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The Environmental Factors Enhance the Growth and Survival of Rhizophora Seedlings

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Abstract. Coastal ecosystems are synonymous with mangrove trees. Threatened mangrove existence is tantamount to reduced biological existence, weakened coastal protection, and reduced carbon storage. Moreover, mangrove growth naturally depends on the quality of seedlings supported by proper nursery management. This study aims to evaluate the impact of four environmental factors (light intensity, salinity, pH, and tidal frequency) on the growth and survival of Rhizophora: *Rhizophora mucronata, Rhizophora stylosa*, and *Rhizophora apiculata* cultivated in nurseries with a water system integrated with the Internet of Things (IoT). A single-factor design was applied, with each treatment bed containing 600 seedlings (200 per species) and an uncontrolled bed as a control. Weekly random sampling of 20% of seedlings from each treatment for eight weeks. Data were analyzed using a two-way ANOVA with treatment and species as fixed factors, followed by the Tukey HSD test at a significance level of 5%. The results showed that *R. mucronata* in the light treatment (without shade net) achieved the highest survival rate (98.8%) and the largest stem diameter, which was 0.698 cm. In addition, the highest seedling height was *R. mucronata* in the high-yield salinity treatment, reaching 56.78 cm, and the highest number of leaves was recorded in *R. apiculata* in the salinity treatment, with an average of 6.92 leaves per seedling. IoT-integrated nurseries stabilize water supply and tidal cycles, reduce mortality to below 10%, and enhance seedling quality. These findings indicate that nursery environmental management can improve the consistency of mangrove seedling growth and support large-scale restoration.

Keywords: Mangrove; *Rhizophora spp.;* Nursery; Survival Rate, Growth Rate

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INTRODUCTION

Mangroves are highly productive and ecologically vital coastal ecosystems, providing services such as shoreline stabilization, nutrient cycling, fisheries support, and carbon sequestration (Alongi, 2022; Friess et al., 2019). Their capacity to capture and store “blue carbon” makes them crucial for global climate change mitigation (Bourgeois et al., 2024). However, rapid coastal development, aquaculture expansion, and pollution have driven widespread mangrove degradation, particularly in South Asia (Goldberg et al., 2020; Hamilton & Casey, 2016). Indonesia, which holds the world’s largest mangrove area, has lost nearly half of its original cover due to land conversion and unsustainable resource use, equivalent to approximately 800,000 hectares over the past three decades, with an annual loss rate ranging from 0.26 to 0.66 percent (Friess et al., 2019; Ilman et al., 2016; Irsadi et al., 2025).

The success of mangrove restoration relies not only on planting density or site selection but also on the availability of a high-quality nursery. The nursery stage is the most vulnerable phase in mangrove propagation, as fluctuations in salinity, tidal inundation, pH, and light intensity directly influence physiological processes such as osmoregulation, chlorophyll synthesis, and tissue lignification (Chen et al., 2022; Lv et al., 2019;

Zhou et al., 2024). Previous studies have shown that Rhizophora species, widely used in rehabilitation programs, display varying tolerance to abiotic stresses, resulting in inconsistent growth performance (Hsiung et al., 2024; Zhou et al., 2024).

Growth responses in tropical nurseries can vary sharply with local environmental conditions. Lisdayanti et al. (2024) reported that *R. mucronata* seedlings in West Aceh experienced a survival rate after the third week when salinity fluctuated beyond 25 ppt, while seedlings in stable nursery conditions maintained a survival rate above 95%. Similarly, Hilmi et al. (2022) found that mangroves in aquaponic systems in North Jakarta reached 96.9% of survival, demonstrating that controlled water and nutrient circulation enhance early establishment. These findings underscore that even small differences in nursery microenvironments can determine restoration success, emphasizing the need for standardized and adaptive management.

Recent advances in the Internet of Things (IoT) have enabled precision nursery management by integrating environmental sensors and automated controls to monitor and regulate parameters such as light intensity, temperature, salinity, and pH (Foysal et al., 2025; Kumar et al., 2024; Soetedjo & Hendriarianti, 2023). Hilmi et al. (2017) showed that microclimate and irrigation frequency in coastal nursery areas can significantly influence the seedling growth. This suggests that an automated and manipulable IoT-based irrigation system can be effective in improving survival and quality of the mangrove seedlings.

