisms such as rhizosphere bacteria. Rhizosphere bacteria inhabit the root zone and interact symbiotically with the plants. The bacterial microbes used in this study were *Bacillus* sp., *Azotobacter* sp., *Pseudomonas* sp., and *Azospirillum* sp. *Pseudomonas* sp. and *Bacillus* sp. are phosphate-solubilizing microbes. At the same time, *Azotobacter* sp. and *Azospirillum* sp. are non-symbiotic nitrogen-fixing microbes. In addition to bacteria, AMF is widely applied to support growing forestry seedlings.

AMF are fungi that associate or form symbiotic relationships with plants, typically occurring within the root system. AMF can enhance plant growth by enriching the soil by absorbing essential nutrients required by plants. These fungi establish a mutualistic symbiosis with plant roots, aiding in nutrient uptake, particularly phosphate, and improving plant stress tolerance conditions, such as aridity and elevated saline concentrations. AMF play a role in enhancing plant productivity, protecting roots from pathogen attack, strengthening plant resistance to drought and salinity stress, and assisting in the absorption of water and nutrients to support growth [3]. This study examined how different types of rhizosphere bacteria and AMF influence the growth of mindi (*Melia azedarac*h L.) seedlings.

2 Methodology

2.1 Research location

The research was undertaken at the Silviculture Laboratory, Forest Pathology Laboratory, and the Greenhouse belonging to the Silviculture Division, Faculty of Forestry and Environment, Institut Pertanian Bogor. Indole acetic acid (IAA) was analyzed at the Testing Laboratory, Bogor Unit of the Palm Oil Research Center.

2.2 Tools and materials

The tools utilized in this research consisted of a laminar airflow cabinet, autoclave, glass jars, measuring cylinders, microscope slides, cover glasses, Erlenmeyer flasks, tweezers, microscope, test tubes, Petri dishes, Bunsen burner, sieves, trowel, thermometer, analytical balance with 10^{-4} precision, inoculating loop, stove, Styrofoam blocks (11 cm × 11 cm), shaker, spectrophotometer, centrifuge, ruler, bucket, polybags (15 cm × 20 cm), watering can, camera, stationery, flash drive, SAS 9.1.3 software, Microsoft Word software, and Microsoft Excel software.

The materials applied in this research included mindi seedlings, bacterial isolates of *Bacillus* sp., *Azotobacter* sp., *Pseudomonas* sp., and *Azospirillum* sp., Tryptic Soy Agar (TSA) medium, King's B medium, semi-solid Nitrogen-Free Bromthymol Blue (NFB)

medium, Ashby's mannitol agar, 70% alcohol, 10% KOH, 2% HCl, lacto-glycerol, acid fuchsin, tryptophan amino acid, Millipore filter paper (0.2 μm), $FeCl_3$, 95% H_2SO_4, distilled water, soil, coir, and rice husk charcoal.

2.3 Research procedures

2.3.1 Seedling preparation

The mindi fruit was harvested from a 12-year-old mindi tree (F1), with the parent tree (F0) sourced from PT Hendratna Plywood, located in Martadah, South Kalimantan. The fruit was extracted to obtain mindi seeds. The mindi seeds were then planted in a planting medium consisting of soil in germination trays. After developing two pairs of leaves (± 55 days after sowing), the mindi seedlings were ready for weaning.

2.3.2 Preparation of growth medium

The growth medium used for weaning the mindi seedlings was a blend of soil, coir, and rice husk charcoal. Soil, coir, and rice husk charcoal were combined in a volume ratio of 2:1:1. The mixture was subsequently sterilized in an autoclave at 121°C and 1 atm pressure for 15 minutes. Once sterilized, the medium was transferred into 15 cm × 20 cm polybags.

