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Original Article

Integrating shade and microbial biostimulants in mangrove seedling nurseries: A field-based evaluation of *Bruguiera gymnorrhiza* for tidal rehabilitation success

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Abstract

This study examined the growth performance of *Bruguiera gymnorrhiza* seedlings in a tidal nursery, employing a split-plot design that incorporated three shade levels (0%, 45%, 80%) and three varying biostimulant doses (0, 10, 30 g). Physiological indicators, morphological traits, biomass allocation, seedling quality, and microclimate variables were evaluated. Shading markedly improved seedling performance compared to non-shaded conditions, enhancing leaf greenness, photosynthetic efficiency, height growth, leaf development, and overall quality index. Among the treatments, 45% shade yielded the highest growth responses, though its performance was statistically similar to that of 80% shade. Biostimulants demonstrated a restricted impact, affecting solely the photosynthetic rate, with 10 g and 30 g doses exhibiting comparable enhancements compared to the control group. No interaction between shade and biostimulants was observed, indicating that the effects of biostimulant application had not manifested during the brief nursery period. The findings indicate that moderate shading significantly influences the early vigor of *B. gymnorrhiza* seedlings in tidal environments, offering practical recommendations for enhancing nursery protocols to facilitate cost-effective mangrove rehabilitation.

Keywords: *Bruguiera gymnorrhiza,* Shading, Biostimulants, Rehabilitation, Mangrove

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Introduction

Indonesia has approximately 3.36 million hectares of mangrove forests spread across forest and non-forest areas (Ministry of Environment and Forestry, 2021). Indonesia, recognized as a megadiverse country, possesses the highest diversity of mangrove species globally, predominantly located in Java, Sumatra, Kalimantan, Sulawesi, Maluku, the Lesser Sunda Islands, and Papua (Kusmana, 2014; Basyuni et al., 2022). The biodiversity of mangroves faces significant threats from land conversion for aquaculture, logging, mining, and reclamation, resulting in the degradation of approximately 637,000 ha or 10–33% of mangrove areas (Kusmana, 2014). This condition jeopardizes the sustainability of mangrove ecosystems, necessitating rehabilitation efforts to restore their ecological functions.

Bruguiera gymnorrhiza is a prevalent mangrove species recognized for its significant potential in ecosystem rehabilitation. This species exhibits a broad distribution across the Indo-Pacific region and demonstrates significant tolerance to salinity as well as adaptability to diverse substrate types (Allen and Duke, 2006). *B. gymnorrhiza* functions as a crucial habitat for marine organism, such as fish and crabs (Krauss and Allen, 2003a), underpins the marine food web (Owuor et al., 2024), contributes to coastal protection against erosion and waves within the mangrove ecosystem (Asari et al., 2021) and enhances the potential for ecotourism in mangrove regions (Blanton et al., 2024).

Bruguiera gymnorrhiza exhibits significant physiological adaptations, such as the capacity to modulate osmotic pressure through the accumulation of Na^+ and Cl^- ions (Zhu et al., 2012) and the regulation of nutrients and ions during decomposition of leaves (Wang and Lin, 1999). On the other hand, based on allometric estimations, the distribution of internal carbon between stems, roots, and leaves in *B. gymnorrhiza* indicates that this species stores substantial carbon in its vegetative biomass (Carbon Partitioning & Allometric Relationships). Additionally, field surveys in Sulawesi, Indonesia, show that *B. gymnorrhiza* holds the highest position in biomass carbon stock compared to other local mangrove species (Malik et al., 2022).

Mangrove ecosystem rehabilitation typically employs plants cultivated in nurseries or through nursery methods. Nursery cultivation is a vital phase in the rehabilitation of the mangrove ecosystem to enhanced survival rate and adaptation of seedlings to variable environmental conditions. Optimal nursery techniques not only accelerate seedling growth rate, but also increase resistance to various environmental stresses, such as high salinity, waterlogging, and nutrient limitations (Liu et al., 2012).

Mangrove nurseries can be conventional or modern/modified conventional. Conventional nurseries generally use soil or mud media without additional treatment and are often applied on a small scale at the local level. Meanwhile, modern/modified conventional nurseries are nursery techniques developed to overcome various obstacles encountered in conventional nurseries, such as extreme temperatures, water availability, pest and disease attacks, and soil nutrient deficiencies. Modifications to traditional nursery techniques are implemented to enhance growth success and resource efficiency. Conventional methods remain prevalent; however, challenges such as low growth success rates, pest and disease infestations, and inadequate seedling adaptation to extreme environmental conditions frequently hinder rehabilitation efforts. Such constraints are frequently observed in degraded regions and those affected by invasive species (Gatt et al., 2024). Therefore, modifications to conventional nurseries that combine technology and ecology are needed to improve the success of mangrove rehabilitation (Charoenlerkthawin et al., 2024). After 2025, the future of mangroves will depend on technological and ecological advances in multi-species silviculture, genetics, and forestry modeling (Alongi, 2002).

Research on nursery procedures for *B. gymnorrhiza* predominantly emphasizes traditional approaches, including the utilization of natural growing media and water management, while often neglecting technological innovations that could enhance seedling performance. Conversely, numerous studies have demonstrated that the use of microorganism-based biostimulants, including *Azotobacter* sp., *Azospirillum* sp., and *Bacillus* sp., enhance plant growth and resilience to environmental stress (Mojumdar et al., 2022). However, the application of biostimulants on mangrove seedlings, especially *B. gymnorrhiza*, is still very limited. In addition, in line with the increasing global temperature, the role of shade becomes very important in plant growth. Shade can directly limit photosynthesis but can also indirectly affect carbon acquisition potential through morphological and physiological acclimation responses (Mathur et al., 2018). Research on the ideal level of shade for plant growth generally states that 25–50% shade treatment has an effect on increasing plant mass, but this does not occur at 90% shade where light is very scarce (Semchenko et al., 2012; Basyuni et al., 2020). In 3-month-old *B. gymnorrhiza*, 0% shade intensity yielded the optimal growth response for seedlings (Kusmana and Sukaesih, 2021). The research findings offer a basis for identifying the optimal shade level for the growth of *B. gymnorrhiza* seedlings. One potential strategy is the development of biostimulant-based seedling techniques (Mojumdar et al., 2022) and shading to improve growth success (Semchenko et al., 2012).