This study aimed to evaluate the effects of four key environmental factors, which are light intensity, salinity, tidal inundation, and pH, on the growth and survival of three Rhizophora species: *R. mucronata, R. stylosa,* and *R. apiculata*, in controlled nurseries. We collected 20% of seedlings randomly every week for eight weeks from each treatment bed to capture the growth dynamics and survival trends. By integrating controlled environmental treatments with continuous monitoring, these findings are expected to support the development of evidence-based, technology-assisted, scalable, and climate-resilient mangrove restoration strategies in Indonesia, starting from good nurseries.

The results of this study are expected to strengthen scientific understanding of the influence of environmental factors on the physiology of mangrove seedlings and provide the basis for the development of a seedbed system integrated with IoT in terms of consistent aeration systems and automation. Overall, these findings will be beneficial for communities and coastal management institutions for increasing the success of mangrove rehabilitation through the provision of high-quality seedlings.

METHODS

Research Site and Design

This research was conducted in a mangrove nursery equipped with an Internet of Things (IoT)-based water control system located in the Pantai Indah Kapuk (PIK) Mangrove Ecotourism Park. The nursery had five beds, each containing 600 seedlings of three Rhizophora species: *R. mucronata, R. apiculata*, and *R. sylosa*, with 200 seedlings per species. Four beds were used as treated groups by varying environmental conditions, which were light, salinity, pH, and tidal frequency. One bed was used as a control group by placing it outside the IoT water system with uncontrolled natural conditions.

Figure 1. Nursery beds mangrove cultivation experiment

Treatment Conditions and Measured Parameters

Five different treatments were used in the nursery experiment to evaluate the effect of environmental factors on Rhizophora seedling growth. The light treatment was applied by removing the parent shade to allow full sun exposure (Wijayasinghe et al., 2019). The salinity was maintained at 15 to 20 parts per thousand (ppt) by mixing NaCl with brackish water (Chen et al., 2022). The pH was adjusted between 7.5 and 8.5 using Dolomit (Haseeba et al., 2025). Tidal conditions were simulated with an automated water level gauge system designed to mimic the natural tidal cycle with the IoT. The control group consisted of seedlings outside the IoT-controlled nursery, exposed to normal environmental conditions without any intervention(Abrogueña et al., 2022).

Observed parameters included seedling height, measured with a ruler; stem diameter, measured using a digital caliper; and leaf number, counted manually. Survival rate was calculated as the proportion of surviving seedlings relative to the initial number at the start of the experiment.

Data Collection and Analysis

Rhizophora seedlings were monitored weekly for eight weeks using a random sampling method with an intensity of 20% in each treatment bed. Parameters assessed included stem diameter, plant height, leaves number, and survival rate, which reflect the physiological performance of the seedlings in various treatments. Data were analyzed using two-way ANOVA to evaluate the effect of treatment and species on each growth measure and survival rate. A Tukey's HSD post hoc test was performed at a 5% significance level if significant differences were found. R version 4.3.2 program was used for all analyses.

RESULTS AND DISCUSSION

Survival Rate

Seedling survival remained consistently high across treatments, reflecting the strong adaptability of Rhizophora species under controlled nursery conditions. As shown in Table 1, the light treatment produced the highest survival rate across all species, reaching 98.8% in *R. mucronata*, 97.5% in *R. stylosa,* and 96.9% in *R. apiculata*. The lowest survival (82,3%) occurred in the control group outside the controlled environmental nursery. This pattern indicated that the environmental regulation, particularly through controlled light and salinity, greatly enhances seedling viability. The raised mud-bed design and regulated microclimate contributed to stable oxygen and moisture availability, reducing stress and sustaining survival rate above 95% across treatments.

The nursery was established within the Mangrove Ecotourism area of Pantai Indah Kapuk (PIK), using natural coastal mud as the substrate. The mud-based beds were constructed as raised structures positioned above the water surface, allowing sufficient moisture through capillary action while preventing constant inundation. This setting balanced aeration and humidity, maintaining adequate oxygen availability for root respiration and minimizing prolonged anoxic stress that often occurs in fully submerged seedbeds. In contrast to traditional community nurseries, where seedlings are grown in shallow shrimp farming with unpredictable tidal flushing, the raised-bed design ensured stable microenvironmental conditions and prevented sediment compaction, thereby sustaining high survival across all species.