2.3.3 Transplanting and seedling selection

The mindi seedlings that were ready for weaning were selected based on the criteria of having a height ranging from 2 cm to 11 cm and being in healthy condition. The weaning process was carried out in the afternoon, and the weaned seedlings were initially placed in a shaded area for 2 weeks to allow them to adapt to the new planting medium. After 2 weeks, the mindi seedlings were then transferred to the greenhouse.

2.3.4 Isolate of bacteria and AMF

The bacterial isolates used in this study included *Bacillus* sp., *Azotobacter* sp., *Pseudomonas* sp., and *Azospirillum* sp. Each bacterial isolate was rejuvenated on different culture media. *Bacillus* sp. was rejuvenated on TSA medium, *Pseudomonas* sp. cultured on King's B medium, *Azospirillum* sp. on NFB medium, and *Azotobacter* sp. was cultured on Ashby's mannitol agar. Each bacterium was rejuvenated on solid culture media and incubated at room temperature for a duration of 7 days. Following the incubation period, the bacterial isolates were propagated in a liquid culture medium and placed on a shaker for 2 days.

The AMF inoculum used in this study was in the form of Mycofer, which consists of spores and infected root segments mixed with zeolite as a carrier medium. The Mycofer contains spores of *Gigaspora margarita*, *Acaulospora spinosa*, and *Glomus manihotis*. Mycofer were obtained from the Biotech Center Laboratory at IPB. Before application to the mindi seedling roots, Mycofer was weighed to 3 g (containing = ± 637 AMF spores). It was then placed in a clip plastic bag to facilitate its application to the seedling roots.

2.3.5 Application of bacteria and AMF

Bacterial isolates were applied by watering a bacterial suspension on the soil surface near the base of the stem. Each seedling received 15 mL of the suspension containing bacteria, whereas the control seedlings were treated with 15 mL of distilled water. One day after the

bacterial application, AMF inoculation was performed. AMF was applied by slightly digging the soil surface until mindi seedling roots were visible. The mycofer was then sprinkled into the hole, and the hole was covered backup.

2.3.6 IAA Production ability test

The ability of *Bacillus* sp., *Azotobacter* sp., *Pseudomonas* sp., and *Azospirillum* sp. to produce IAA was analyzed using the method described by Glickman and Dessaux (1995). Each bacterial isolate was cultured in the appropriate medium, as described for the section *Isolate of Bacteria and AMF*. Tryptophan amino acid (0.5 g. L^{-1}) was added to each medium as a precursor for auxin synthesis. The bacterial cultures were then centrifuged at 10,000 rpm for 10 minutes to collect the supernatant, which was separated from the bacterial pellet by filtration using Millipore filter paper (0.2 μm). The IAA content in the filtrate was analyzed by adding $FeCl_3$ (12 g. L^{-1} in H_2SO_4 7.9 M) as a reagent. A 1 mL aliquot of the bacterial culture filtrate and 1 mL of $FeCl_3$ reagent were combined in a 2 mL Eppendorf tube and incubated in the dark at 26 °C for a duration of 30 minutes. Following incubation, the absorbance of the mixture was determined using a spectrophotometer set to a wavelength of 550 nm.

2.3.7 Maintenance, observation, and data collection

The upkeep of mindi seedlings took place over 5 months, with each plant watered individually during the morning hours, and weed control was performed. Data collection and observations were conducted throughout the 5-month maintenance period. The observed parameters included height, diameter, number of leaves, dry weight of shoots and roots, total dry weight (TDW), and colonization of AMF in the plant roots.

The height of the seedlings was measured weekly, starting after the treatment phase and continuing for 5 months. A 60 cm ruler was used to measure the height from 1 cm above the soil surface at the base of the stem to the tip of the apical shoot. A caliper was used to measure stem diameter at the base, 1 cm above the soil surface. The measurements were performed every 4 weeks over a 5-month period. The number of leaves was counted manually once a week by counting the leaves on each seedling. This measurement was performed after 5 months of treatment. The dry weights of the roots and shoots were assessed after a 5-month observation period. The plants were harvested, the roots and shoots diverged, then wrapped in paper and dried in a controlled oven environment at 80°C for 72 hours until a constant weight was achieved. After drying, the plant parts were balanced and the roots and shoots were separated. The dry weight of the roots was recorded as the dry weight of the roots and the dry weight of the shoots was recorded as the dry weight of shoots. The total dry weight (TDW) was calculated by summing the dry weight of the roots and shoots.