This study evaluates the growth of *B. gymnorrhiza* seedlings in tidal-affected nursery areas with modified shade and biostimulant treatments. Based on these research (Semchenko et al., 2012; Basyuni et al., 2020) insights, 45% shade was selected to represent an empirically supported moderate-light environment that promotes biomass accumulation, while 80% shade was included to simulate low-light conditions commonly encountered under dense mangrove canopies. This research addresses a key gap between controlled nursery optimization and field-scale mangrove rehabilitation. We hypothesize that moderate shading will substantially improve physiological performance and seedling vigor relative to non-shaded conditions. That microbial biostimulants will provide additional, physiological benefits; and that interaction effects will remain limited under short-term tidal nursery conditions. The outcomes are expected to inform restoration programs in Indonesia and offer a scalable model for climate-resilient seedling production in tropical coastal regions.

Material and Methods

Study site

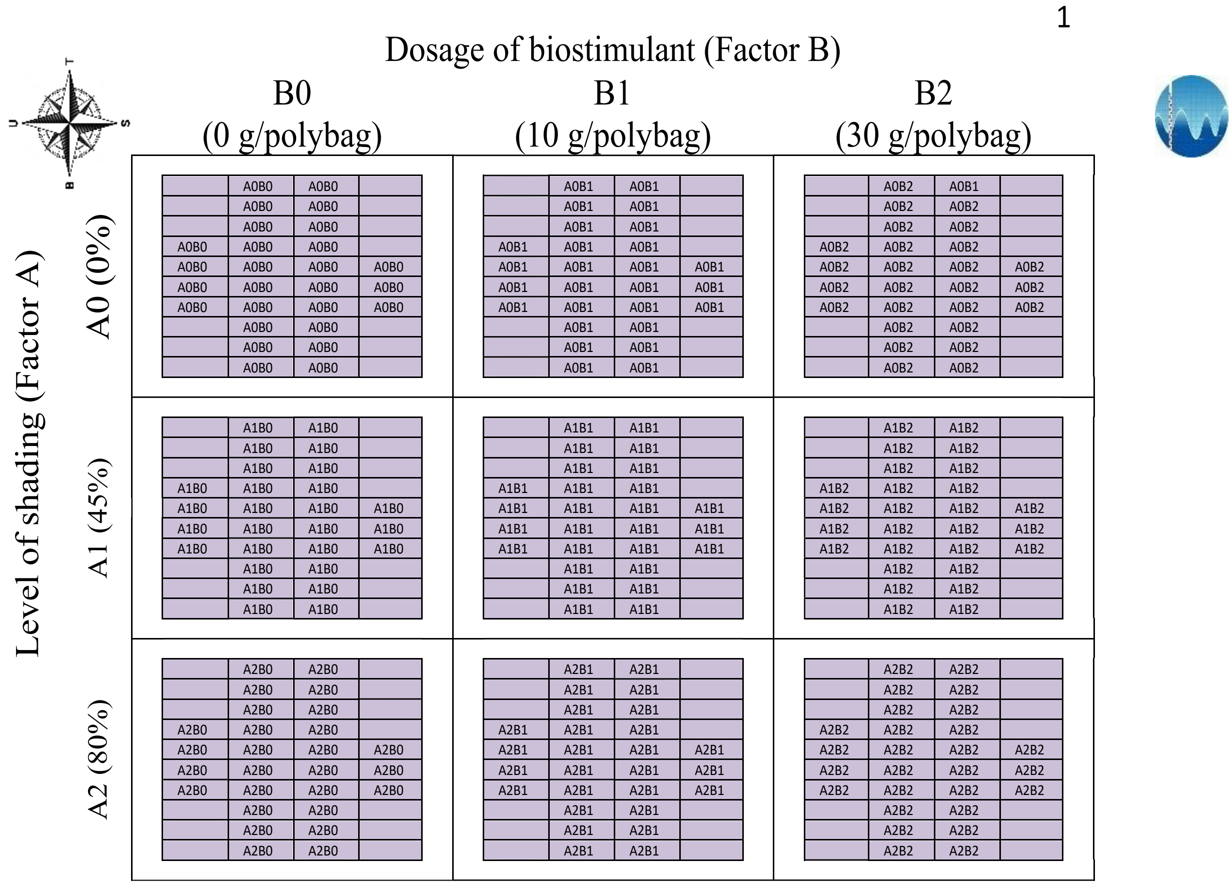
The study was carried out in the mangrove nursery zone of the Krida Wana Lestari Farmer Group. The research site is located in the Segaran Anakan Mangrove Ecosystem in Lempong Pucung Village, Kampung Laut Sub-District, Cilacap Regency, Central Java Province, Indonesia. This region is at 7°42'57.25" S and 108°52'29.21" E and it has a dynamic mangrove habitat. Over the past ten years, annual rainfall in Kampung Laut Sub-District has received an average of 2,940 mm of rain each years (Kresnasari et al., 2022). The data was gathered from February to November 2024, which is the rainy season.

Planting material preparation

The planting material used was *B. gymnorrhiza* propagules taken from parent trees more than 10 years old in Binangun Baru Village, Cilacap Regency, Central Java Province, Indonesia. The propagules used had a diameter of 1.7–2.0 cm and a length of 15–20 cm. The propagules were then planted in 20 x 15 cm polybags containing 750 g mud medium per polybag. The propagules planted in the polybags were then placed in a nursery area exposed to tidal fluctuations without using gabions (to protect the seedlings from being washed away). Another material prepared is a formula biostimulant containing a consortium of six bacteria (*Rossellomorea vietnamensis* strain Azt 8.1, *Bacillus altitudinis* strain PK 5.1, *B. tequilensis* strain Azp 4.1, *B. cereus* strain Azt 5.2, *B. cereus* strain Azt 7.1, *B. cereus* strain Azt 9.1, *and Aspergillus niger*) from the mangrove ecosystem in Suwung, Bali. These bacteria have the potential to stimulate plant growth because they are active in dissolving phosphate, fixing nitrogen, producing IAA growth hormones, ACC deaminase, siderophores, and cellulase. Biostimulants are applied by mixing the biostimulant liquid with a binding medium consisting of a combination of fine zeolite, coarse zeolite, and cocopeat in a ratio of 3:1:1. For every 10 kg of binding medium, 1.5 L of biostimulant is mixed in. A solid biostimulant is applied to *B. gymnorrhiza* seedlings once when they are 2 months old (or have two pairs of leaves).