Table 1. Survival rate (%) of Rhizophora seedlings in week 8 based on treatment

Treatment	*R. mucronata*	*R. stylosa*	*R. apiculata*
Light	98.8 ± 1.2^a	97.5 ± 1.9^a	96.9 ± 2.4^a
Salinity	96.4 ± 1.5^a	94.8 ± 2.3^a	94.5 ± 2.0^a
pH	90.5 ± 2.8^b	89.4 ± 2.6^b	91.2 ± 2.7^b
Tide	88.1 ± 3.1^b	86.9 ± 3.5^b	87.5 ± 3.2^b
Control	82.3 ± 3.9^c	84.0 ± 3.6^c	83.2 ± 3.4^c

*Numbers in the same column followed by different letters indicate significant differences at the 5% level (Tukey HSD test).

The three species of Rhizophora show morphological differences that reflect their respective adaptation strategies. *R. mucronata* has the largest hypocotyl and is dark green in color with thick, elliptical leaves, indicating a high tolerance to salinity. *R. stylosa* has a slender, elongated hypocotyl with narrower leaves, usually adapting to more open and oxygen-rich coastal locations. Meanwhile, *R. apiculata* has oval leaves with pointed tips as well as reddish-colored stems, which indicate stronger adaptation to stable, muddy environments. These visible morphological differences reinforce the interpretation that each species responds differently to light and salinity settings during the growth phase.

Light intensity emerged as a dominant factor influencing seedlings' viability. Exposure to sufficient light enhanced photosynthesis activity, enabling greater carbohydrate accumulation that supported energy-demanding processes such as osmotic regulation and root establishment (Basyuni et al., 2020; Taiz & Zeiger, 2012; Walter & Kromdijk, 2022; Wasilewska-Dębowska et al., 2022). The superior performance of *R. mucronata* under light treatment reflects its high tolerance to irradiance and ability to maintain photochemical efficiency through balanced electron transport (Arachchilage et al., 2020). Moderate salinity (15–20 ppt) also contributed positively to survival by maintaining osmotic balance and preventing ionic toxicity. Under this condition, seedlings retained turgor through the compartmentalization of sodium and chloride ions into vacuoles and the synthesis of compatible solutes such as proline and glycine betaine, sustaining cell integrity and metabolic stability (Mansour, 2023; Reef & Lovelock, 2015).

The survival rate recorded in this study was notably higher than that of conventional nursery systems. Lisdayanti et al. (2024) reported that *R. mucronata* seedlings in West Aceh experienced survival declines after the third week once salinity exceeded 25 ppt, while Hilmi et al. Hilmi et al. (2022) found that aquaponic-based nurseries maintained survival around 96.9%, suggesting that stable water and nutrient regimes are key determinants of early growth. The results of the present research surpassed both references, indicating that environmental regulation focusing on light and salinity stability can achieve comparable or even superior survival without requiring complex system modifications. These findings confirm that mortality in conventional nurseries largely arises from fluctuating salinity and oxygen limitation in waterlogged substrates, whereas raised mud beds under controlled exposure can effectively eliminate these stressors.

Lower survival observed in pH and tidal treatments further highlights the importance of maintaining chemical and hydrological equilibrium. Fluctuations in pH can alter the solubility of essential micronutrients, affecting chlorophyll formation and enzymatic activity, while excessive tidal simulation promotes hypoxic stress, reducing root vitality and carbohydrate translocation (Goldberg et al., 2020; Ma et al., 2024). The control seedlings grown outside the regulated environment experienced combined stress from the variation of temperature, humidity, and salinity, resulting in a lower survival rate compared the the treatments.

Overall, the combination of natural mud substrate, raised-bed structure, and regulated environmental conditions formed an optimal balance for Rhizophora seedlings' establishment. The consistent survival rate above 95% across most treatments demonstrates that physical and environmental stability can effectively support early mangrove development, even in the absence of technological control systems. These findings provide a practical framework for designing semi-controlled nursery models that integrate natural substrate properties with stable microclimate management, ensuring robust seedling survival and uniform quality for large-scale restoration. The novelty of this research lies in demonstrating that high survival can be achieved through fundamental environmental regulation rather than mechanized intervention, contributing scientific evidence for cost-effective and ecologically grounded mangrove nursery management.