Mycorrhizal colonization in the roots of the mindi seedlings was observed after the observation period. (week 20 post-inoculation). The colonization rate of AMF in the roots was determined by examining the roots using the root staining technique. The first step was to select fresh root segments, approximately 3-5 cm long, and wash them thoroughly with running water. The roots were then placed in a 10% KOH solution and left for 24 hours until the exhibited a white or pale coloration. The KOH solution was disposed of appropriately and the roots were rewashed with flowing water for 5-10 minutes. The roots were immersed in 2% HCl solution for 24 hours. The HCl solution was slowly drained, and the roots were immersed in an acid fuchsin staining solution for 3 minutes. Afterward, the roots were transferred to a lacto-glycerol solution to reduce the color for 24 hours. The fungal structures within the roots were observed by placing the stained root segments on a slide, covered with

a glass slide, and observed under a microscope. The root colonization percentage was calculated using the formula proposed:

$$Colonization\ (\%) = \frac{\sum colonized\ fields\ of\ view}{\sum total\ fields\ of\ view} X\ 100\% \quad (1)$$

2.3.8 Design of experiments and analysis of data

The experiment to test the IAA production ability of the bacterial microbes was carried out using a Completely Randomized Design (CRD) with one factor, which refers to the bacterial type, with each treatment repeated thrice. The bacterial and AMF application experiment used a Nested Completely Randomized Design (CRD), where the main plot was bacterial inoculation and the subplot was the application of AMF. The experiment was conducted in 4 replicates, each consisting of 4 units of mindi seedlings. Bacterial inoculation consisted of 6 levels: control (T0), inoculation with *Bacillus* sp. (T1), inoculation with *Pseudomonas* sp. (T2), inoculation with *Azotobacter* sp. (T3), inoculation with *Azospirillum* sp. (T4), and bacterial consortium (T5). AMF application consisted of 2 levels: mindi seedlings without AMF (F0), and mindi seedlings with AMF (F1).

The measurements were analyzed using analysis of variance. Data were processed using SAS 9.1.3 software. Decision-making based on the hypothesis test was made by rejecting H0 if ρ-value < 0.05, and failing to reject H0 if ρ-value ≥ 0.05. If significant differences were found between the treatments, subsequent analysis was performed using Duncan's Multiple Range Test (DMRT) at a 5% significance level.

3 Results and discussion

3.1 The effect of bacterial application on the growth of mindi seedlings

The parameters observed in this study were plant height (cm), diameter (mm), number of leaves, and biomass (g). Table 1 shows that bacterial treatments significantly influenced the height, diameter, biomass, and AMF colonization of mindi seedlings, while there was no significant effect on the number of leaves. ANOVA results reflect the growth data of the mindi seedlings subjected to bacterial treatments over the 5-month observation period.

Table 1. Effect of rhizosphere bacteria on growth parameters of mindi (*Melia azedarach* L.) seedlings.

Variable	Species of Bacteria[a]	ρ-value
Height	*	0.0419
Diameter	*	0.0006
Number of leaves	ns	0.1622
Biomass	*	0.0501
AMF Colonization	*	0.0051

[a](ns): not significantly affected (*): significantly affected at the 5% significance level

In the observed variable of plant height under the bacterial inoculation treatments, the bacterial consortium treatment (T5) resulted in the highest plant height, which was significantly different from the other treatments (Table 2). The treatments with *Pseudomonas* sp. (T2) and *Azotobacter* sp. (T3) did not exhibit significant differences compared with the

control (T0). Treatments with *Bacillus* sp. (T1) and *Azospirillum* sp. (T4) were not significantly different, with *Azospirillum* sp. (T4) producing the lowest plant height among the bacterial treatments.