Experiment design

The study was conducted using a split plot design with two factors (Figure 1). The main plot was the shade level (Factor A), consisting of three levels: A0 = 0%, A1 = 45%, and A2 = 80%. The subplot was the biostimulant dose (Factor B), consisting of 3 levels, namely B0 = 0 g/polybag, B1 = 10 g/polybag, and B2 = 30 g/polybag. This research design produced 9 treatment combinations carried out with 3 replicates, resulting in 27 experimental units. Each experimental unit used 27 seedlings. Seedlings were randomly assigned within each experimental unit, and treatment allocation to plots was conducted using a randomization scheme to minimize positional bias. The total number of seedlings used in this study was 27 experimental units x 27 seedlings = 729 seedlings. The observation of the effects of shade and biostimulants was conducted over a period of 10 months.



Remark: The blue circle on the top right indicates the direction of tidal flow.

Figure-1. Experimental design layout of *Bruguiera gymnorrhiza* seedlings under shading and biostimulant treatments.

Data collection and sample analysis

Data collection was carried out on physiological parameters, morphology, nursery environment and components of nursery costs. In this study, the biostimulant consortium was applied as a commercially formulated product, and information on microbial survival, viability, and population density at the time of application was not available. In comparison, morphological data were also collected from the mangrove nursery at Tahura Ngurah Ray, which operates without shade but with a controlled water system.

Physiological observation

Physiological parameters include chlorophyll (leaf greenness index) and gas exchange (photosynthesis rate, transpiration rate, stomatal conductance). The measurements of leaf greenness index were taken on the 3rd, 4th, and 5th leaves at 4, 6, 8, and 10 months after planting using a Soil and Plant Analysis Development (SPAD) 502Plus optical device (Minolta). Gas exchange parameters were measured 10 months after planting between 9:00 and 10:00 a.m. during the rainy season (November 2024) using the LI-6800 Portable Photosynthesis System (LI-COR Biosciences, Lincoln, NE, USA). This study was limited by the observation timeframe. Gas exchange data observations were conducted on the 10th month, November 2024. According to data from the Indonesian Meteorology, Climatology, and Geophysics Agency (BMKG), November 2024 is the rainy season. The observation timeframe can be used as a basis for drawing conclusions from the gas exchange data.

Morphological observation

Morphological parameters include: survival rate, seedling height, leaf morphology (leaf length, leaf width, and leaf area), seedling quality index (ISQ), and biomass. Seedling height was measured from bud emergence to the top of the bud using a tape measure. Leaf morphology measurements, including length, width, and area, were taken on the 3rd, 4th, and 5th leaves using a meter stick. Three seedlings samples were selected for biomass measurement for each treatment. Shoot and root biomass samples were oven-dried at 85°C for 24 hours, and then weighed using a digital balance. Biomass parameters included shoot dry weight (SDW), root dry weight (RDW), total dry weight (TDW), and seedling quality index (ISQ).

The Seedling quality index (ISQ) was calculated vertically or horizontally based on seedling height, seedling diameter, and total dry weight which describes the entire seedling, as shown in the equation below:

$$\mathrm{ISQ} = \frac{(\mathrm{SDW\ (g)} + \mathrm{RDW\ (g)}}{\frac{\mathrm{H\ (cm)}}{\mathrm{D\ (cm)}} + \frac{\mathrm{SDW\ (g)}}{\mathrm{RDW\ (g)}}}$$

Remark:
ISQ = Seedling quality index,
SDW= Shoot Dry Weight (g),
RDW= Root Dry Weight (g),
H=Heigh (cm),
D= Diameter (cm).

Seedlings height and number of leaves were measured periodically at 4, 6, 8, and 10 months, and leaf length and width were measured at 8 and 10 months after planting. while leaf area and biomass measurements were taken at 10 months after planting. Leaf area (LA) was calculated using the ellipse formula used on *Shorea* sp. leaves (Rosdayanti et al., 2019), as an approximation as follows:

$$\mathrm{LA} = \frac{1}{2} x (3.14) x (\mathrm{LWxLL})$$

Remark:
LA= Leaf area (cm),
LW=Leaf Width (cm); measured at the widest point of the leaf,
LL= Leaf Length (cm); length from the base of the leaf to the tip of the leaf.

Climatic and edaphic observation

Microclimate measurements, including air temperature, humidity, tidal height, and soil nutrient content, were taken in the nursery area. Microclimate conditions were measured using a thermometer, while tidal height was measured using high tide data throughout the study period. Soil nutrient content was measured at the beginning and end of the study using the composite method. The weight of the soil sample analyzed was 500 g. Soil physical and chemical parameters included acidity (pH), organic carbon, total nitrogen, Bray phosphorus, total phosphorus, potassium, cation exchange capacity, and C/N ratio. Analysis of physical and chemical parameters of the soil was carried out at the Laboratory of the Department of Soil Science and Land Resources, IPB University, Indonesia. The study is limited by the lack of direct measurements of microbial activity or soil biological indicators.

Cost component measurement

Cost components include investment and operational costs incurred during the nursery period. These costs assume a production of 10,000 seedlings on communally managed land and relatively stable labor inputs over a short production cycle. The use of assumptions represents a limitation of this study; therefore, the economic values should be interpreted as comparative rather than absolute values and should not be directly applied across different locations or business scales.

Statistical analysis

Data analysis was performed using Microsoft Office Excel and SAS (Statistical Analysis System) 9.1.3 Portable software (SAS Institute, 2005). The measurement data were analyzed using analysis of variance (ANOVA), followed by Duncan Multiple Range Test (DMRT) at a 5% level when significant differences were observed (Gomez, 2007). DMRT was used because it is more sensitive in detecting differences between means, especially when treatment responses show very little biological variation and differences between treatments are relatively small.

Results and Discussion

The impacts of shading and biostimulants on *B. gymnorrhiza* seedlings' physiology