Stem Diameter

Stem diameter varied significantly among treatments, highlighting that light and salinity settings affect secondary growth in Rhizophora seedlings. As shown in Table 2, seedlings exposed to light had the largest diameters, with *R. mucronata* reaching 0.698 ± 0.119 cm, *R. apiculata* 0.590 ± 0.098 cm, and *R. stylosa* 0.405 ± 0.064 cm. Under salinity treatments, diameters remained relatively high, averaging 0.562 ± 0.067 cm for *R. mucronata* and 0.500 ± 0.061 cm for *R. apiculata*, while control and pH treatments showed smaller values, mostly below 0.55 cm. These data indicate that adequate light intensity and moderate salinity promote secondary thickening, indicating carbon allocation and tissue lignification.

Table 2. Average stem diameter (cm) ± SD Rhizophora seedlings at week 8.

Treatment	*R. mucronata*	*R. stylosa*	*R. apiculata*
Light	0.698 ± 0.119[a]	0.405 ± 0.064[b]	0.590 ± 0.098[a]
Salinity	0.562 ± 0.067[ab]	0.410 ± 0.055[b]	0.500 ± 0.061[ab]
pH	0.622 ± 0.112[b]	0.390 ± 0.059[b]	0.508 ± 0.106[b]
Tide	0.503 ± 0.092[b]	0.405 ± 0.064[b]	0.503 ± 0.137[b]
Control	0.539 ± 0.059[b]	0.412 ± 0.046[b]	0.435 ± 0.049[b]

*Numbers in the same column followed by different letters indicate significant differences at the 5% level (Tukey HSD test).

Optimal stem development can be achieved when the plant receives enough direct light. This can happen because the increase in electron photosynthesis and energy production requires light for carbon fixation as well as lignin biosynthesis (Basyuni et al., 2020; Falcioni et al., 2018; Walter & Kromdijk, 2022). Sufficient increase in direct light exposure makes the seedlings increase the activity of the cambium and causes the stems to become thicker by accumulating structural carbohydrates. The response of the seedlings to the beds in the treatment without shade proves this; the seedlings were recorded to have the highest average diameter width compared to other treated beds. *R. mucronata* showed the most efficient photosynthesis results in the development of the diameter (Arachchilage et al., 2020). In contrast, *R. stylosa* has the smallest average diameter development compared to others. This reflects a conservative resource utilization strategy that prioritizes ionic balance and osmoprotective production rather than structural growth (Akaji et al., 2019).

The average diameter of the rod obtained is higher than the research has ever recorded by Lisdayanti et al. (2024) he reported that there was a decrease in the growth of *R. mucronata* seedlings exposed to unstable salinity levels. Similarly, Hilmi et al. (2022) found that smaller diameters were in aquaponic nurseries despite the high survival of seedlings. This suggests that sufficient light conditions and stable salinity can increase the diameter of the seedling stem, and maintaining environmental stability with minimal intervention can significantly increase secondary growth in seedlings.

Plant Height

Seedling height showed a significant impact of light and salinity on the vertical growth. As shown in Table 3, *R. mucronata* had the highest average height, at 56.78 ± 8.45 cm, under salinity conditions, followed by 53.28 ± 4.64 cm under light exposure conditions. In contrast, seedlings exposed to pH and tidal treatments were significantly shorter, with an average height below 50 cm, while the control group only reached 47.00 ± 5.12 cm.

The strong height performance under salinity treatments indicates that moderate ionic stress in the range of 15–20 ppt induces osmotic adjustment without triggering growth inhibition. Under such conditions, Rhizophora seedlings accumulate compatible solutes, maintain cell turgor, and allow elongation to continue efficiently. Meanwhile, salinity control also maintains chlorophyll stability, ensuring that photosystem activity is not suppressed. This physiological response likely contributes to sustained vertical growth, allowing *R. mucronata* to invest assimilated carbon into shoot elongation rather than stress recovery (Moslehi et al., 2021). Consistently high values under light treatments further support the idea that sufficient irradiation facilitates carbohydrate supply for cell wall expansion, reinforcing the role of light-driven photosynthesis in maintaining (Taiz & Zeiger, 2012; Walter & Kromdijk, 2022).

Table 3. Average plant height (cm) ± SD Rhizophora seedlings at week 8.

Treatment	*R. mucronata*	*R. stylosa*	*R. apiculata*
Light	53.28 ± 4.64[a]	39.25 ± 6.12[b]	45.92 ± 5.75[a]
Salinity	56.78 ± 8.45[a]	37.90 ± 5.44[b]	43.30 ± 4.25[a]
pH	49.80 ± 5.19[b]	33.75 ± 4.39[b]	43.05 ± 4.61[b]
Tide	43.42 ± 3.85[b]	35.40 ± 4.60[b]	40.40 ± 5.57[b]
Control	47.00 ± 5.12[b]	38.02 ± 5.89[b]	42.10 ± 4.82[b]

*Numbers in the same column followed by different letters indicate significant differences at the 5% level (Tukey HSD test).