The highest value for stem diameter was recorded in the *Bacillus* sp. treatment, demonstrating a notable difference from the other treatments (Table 2). The *Pseudomonas* sp. treatment also exhibited a significant difference compared with the other treatments, although the diameter achieved was smaller than that of *Bacillus* sp. The treatments with *Azotobacter* sp., *Azospirillum* sp., and the bacterial consortium did not exhibit significant differences, whereas the control plants showed the lowest diameter value compared to plants inoculated with bacteria.

All bacterial application treatments resulted in higher biomass values than those of the control. According to Duncan's post hoc test results on the impact of bacterial inoculation on the root biomass of mindi seedlings, *Azotobacter* sp. treatment yielded the highest biomass value among all treatments (Table 2). The biomass of mindi seedlings inoculated with *Azotobacter* sp. was significantly different from that of the control, *Bacillus* sp., *Azospirillum* sp., and bacterial consortium. The biomass of mindi seedlings treated with *Pseudomonas* sp. was also higher than that of the control, *Azospirillum* sp., and *Bacillus* sp seedlings. In contrast, the control, *Bacillus* sp., *Azospirillum* sp., and bacterial consortium treatments did not show significant differences.

For the AMF colonization variable, the *Azotobacter* sp. treatment exhibited the highest colonization value, which was significantly different from the other treatments (Table 2). The treatments with inoculation of *Pseudomonas* sp., *Bacillus* sp., and the bacterial consortium did not show significant differences. The control seedlings exhibited the lowest colonization values compared with the plants treated with bacterial inoculation.

Tabel 2. The effect of *Bacillus* sp., *Azotobacter* sp., *Pseudomonas* sp., *Azospirillum* sp., and bacterial consortium on mindi seedlings.

Treatment	Variable[a]			
	Height (cm)[a]	Diameter (cm)[a]	Biomass (g)[a]	AMF colonization (%)[a]
Control (T0)	8.29^{ab}	0.9^{c}	0.68^{b}	33.75^{c}
Bacillus sp. (T1)	8.00^{b}	1.66^{a}	0.71^{b}	65.63^{ab}
Pseudomonas sp. (T2)	8.32^{ab}	1.33^{b}	0.83^{ab}	58.13^{ab}
Azotobacter sp. (T3)	8.49^{ab}	1.27^{bc}	0.87^{a}	74.38^{a}
Azospirillum sp. (T4)	7.83^{b}	1.05^{bc}	0.68^{b}	46.88^{bc}
Bacterial Consortium (T5)	9.21^{a}	1.11^{bc}	0.71^{b}	66.25^{ab}

[a]Numbers accompanied by the same letter within the same row indicate no significant difference at the 5% significance level, according to Duncan's Multiple Range Test.

A notable influence on plant growth was observed for plant height, biomass, diameter, and mycorrhizal colonization parameters. Each treatment produced different effects, depending on the type of microorganism applied.

For plant height, the results of the research demonstrated that treatments with various types of microorganisms had variable effects, and the bacterial consortium treatment produced the highest results compared to other treatments. The increase in plant height observed with the bacterial consortium treatment may be attributed to the mechanisms of rhizosphere bacterial mixtures that provide mutually positive effects on the plant. Bacterial consortium can enhance the availability and absorption of essential nutrients like potassium, nitrogen, and phosphorus and through nitrogen fixation and enzyme secretion, thereby

supporting plant height growth. The use of bacterial consortium, which comprise a mixture of various bacterial strains with distinct functions, can substantially enhance plant growth, crop yield, and the overall health of agroecosystems [4].

For the plant diameter variable, the treatment with *Bacillus* sp. inoculation resulted in the highest stem diameter value for mindi seedlings compared with the other treatments. *Bacillus* sp. produces hormones like auxins and cytokinins, which strengthen plant structures, including the enlargement of seedling stem diameter. Cytokinins produced by *Bacillus* sp. and absorbed by the plant can stimulate cell division and differentiation in the cambium region, thus contributing to improved growth [5].