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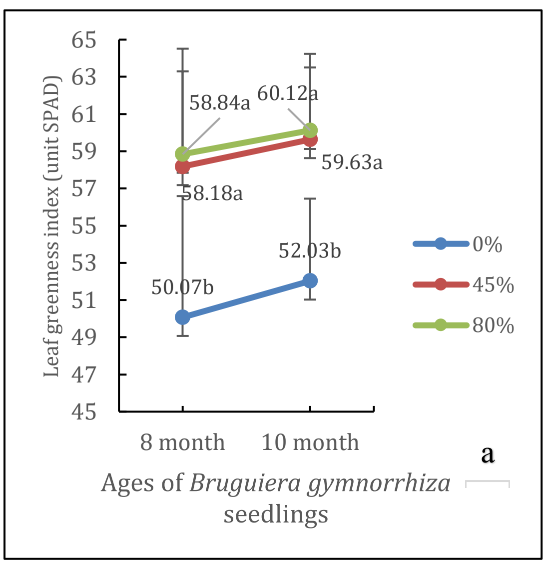
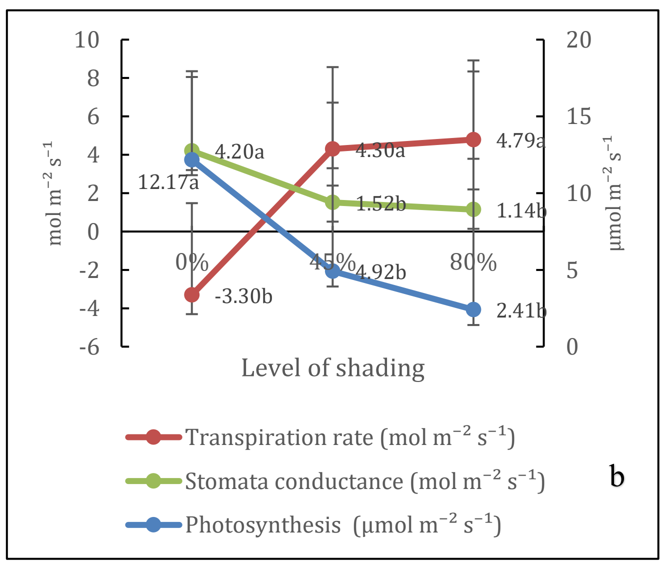
The interaction between shade and biostimulant had no significant effect on all physiological parameters. Shading alone significantly affected the leaf greenness index at 8 months (f=23.61*) (p=0.006) and 10 months (f=6.95*) (p=0.049), photosynthetic rate (f=31.52*) (p=0.004), transpiration rate (f=6.95*) (p=0.050), and stomatal conductance (f=9.58*) (p=0.0003). Meanwhile, biostimulants application alone significantly affected the photosynthetic rate (f=11.19*) (p=0.05). This condition shows that the physiological response of *B. gymnorrhiza* is affected by light stress. Shading acts as a stress reducer that can affect plant physiological activity, and biostimulants can influence photosynthetic rates. Interventions such as shading, humidity management, or optimal temperature are needed in conjunction with biostimulants application to increase physiological effects more significantly (García-Cano et al., 2025). Biostimulants can have positive or negative effects on plant growth and production, which are determined by several factors, such as the dose or concentration of biostimulants administered (Nardi et al., 2016). Proper use of biostimulants can increase plant metabolism and endogenous plant hormone activity, thereby accelerating plant growth (Du Jardin, 2012). The application of biostimulants to *Brugueria* sp. mangrove seedlings in mangrove ecosystems is considered to enhance CO_2 exchange and photosynthetic efficiency through increased nitrogen uptake. The beneficial effects of microbe-based biostimulants depend on the species (Rouphael and Colla, 2020). The role of bacterial consortia, especially *B. cereus*, in biostimulant formulation may influence photosynthetic rate. In addition, the application of microbial and non-microbial plant biostimulants can modify both primary and secondary plant metabolism (Colla and Rouphael, 2015).

Physiological parameters of *B. gymnorrhiza* indicated no significant differences between 45% and 80% shading, whereas both differed significantly from 0% shading (Figure 2). The leaf greenness index and transpiration rate parameters are highest at 45% and 80% shade. Meanwhile, the photosynthetic rate and stomatal conductivity were highest without shade (0%) (Figures 2a and 2b). Biostimulants at 10 g and 30 g showed the same effect on the photosynthesis rate and differed from the 0 g dosage (Figure 2c).





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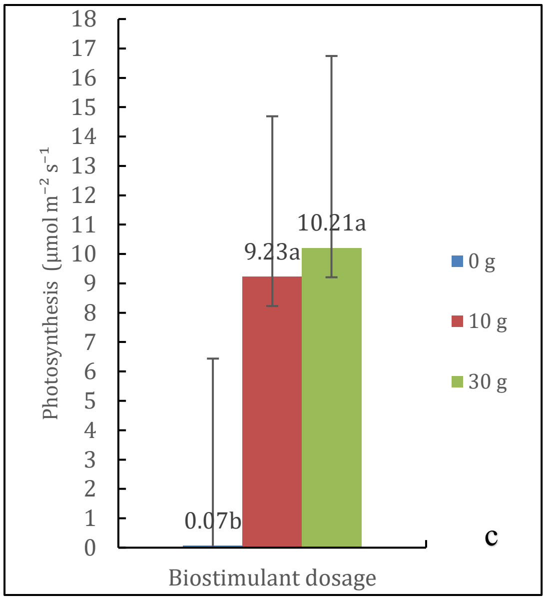

Figure-2. Effects of shading level on the physiological parameters of *B. gymnorrhiza* seedlings: a) Leaf greenness index; b) Gas exchange of *B. gymnorrhiza* at the age of 10 months; c) Effects of biostimulant dosage on Photosynthesis rate of *B. gymnorrhiza* at the age of 10 months. The letters indicated the significance level according to ANOVA and DMRT at $p \leq 0.05$.

The leaf greenness index tended to be lower under 0% shading and highest under 45–80% shading, presumably due to environmental stress conditions caused by direct exposure to sunlight, which caused degradation of the leaf greenness index through photo-oxidation. Other studies have shown that direct exposure to sunlight without shade causes a decrease in chloroplast stability in mangrove species, including *B. gymnorrhiza* (Li et al., 2021). Shade levels of 45% and 80% at 8 and 10 months of age had a positive effect on leaf greenness index values, thereby reducing damage to leaf greenness index due to excessive solar radiation.

The rate of photosynthesis and stomatal conductance showed a decrease in line with increasing shade, whereas the transpiration rate increased in line with increasing shade (Figure 2b). The low transpiration rate in the treatment without shade (0%) indicated low dehydration potential. Under no shading conditions (0%), *B. gymnorrhiza* at 10 months of age had higher stomatal conductivity (4.20 mmol m^{-2} s^{-1}) compared to 45%-80% shading levels (1.52 mmol m^{-2} s^{-1}; 1.14 mmol m^{-2} s^{-1}). This allows more CO2 diffusion into the leaves, resulting in an increased photosynthetic rate (12.27 µmol m^{-2} s^{-1}). At 10 months after planting, *B. gymnorrhiza* seedlings under 0% shading exhibited optimized photosynthetic efficiency, indicating adaptation to high-light conditions. These results are in line with greenhouse-based studies, which evaluated the growth of *B. gymnorrhiza* in greenhouse conditions without shading, which reported a maximum photosynthetic rate approximately 9–10 µmol m^{-2} s^{-1}, with an average leaf greenness index (SPAD unit) of 50.3 (Kusmana et al., 2018). These findings reinforce the concept that the balance between light intensity, nutrient availability, and the ability of plants to regulate stomatal opening is key to optimizing the physiological performance of mangrove plants in the early growth phase (Loudari et al., 2022). In comparison to other mangrove species, *Rhizophora mucronata* cultivated under 50% shadowing showed a maximum photosynthetic rate of 12.2 µmol m^{-2} s^{-1} and a SPAD value of 56.1 (Xue et al., 2023) which is higher than other mangrove species. On the other hand, *Rhizophora mangle* that was grown in 25% photosynthetically active radiation (PAR) and 25 salinity had a photosynthetic rate of 8.8 µmol m^{-2} s^{-1} (López-Hoffman et al., 2006). Under moderate light intensity, *Avicennia marina* had a photosynthesis rate of about 10–11 µmol m^{-2} s^{-1}, but this rate dropped sharply during the day because the stomatal closed (Ball, 2002; Krauss et al., 2008). Basyuni et al. (2020) found that *B. gymnorrhiza* had more steady physiological performance than