By comparison, the height reduction under pH and tidal treatments can be related to environmental imbalances that limited growth efficiency. Unstable pH conditions might affect nutrient solubility, particularly nitrogen and iron, which are essential for chlorophyll synthesis, while excessive tidal inundation likely induced temporary oxygen deficiency around the roots, constraining aerobic respiration and energy production (Gobler & Baumann, 2016; Ma et al., 2024). In contrast, stable aeration in controlled treatments maintained oxygen supply and prevented diversion of assimilates toward adaptive structures such as aerenchyma, thereby sustaining upward growth.

The height data obtained in this research were higher than those reported by Lisdayanti et al. (2024), where *R. mucronata* seedlings under fluctuating coastal salinity reduced elongation after three weeks. Similarly, the vertical growth recorded here surpassed the previous research by Hilmi et al. (2022), that aquaponic-grown seedlings reached approximately 45–50 cm under mixed water conditions. These comparisons indicate that maintaining stable salinity and light within the tolerance range of Rhizophora species provides a more reliable outcome for height development than systems relying on periodic tidal flow.

From a practical perspective, increasing the elongation of shoots during the nursery stage has a direct impact on growth success. Tall seedlings and strong stems indicate efficient photosynthesis in seedlings. Therefore, the seedlings are expected to have good resistance and develop a crown to bear the initial pressure when planting in nature. These findings highlight that environmental control of seedlings, such as salinity consistency, can increase the vigor of mangrove seedlings, thereby supporting restoration efforts.

Leaf Number

Leaf production varied significantly among treatments, demonstrating the sensitivity of Rhizophora species. Based on Table 4, the salinity treatment produced the highest average leaf number for all species, with *R. apiculata* reaching 6.92 ± 1.01 leaves, followed by *R. mucronata* at 6.40 ± 0.81 leaves, and *R. stylosa* at 6.00 ± 0.00 leaves. Seedlings treated with pH, tidal, and light produced fewer leaves, typically between three and five leaves per plant, whereas control plants exhibited similarly low leaf numbers. These results indicated that moderate salinity levels (15–20 ppt) are sufficient for leaf initiation and growth, while pH variation and flooding tend to inhibit leaf development.

The moderate salinity (15-20 ppt) caused good leaf growth, indicating adaptive Rhizophora seedlings that can support an osmotic adjustment. It suggests that the physiological balance in the seedlings might be due to sodium and chloride ions compartmentalized into vacuoles to stabilize the osmotic potential, leading to the maintenance of cell turgor. This supports leaf development and prevents leaf breakage (Mansour, 2023; Reef & Lovelock, 2015). This balance explains the good response of the seedlings to consistent and controlled salinity levels by showing active bud growth and good leaves. In addition, mangrove seedlings in this study produced greener and thicker leaves and a consistent number of leaves number (Chan-Keb et al., 2025).

The average number of leaves obtained in the observation showed that the beds with light treatment (without shade) were lower. This might be because of widespread irradiation caused by mild photooxidative stress that limits leaf initiation while promoting stem thickening. In addition, pH fluctuations and tides interfere with nutrient absorption and oxygen supply, which inhibits the initiation of new leaf growth. These findings are consistent with the mechanism described by Ma et al. (2024) and Gobler & Baumann (2016), where acid-base imbalance and hypoxia inhibit metabolic activity in photosynthetic tissues. It can be seen in the control group showing a similar decrease due to the combination of temperature, wetness, and salinity, hence the need to maintain the chemical and hydrological stability of the nursery during the cultivation period.

Table 4. Average number of leaves ± SD Rhizophora seedlings at week 8.

Treatment	*R. mucronata*	*R. stylosa*	*R. apiculata*
Salinity	6.40 ± 0.81[a]	6.00 ± 0.00[a]	6.92 ± 1.01[a]
Tide	4.60 ± 1.02[b]	4.00 ± 0.00[b]	4.80 ± 1.05[b]
pH	3.50 ± 0.90[b]	3.80 ± 0.95[b]	3.25 ± 0.85[b]
Light	2.70 ± 0.97[b]	4.00 ± 0.00[b]	2.70 ± 0.97[b]
Control	3.05 ± 1.01[b]	4.00 ± 0.00[b]	3.05 ± 1.01[b]

*Numbers in the same column followed by different letters indicate significant differences at the 5% level (Tukey HSD test).