For the biomass variable, the bacterium that significantly influenced the biomass of mindi plants was *Azotobacter* sp. This finding is consistent with a previous study [6], which reported that *Azotobacter* sp. helps in the absorption of nitrogen (N) in non-leguminous plants, produces substances that promote plant growth, and enhances the availability of additional nutrients (P, K, and Zn) for improved plant nutrition. *Azotobacter* sp. has been widely used for Leguminosae plants, but its application to mindi plants is rarely reported. Furthermore, rhizosphere bacterial inoculation can improve nutrient availability and enhance root growth in plants [7], including increasing plant resistance to environmental stresses like aridity, salinity, and heavy metals.

The research results indicated that *Azotobacter* sp. resulted in the highest percentage of AMF colonization compared to the other bacterial species. This finding is supported by research [8], which showed that *Azotobacter* sp. rhizosphere bacteria have a superior ability to compete with other microorganisms in the soil, thereby allowing AMF to colonize without significant interference from competing microorganisms. Additionally, rhizosphere bacteria can enhance the availability of nutrients required by AMF, such as phosphate and nitrogen, through processes like nitrogen fixation and the ability to solubilize phosphate.

3.2 IAA synthesis capability by bacterial microorganisms

The results of the IAA concentration test produced by each bacterium showed that *Azotobacter* sp. produced the highest IAA levels compared to the other bacteria (Fig.1). *Bacillus* sp. and *Pseudomonas* sp. exhibited the same average values, whereas *Azospirillum* sp. had the lowest average value among the four bacterial species. The distribution of IAA concentrations for each bacterium was also statistically analyzed, and the results revealed significant differences between the bacterial groups.

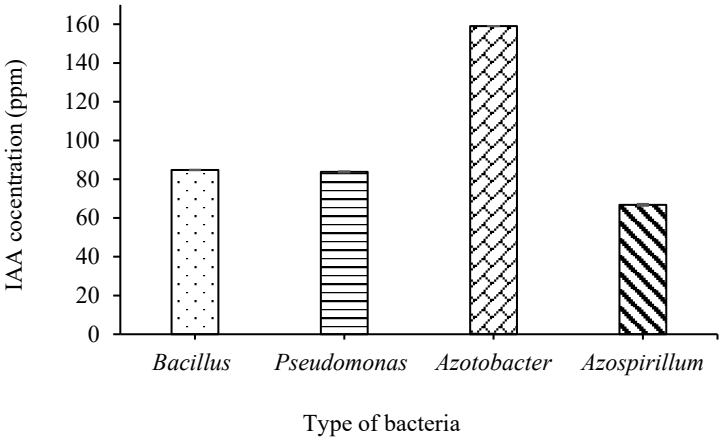

Fig. 1. Indole Acetic Acid (IAA) production by different rhizosphere bacteria

The results of this study demonstrated that one of the rhizosphere bacteria inoculated into mindi plants with the highest IAA content was *Azotobacter* sp. *Azotobacter* sp. is a rhizosphere bacterium classified as a plant growth-promoting rhizobacterium, that plays a crucial role in enhancing nitrogen nutrition in plants and can directly influence plant progression, as it is capable of synthesizing plant growth hormones, including IAA, cytokinins, and gibberellins [9]. IAA is a type of natural Plant Growth Regulator (PGR) classified as an auxin, which, at optimal concentrations, provides a positive response by accelerating plant growth [10]. IAA produced by *Azotobacter* sp., indicating the presence of exogenous IAA, can be utilized by plants in processes such as promoting plant parts and enhancing root number.