Sonneratia caseolaris when it came to adapting to tidal conditions. In this work *B. gymnorrhiza* exhibited a high leaf greenness index (SPAD unit > 58) even under 80% shade, demonstrating significant morphological-physiological flexibility to fluctuations in light intensity.

The optimum shading and biostimulant for mangrove growth

The growth of *B. gymnorrhiza* shows that shade, biostimulants, and their interaction had no significant effect on survival rate, biomass, and seedling quality index. The survival rate was not affected by shade and biostimulants but decreased with increasing seedling age. This decline was caused by crab beetle attacks that damaged young plant shoots, which are more likely to be harmed by pests (Erickson et al., 2003; Davidson et al., 2022). There was no pest control during the trial, consequently, therefore the unshaded area had more crab beetles, which made *B. gymnorrhiza* less likely to survive (53.09%) than in the shaded area (83.95%).

The largest biomass of *B. gymnorrhiza* was seen in 45% shade (233.50 g), while the lowest was seen in 0% (165 g). The highest seedling quality index was also discovered under 45% shade without biostimulant, while the lowest was reported under 0% shade without biostimulant (Figure 3d). Shade generally reduces the intensity of light received by plants, thereby decreasing the rate of photosynthesis and inhibiting biomass accumulation and seedling quality (Lopes et al., 2013; Basyuni et al., 2020).

Shade had a very significant effect on the growth of *B. gymnorrhiza* in terms of height (f=22.43*) (p=0.007), number of leaves (f=11.22*) (p=0.023), leaf length (f=16.43*) (p=0.012), leaf width (f=14.33*) (p=0.015), and leaf area (f=18.83*) (p=0.009). Meanwhile, the application of biostimulants on all growth parameters did not show a significant effect. The interaction effect of shade and biostimulant was very significant on plant height parameters at 6 and 10 months of age.

The effect of shade on growth parameters showed that there was no difference between 45% and 80% shade, but there was a difference compared to 0% shade. The 45–80% shade range provided the best growth in shoot height (38.6–39.84 cm), number of leaves (15–17 leaves), leaf length (11–12 cm), leaf width (4.38–4.47 cm), and leaf area (80.25–85.96 cm^2) (Figure 3). Previous research has shown that *B. gymnorrhiza* seedlings grew better in the shade than in full sunlight (Krauss and Allen, 2003b), which supports our finding. Some mangrove seedlings can grow best in mild shade (30-50%) since it reduces light stress (Silva and Maia, 2019; Smith and Lee, 1999). Photooxidation can happen when there is too much sunlight, which can damage leaves (Li et al., 2022). Nonetheless, a trend for optimal development parameters was observed at a shade level of 45% (Figures 3). In addition, the biostimulant dose used in this study showed limited effects on physiological aspects however, Mojumdar et al. (2022) documented enhanced photosynthetic rates under specific conditions, as evidenced by the application of 10 g and 30 g doses, which proved more beneficial than the absence of biostimulant.

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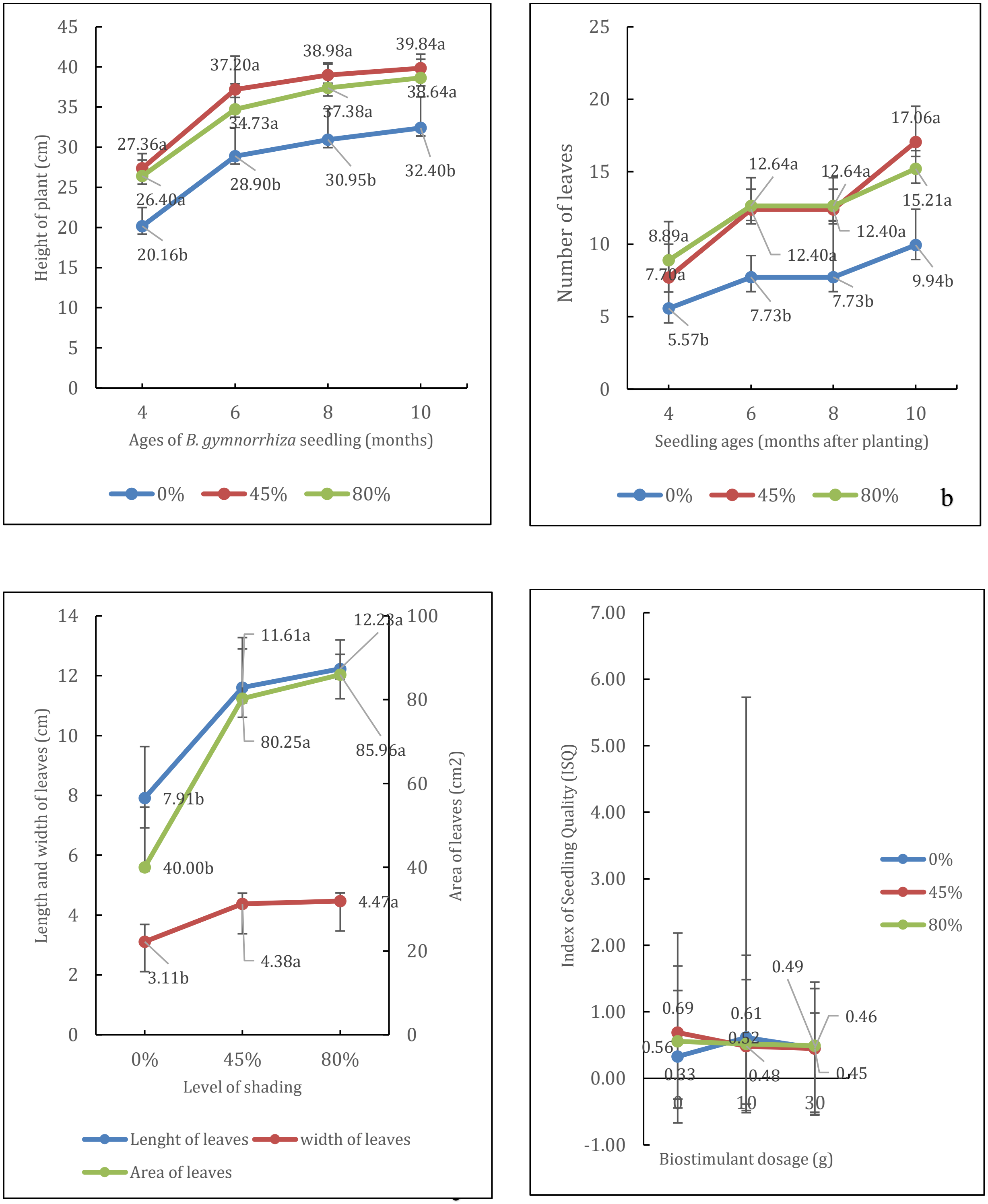