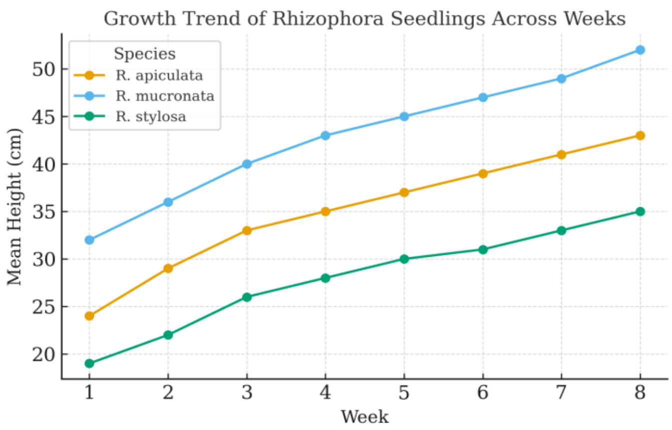

Figure 2. Growth trend of Rhizophora seedlings across weeks

Compared to previous studies, the study noted that the number of leaves was slightly higher. Lisdayanti et al. (2024) reported that fewer than five leaves after eight weeks in *R. mucronata* seedlings were exposed to unstable salinity. Similarly, Hilmi et al. (2022) found that about six inbreeding leaves in aquaponic systems require constant and stable water cycles. However, controlled salinity conditions produce seedling-comparable yields from nursery yields with simple environmental management. This underscores that maintaining ion balance is more important than using more complex technologies for optimal leaf productivity. Increased leaf count is best produced by beds with stable moderate salinity treatment. Seedlings with good leaf growth are expected to become mangrove trees that have a wide crown area, and thereby they are expected to absorb more carbon. Mangrove seedlings that can absorb carbon better are certainly optimal for restoration efforts and reducing global pollution.

Physiological Growth and Integration Trends

The average height of seedlings in all Rhizophora species grew stably from week 1 to week 8 (Figure 2). Trends show that *R. mucronata* shows the fastest and consistent propagation of seedlings, followed by *R. apiculata* and *R. stylosa,* which experience rather slow but stable growth. This nearly identical pattern shows consistent and sustained carbon assimilation and a balanced distribution for bud growth throughout the cultivation period in the nursery. This trend confirms the effective physiological coordination between photosynthesis and osmotic regulation that allows seedlings to retain turgor and grow without any significant stress constraints. The consistent patterns that occur indicate that stable light and salinity conditions support sustainable vegetative growth, help produce uniform and quality seedlings, and are expected to absorb carbon more optimally to support efforts to reduce global pollution through restoration projects.

The results of this research represent the growth dynamics of Rhizophora seedlings in the controlled environmental conditions, thus making this research have a novelty value. Unlike previous research that focused on the aspect of environmental stability on seed growth. These findings are expected to strengthen scientific knowledge of the growth adaptation mechanisms in mangroves and provide practical benefits in the high-quality seeds production for large-scale coastal restoration and climate change mitigation efforts.

CONCLUSION

Controlled environmental management significantly improves the growth and survival of Rhizophora seedlings compared to a conventional nursery. Light treatment has the most positive impact, especially on the survival and growth of stem diameter in *R. mucronata* seedlings. Meanwhile, salinity treatment showed good results for plant height in *R. mucronata* seedlings and leaves number in *R. apiculata* seedlings. These results confirm that stable conditions can improve the quality of seedlings for restoration activities. Further research, such as planting observation is recommended to assess the long-term response.

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AUTHOR CONTRIBUTION STATEMENT

NDMS conceptualization, data curation, formal analysis, investigation, methodology, visualization, project administration, and writing – original draft. AP validation, review, feedback, and adjustment of the draft manuscript. NKTM review, editorial input, and refinement of the final manuscript. MAJ validation, review, feedback, and adjustment of the draft manuscript. IDS review and proofreading of the manuscript.

INFORMED CONSENT STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this paper.

CONFLICT OF INTEREST STATEMENT

This research did not involve humans or animals. All procedures were performed using plant material (Rhizophora spp.) in accordance with breeding standards and research ethics.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no AI-assisted technologies were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-assisted technologies.

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