An increase in the number of roots in plants can enhance root exudation, which is the activity of roots releasing organic substances like organic acids, sugars, and enzymes into the surrounding environment. These exudates function as nutrients for soil microbes, including bacteria. With greater exudation, the population of microorganisms around the roots, particularly rhizosphere bacteria, can increase, thereby supporting plant growth by assisting nutrient cycling, improving soil structure, and providing protection against pathogens. According to previous research [11], *Azotobacter* sp. is known to possess highly efficient metabolic capabilities and can synthesize large amounts of IAA. This is because of the presence of specific enzymes involved in the IAA biosynthesis pathway, which are highly active in this bacterium, especially under optimal environmental conditions. Furthermore, *Azotobacter sp.* can adapt to different environmental conditions, enabling it to thrive in various environments, while enhancing its enzymatic activity, including IAA production. This adaptive capacity enables *Azotobacter* sp. to remain efficient in IAA production even when environmental conditions fluctuate.

3.3 The effect of AMF application on mindi seedling growth

Based on the data presented (Fig. 2), a comparison of treatments on mindi seedlings inoculated with AMF and those without AMF showed similar responses for each growth parameter tested, including plant height (cm), diameter (mm), number of leaves, and biomass (g). Average values were not significantly different between the two treatments. AMF inoculation did not significantly increase the height of mindi seedlings. The height of the seedlings without AMF and with AMF inoculation showed no significant differences. Mindi seedlings inoculated with AMF showed a slight increase in plant height compared to the seedlings without AMF, although the difference was small (Fig. 2A). The same trend was observed for the diameter variable, where the AMF treatment showed higher values than the nonAMF treatment, although the difference was not statistically significant (Fig. 2B). The leaf number also showed higher values for the seedlings with AMF treatment than for the nonAMF treatment, but the difference was not statistically significant (Fig. 2C). For the biomass variable, the results of both treatments were nearly identical, indicating that AMF had no significant effect on this parameter (Fig. 2D).

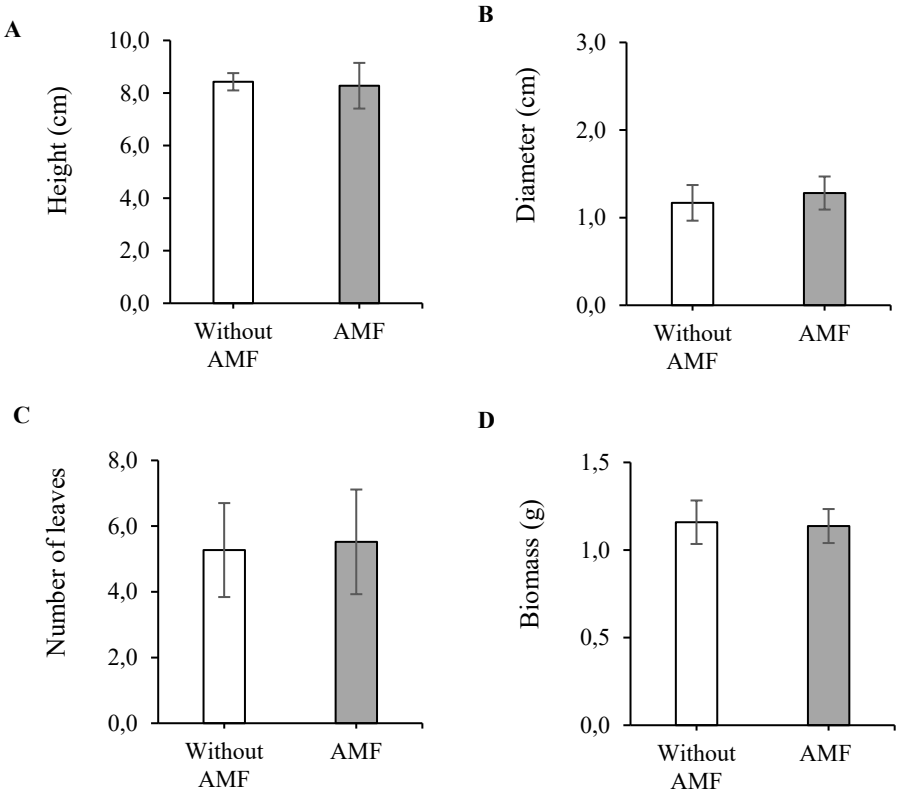

Fig. 2. The effect of treatment without AMF and with AMF on growth test; (A) height, (B) diameter, (C) number of leaves, (D) biomass.