Figure-3. Effects of shading levels on the morphological parameters of *B. gymnorrhiza* seedlings: a) Stem height of *B. gymnorrhiza* seedling at age of 4, 6, 8 and 10 months; b) Number of leaves of *B. gymnorrhiza* seedlings at age of 4, 6, 8 and 10 months; c) length, width and area of leaves *B. gymnorrhiza* seedlings at age of 10 months; d) Index of seedling quality (ISQ). The letters indicated the significance level according to ANOVA and DMRT at p≤ 0.05.

Earlier studies on the impact of shadowing in various species indicate that *Ceriops tagal*, *Rhizophora mucronata*, and *Sonneratia caseolaris* demonstrate optimal height growth and stem diameter under 50% shade intensity. Seedlings of *Avicennia schaueriana* and *Laguncularia racemosa* exhibit superior quality under 50% shade relative to conditions without shade, and *L. racemosa* under full sunlight suffers a 50% reduction in mortality (Silva and Maia, 2019; Basyuni et al., 2020). Nevertheless, these findings contrast with the study conducted by Kusmana and Sukaesih (2021), which indicated that three-month-old *B. gymnorrhiza* exhibit optimal growth in the absence of shade. This discrepancy is probably because of where the nursery is located. The experiment took place in a greenhouse where light intensity was controlled and kept at the best values for the initial growth of *B. gymnorrhiza*. Mangrove plants in the field need a lot of direct sunlight, whereas young mangrove seedlings need shade. For instance, a light intensity of 50% has been reported to increase the germination rate of *R. apiculata* seedlings (Kusmana and Lestari, 2021).

Adding biostimulants in amounts of 10 g and 30 g to the growing media of *B. gymnorrhiza* did not have a significant effect on overall growth characteristics. When put in zeolite and cocopeat carrier media, the bacteria *Azospirillum, Azotobacter*, and *Bacillus*, which are the primary parts of biostimulants, did not grow as well as they could have. According to Hindersah et al. (2021), *Azotobacter* sp. can enhance plant growth through four main mechanisms, namely nitrogen fixation, phytohormone synthesis, exopolysaccharide production, and pathogens supression. Meanwhile, *Azospirillum* sp. has the ability to decompose organic materials such as cellulose, amylose, andlipid-containing biomass (Widawati and Muharam, 2012).

Hadi et al. (2024) found that using compost, rice bran, and *Azolla* flour as carrier materials for nitrogen-fixing bacteria made upland rice grains heavier on average than they were in the control group. In this study, biostimulants composed of zeolite and cocopeat carriers did not promote the growth of *B. gymnorrhiza*. This is probably because the seedling medium did not have enough organic matter, which stopped the microbes from working and breaking down organic matter. Furthermore, it is hypothesized that the observation duration may have been inadequate to fully assess the possible impacts of biostimulants on growth metrics.

Other studies show that biostimulant applications generally have the highest impact on crop yields when applied in less favorable environmental conditions, such as soils with low organic matter content, non-neutral soils, saline soils, nutrient-poor soils, and sandy soils (Bogunovic et al., 2024; Ntanasi et al., 2024). These conditions differ from the substrate conditions at the research site, which is tidal land with high to very high nutrient (N, P, K) content. High nutrient availability causes more stable environmental conditions, which may alter plant growth responses. Therefore, additional nutrients inputs through biostimulants do not trigger growth because there is no nutrient deficiency that needs to be addressed (Alongi, 2021). Furthermore, physiologically, *B. gymnorrhiza* has an adaptive capacity to extreme environments, so the addition of hormones, enzymes, and bacteria is not significant because this plant is already "self-sufficient" to survive in its habitat (Alongi, 2002; Parida and Jha, 2010).

Soil fertility and environmental factors

The findings of this study highlight the significance of environmental factors, including salinity and nutrient availability, in supporting the growth of *B. gymnorrhiza* and influencing the overall health and productivity of mangrove ecosystems (Zheng and Takeuchi, 2022). Environmental conditions during the study showed that the range of daytime temperatures (26–32°C) and nighttime temperature (27–29°C) was within the optimal range for mangrove growth, especially for *B. gymnorrhiza*, which is physiologically tolerant to tropical temperatures (Alongi, 2002). The neutral soil pH (7.43–7.50) shows that there was no excessive acidity disturbance. This means that nutrients are maintained within the right range for root growth and soil enzymes to work (Alongi, 2017; Wakushima et al., 1994). A relative humidity of 60-80% and tidal changes of 25–185 cm shows that the hydrological dynamics are healthy and allow critical biogeochemical activities to happen, like breaking down organic matter and exchanging nutrients in the root zone (Kristensen et al., 2008).

Salinity levels ranging from 14.97 to 15.77 ppt show that the salinity is moderate, which is suitable for the growth of many mangrove species. Moderate salt levels do not impede soil microbial activity, facilitating enhanced mineralization of organic matter and nutrient accessibility (Morrissey et al., 2014; She et al., 2021). Several prior studies have established a correlation between salinity levels and light intensity in connection to mangrove growth (Krauss and Allen, 2003b; López-Hoffman et al., 2006). Compared to high salinity circumstances, low salinity condition with more light seem to have a higher survival rate for mangrove seedlings (López-Hoffman et al., 2006). In low-salinity settings, higher light levels can greatly speed up the growth of mangrove seedlings. In high salinity settings, however, more light does not directly help seedling grow, but it can be a limiting factor since the plants suffer more osmotic pressure experienced by the plants (Ball, 2002; Krauss and Allen, 2003b; López-Hoffman et al., 2006). When salinity is high, mangrove seedlings cannot take in as much water, which makes the concentration even lower. Mangrove plants can handle salt, but they do not need it (Kodikara et al., 2017). Mangrove seedlings grow best when they have access to a lot of nutrients, like more organic carbon and phosphorus (Alongi, 2021). This study underscores the necessity of evaluating the connections among shade, biostimulants, and environmental conditions in formulating appropriate rehabilitation techniques to enhance the long-term sustainability of mangrove ecosystems.