The AMF inoculation treatment of mindi seedlings showed no significant effect on seedling growth, including plant height (cm), diameter (mm), number of leaves, and biomass (g). AMF can be influenced by the host plant's ability to form symbioses with specific fungi. In the present study, mindi seedlings inoculated with AMF did not show significant effects on any of the observed parameters. This may be due to several factors, such as the already high fertility level of the plants, the incompatibility of the AMF species for symbiosis with the plant, and the short duration of the research, which may not have been adequate for detecting the AMF colonization effect. Various types of AMF have been widely used to enhance seedling quality, are capable of forming symbiosis with different plant species, and are potential sources of nutrients for plants [12].

AMF colonization in plants is influenced by three main factors: host plant, AMF diversity, and environment. This symbiotic relationship is generally influenced by the characteristics of the host plant, the type of fungi involved, as well as abiotic and biotic factors, all of which collectively determine the dynamics of this interaction [13]. Certain plant species are compatible with specific AMF species, thereby significantly enhancing their growth.

The next factor is the type and diversity of AMF, which can affect the colonization of the host plant. Different AMF species have varying abilities to form symbiotic relationships with their host plants. Another factor is the environment, as soil conditions (pH, moisture, and nutrient availability) and the presence of other microorganisms in the rhizosphere are crucial

for AMF colonization. Soil characteristics also significantly affect the ability of AMF to form a symbiotic relationship with the host plant. In nutrient-deficient soils, particularly those with low phosphorus content, AMF will be more efficient in colonizing plants.

This study demonstrated that the application of rhizosphere bacteria has a significant positive impact on the growth of mindi seedlings. AMF treatment did not differ significantly from the control seedlings (without AMF) in seedling growth tests. These results suggest that there is no synergistic interaction between AMF and rhizosphere bacteria to support mindi seedling growth. Additional research is required to explore the processes of rhizosphere bacteria and the factors influencing their effectiveness, and to evaluate the potential application of AMF under different environmental conditions and plant species. Thus, optimizing the use of biological agents is expected to contribute to the sustainable management of forest resources while meeting the increasing demand for timber.

4 Conclusion

Rhizosphere bacterial inoculation of mindi seedlings significantly affected the growth indicators tested. Bacterial consortium inoculation resulted in the highest plant height for mindi seedlings. Inoculation with *Bacillus* sp. produced the largest stem diameter compared to the other treatments. Regarding biomass and AMF colonization, *Azotobacter* sp. showed the highest biomass and AMF colonization percentage compared to the control and other bacterial treatments. In the IAA concentration test, *Azotobacter* sp. produced the highest IAA. AMF inoculation in mindi seedlings had no significant effect on the growth indicators assessed.

References

1. B. Liao, Q. Que, X. Xu, W. Zhou, K. Ouyang, P. Li, H. Li, C. Lai, X. Chen, Climate driven adaptive differentiation in *Melia azedarach*: evidence from a common garden experiment. Genes. **13**(11), 19-24 (2022). https://doi.org/10.3390/genes13111924
2. J.P.G. Sutapa, Properties and utilization possibilities of mindi (*Melia azedarach* L.) wood from agroforestry plantation, IOP Conference Series: Earth and Environmental Science, Indonesia, Yogyakarta, October 16-17 **449**, 012026 (2020). https://doi.org/10.1088/1755-1315/449/1/012026
3. M. A. Salim, S. W. Budi, L. Setyaningsih, Iskandar, I. Wahyudi, H. Kirmi, Root colonization by arbuscular mycorrhizal fungi (AMF) in various age classes of revegetation post-coal mine. BIODIVERSITAS. **21**(10), 5013-5022 (2020). https://doi.org/10.13057/biodiv/d211105
4. A. Bargaz, K. Lyamlouli, M. Chtouki, Y. Zeroual, D. Dhiba, Soil microbial resources for improving fertilizers efficiency in an integrated plant nutrient management system. Front. Microbiol. **9**, 1-25 (2018). https://doi.org/10.3389/fmicb.2018.01606
5. S.S. Akhtar, M.F. Mekureyaw, C. Pandey, T. Roitsch, Role of cytokinins for interactions of plants with microbial pathogens and pest insect. Frontiers. **10**(1), 1-12 (2020). https://doi.org/10.3389/fpls.2019.01777
6. A. Sagar, R.Z. Sayyed, P.W. Ramteke, W. Ramakrishna, P. Poczai, S. Al Obaid, M.J. Ansari, Synergistic effect of *Azotobacter nigricans* and nitrogen phosphorus potassium fertilizer on agronomic and yieldtraits of maize (*Zea mays* L.). Front. Plant. Sci. **13**(1), 1-12 (2022). https://doi.org/10.3389/fpls.2022.952212
7. Y. Lee, R. Krishnamoorthy, G. Selvakumar, K. Kim, T. Sa, Alleviation of salt stress in maize plant by co-inoculation of arbuscular mycorrhizal fungi and *Methylobacterium*

oryzae CBMB20. J. Korean Soc. Appl. Biol. Chem. 58(4):533-540 (2015). https://doi.org/10.1007/s13765-015-0072-4

8. C. Zhang, M.G.A. Van der Heijden, B.K. Dodds, T.B. Nguyen, J. Spooren, A.V. Held, M. Cosme, R.L. Berendsen, A tripartite bacterial fungal plant symbiosis in the mycorrhiza shaped microbiome drives plant growth and mycorrhization. Microbiome. **12**(13), 1-13 (2024). https://doi.org/10.1186/s40168-023-01726-4
9. A. Aasraf, A. Bargaz, K. Yaakoubi, A. Hilali, I. Bennis, Y. Zeroual, I.M. Kadmiri, Nitrogen fixing *Azotobacter* species as potential soil biological enhancers for crop nutrition and yield stability. Front. Microbiol. **12**(1), 1-19 (2021). https://doi.org/10.3389/fmicb.2021.628379
10. Suliasih, S. Widawati, Isolation of indole acetic acid (IAA) producing *Bacillus siamensis* from peat and optimization of the culture conditions for maximum IAA production, IOP Conference Series: Earth and Environmental Science, Indonesia, Bogor, October 28-29 **572**, 012025 (2020). https://doi.org/10.1088/1755-1315/572/1/012025
11. G. Chennappa, M.K. Naik, Y.S. Amaresh, H. Nagaraja, M.Y. Sreenivasa, *Azotobacter*: A potential biofertilizer and bioinoculants for sustainable agriculture. Springer, Singapore. **6**(1), 87–106 (2017). https://doi.org/10.1007/978-981-10-6241-4_5
12. Y. S. Harus, S. W. Budi, A. Sukendro, I. Mansur, Growth and physiology responses of *Samanea saman* inoculated with arbuscular mycorrhizal fungi in silica post-mining soil media using biodegradable pots. Journal of Degraded and Mining Lands Management. **12**(1), 6613-6622 (2024). https://doi.org/10.15243/jdmlm.2024.121.6613
13. W. Herman, Iskandar, S. W. Budi, H. B. Pulunggono, Kurniawati, N. Milantara, Dynamics of vegetation diversity and arbuscular mycorrhizal fungi in post-coal mining revegetation land in Sawahlunto, West Sumatra, Indonesia. BIODIVERSITAS. **25**(12), 4627-4641 (2024). https://doi.org/10.13057/biodiv/d251201