Under 80% shade, water temperature was significantly reduced to approach the ideal threshold for mangrove photosynthesis, which often diminishes at temperatures over 32°C (Ball, 2002). The decrease in light intensity reaching the soil surface subsequently influences soil temperature, moisture, and photosynthesis. These factors influence the accessibility of organic matter for soil microbes. Elevated shadow facilitates the proliferation of anaerobic bacteria and fungi, which contributes to the breakdown of organic matter. In contrast low shade promotes microorganism that actively participate in the soil nutrient cycle (Fazhi et al., 2023).

This study assessed soil nutrient content by comparing levels before and after the experimental period. The results indicated a trend of elevated organic carbon (C-organic), total phosphorus (P-total), and C/N ratio from high to extremely high levels (Table 1).

Table-1. Nutrient content in growing medium of *B. gymnorrhiza* before and after shade and biostimulant treatment.

Before Shade and Biostimulant Treatment (Criteria)			After Shade and Biostimulant Treatment								
			0%			45%			80%		
			0 g	10 g	30 g	0 g	10 g	30 g	0 g	10 g	30 g
C-Org (%)	6.99	H	8.91(VH)	8.56(VH)	7.98(VH)	8.91(VH)	8.64(VH)	7.64(VH)	8.42(VH)	8.31(VH)	8.77(VH)
N-Total (%)	0.58	H	0.54(H)	0.51(H)	0.44(M)	0.45(M)	0.61(H)	0.39(M)	0.33(M)	0.37(M)	0.35(M)
P Bray (ppm)	8.42	VH	12.59(L)	9.98(VH)	34.8(H)	8.8(M)	8.24(M)	13.21(H)	6.76(L)	9.57(M)	8.61(M)
P-Total HCL 25% (ppm)	56.8	H	157(VH)	155(VH)	224(VH)	179(VH)	165(VH)	176(VH)	183(VH)	181(VH)	169(VH)
K (cmol/kg)	3.78	VH	12.54(VH)	12.13(VH)	12.4(VH)	11.75(VH)	12.13(VH)	14.3(VH)	20.66(VH)	11.34(VH)	11.28(VH)
KTK (cmol/kg)	63.21	VH	82.62(VH)	87.56(VH)	91.52(VH)	86.48(VH)	87.56(VH)	88.86(VH)	96.87(VH)	88.86(VH)	87.77(VH)
C/N	12.05	M	16.5(H)	16.78(H)	18.14(H)	19.80(H)	14.16(H)	19.59(H)	25.52(H)	22.46(H)	25.06(VH)

Remarks: L=Low, M=Medium, H=High, VH=Very High (Tanah, 1983).

A positive trend was observed in the levels of organic carbon (organic C), total nitrogen (total N), available phosphorus (Bray P), and potassium (K). The increase in organic carbon by up to 8.91% in the control treatment indicates that the accumulation of organic matter continues to occur naturally, which may be driven by the contribution of leaf litter and microbial activity (Alongi, 2014).

The increase in P-Bray to 13.21 ppm and K to 20.66 cmol/kg indicates good nutrient mobilization, most likely supported by adequate aeration and increased phosphate-solubilizing microbial activity due to biostimulant treatment. Phosphorus is an important element in plant energy metabolism, and increasing its availability can have a positive impact on the vegetative growth and photosynthesis of mangroves (Reef et al., 2010). In addition, the KTK value of the soil was categorized as very high, presumably due to the decomposition of organic matter, which can produce humus. Meanwhile, the increase in the C/N ratio indicates the availability of N for plant growth. Soil fertility parameters indicate that 45% shade and a 10 g biostimulant dose have a higher fertility level than without shade.

Nursery establishment costs and mangrove seedling performance

The nursery for *B. gymnorrhiza* with shade and biostimulant treatment in tidal areas requires investment and operational costs (Table 2). Investment costs encompass the physical construction of the nursery, including the purchase of propagules, bamboo poles, shading materials (paranet), and supporting equipment (hoes, binding wire, etc.). Operational costs include field labor wages for nursery construction and maintenance, water, electricity, as well as biostimulant costs. No land rental costs were incurred, and labor costs remained constant. These assumptions are applied to reflect typical conditions in community-based mangrove nurseries.

Investment and operational costs are calculated based on the assumption of seedling requirements per hectare, equivalent to 10,000 seedlings (planting distance 1 x 1 m). To meet the requirement of 10,000 seedlings, a nursery area of 2,500 m² is needed (planting distance 0.5 x 0.5 m). The land rental cost here is assumed to be zero. Nursery cultivation is carried out for a maximum of 6 months (after the first leaves emerge or approximately 10 months after the propagules are planted in the nursery growth medium). At 10 months of age, the seedlings are ready for transplantation, after they have more than 2 pairs of leaves and a shoot height of>40 cm. Based on the investment and operational cost components, investment costs account for a larger proportion than operational costs. The investment cost ratio is greater than the operational cost ratio. Investment costs are incurred in the first year and will be reduced by 50% in the following year (Table 2).

Table-2. Investment and operational costs and morphological parameters of *B. gymnorrhiza* for 10 months.

Treatment	Cost (IDR x 1,000)			Survival rate (%)	Stem height (cm)	Number of leaves	Biomass (g)	Index of Seedling Quality (ISQ)
	Investment	Operational	Total					
Shading level								
0%	47,000	9,000	56,000	67.08	32.4	9.94	165	0.47
45%	55,330	9,000	64,330	74.07	39.84	17.06	233.5	0.54
80%	56,996	9,000	65,996	83.13	38.64	15.21	224.5	0.52
Biostimulant dosage								
0 g	47,000	9,000	56,000	71.6	37.51	14.62	225.11	0.52
10 g	47,000	14,000	61,000	74.07	37.35	14	199.89	0,54
30 g	47,000	24,000	71,000	78.6	36.03	13.58	198	0,47
Shading and biostimulant								
0% 0 g	47,000	9,000	56,000	53.09	35.34	11.18	148	0.33
0% 10 g	47,000	14,000	61,000	72.84	32.38	8.97	156.33	0,61
0% 30 g	47,000	24,000	71,000	75.31	29.47	9.68	190.67	0.46
45% 0 g	55,330	9,000	64,330	77.78	38.95	17.19	293.17	0.69
45% 10 g	55,330	14,000	69,330	66.67	40.18	17.78	214	0,48
45% 30 g	55,330	24,000	79,330	77.78	40.04	16.2	193.33	0.45
80% 0 g	56,996	9,000	65,996	83.95	38.25	15.51	234.17	0.56
80% 10 g	56,996	14,000	70,996	82.72	39.48	15.25	229.33	0.52
80% 30 g	56,996	24,000	80,996	82.72	38.21	14.87	210	0.49

Table-3. Cost structure of nursery establishment with various levels of shade and biostimulants.

Year	0% shading + biostimulant (IDR x 1000)	45% shading + biostimulant (IDR x 1000)	80% shading + biostimulant (IDR x 1000)
1	56,000	69,330	80,996
2	28,000	34,665	40,498
3	28,000	34,665	40,498
Total	112,000	138,660	161,992

The cost of conventional nurseries without shade and biostimulants in the first year is lower (IDR 56,000,000) compared to those using shade and biostimulants (IDR 69,330,000 and 80,996,000) (Table 3). This cost trend persists throughout the second and third years. Previous studies have reported that conventional mangrove nurseries in the tidal areas require IDR 34,629,046 in Suwung Village, Denpasar (Sukanteri et al., 2023) and IDR 41,530,013 without regular maintenance in Bontang City (Alviyani et al., 2023).

Despite the higher cost associated with seedling cultivation utilizing shade and biostimulants, it yields superior seedling performance relative to those grown without these enhancements, with the highest seedling survival rate reaching 83.95%. According to the Minister of Forestry Regulation No. P.70/Menhut-II/2008, which stipulates a minimum survival rate of at least 70% for successful rehabilitation, then the results of this study shows a fairly high seedling survival rate (Arifanti et al., 2022). Meanwhile, the highest plant height (40.18 cm), the highest number of leaves (18), the highest biomass weight (293.17 g), and the highest seedling quality index (0.69) were recorded under this treatment representing superior performance compared with each showing the best values compared to conventional methods or without shade and biostimulants. The shade level of 45% and the biostimulant dose of 10 g showed the best performance in terms of height and number of leaves (Table 2). In comparison, the mangrove nursery at Tahura Ngurah Ray, which operates without shade but with a controlled water system, produced plants with an average height of 29 cm, a diameter of 0.4cm, 7 leaves, and a biomass of 170 g. These conditions indicate that the growth of *B. gymnorrhiza* using shade in tidal areas provides the best value compared to high-tech nurseries in Ngurah Ray Bali (Figure 4).

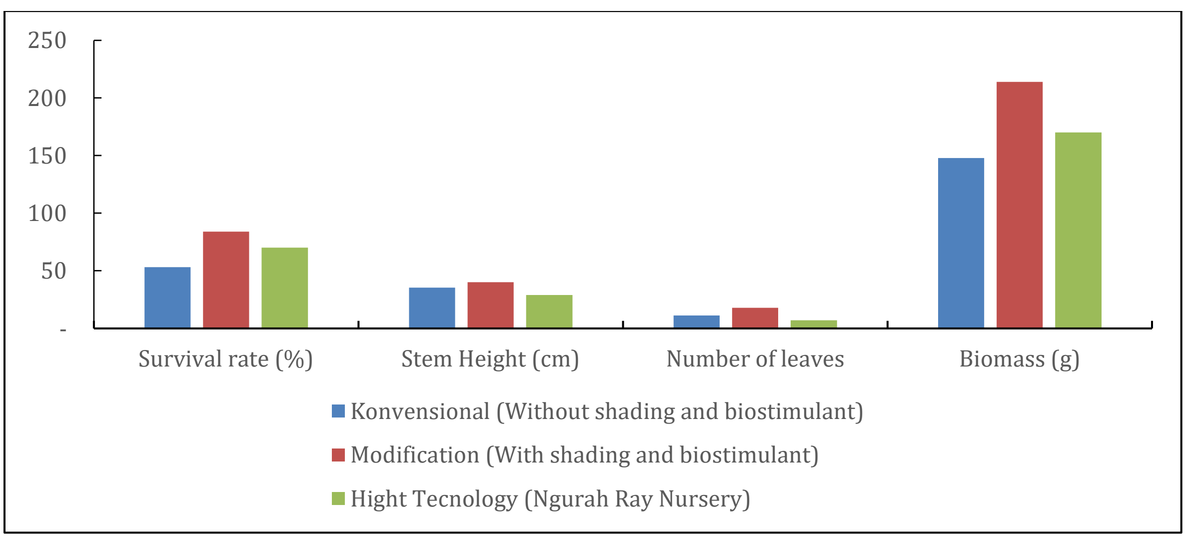

Figure-4. Comparison of nursery methods for *B. gymnorrhiza.*

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Approach to mangrove nursery and rehabilitation

Indonesia has a strong commitment to protect the remaining mangroves and restore those that have degraded. Advancing research, science, technology, and information system is essential for enhancing the sustainable management of mangrove ecosystems and fulfilling global environmental commitments (Arifanti et al., 2022).

This study's results demonstrate that 45–80% shade can markedly enhance the physiological and morphological performance of *B. gymnorrhiza* seedlings. Despite the absence of distinction between 45% and 80% shade, seedlings grown under 45% shade showed exhibited superior performance compared to the 80% shade. This improvement occurs through the role of shade in reducing excessive light pressure, lowering micro-temperature, and optimizing photosynthetic efficiency. Globally, this strategy is highly relevant for application in tropical and subtropical regions facing high radiation intensity and extreme temperatures due to climate change. This concept can be integrated into large-scale mangrove nursery practices to improve the initial growth success of seedlings.

The application of biostimulants in this study had a little effect on physiological parameters and did not significantly influence soil fertility. This underscores the significance of considering the ecological context when applying biostimulants. The efficacy of biostimulants worldwide depends on optimal soil conditions (aeration, pH, moisture) and sufficient reaction durations. Consequently, the application of biostimulants in mangrove rehabilitation worldwide should be judicious and guided by site-specific evaluation, rather than employing a uniform strategy. *B. gymnorrhiza* is a species suited for cultivation in degraded habitats such as former logged-over regions, owing to its capacity to establish mature stands, hence allowing for its utilization without altering existing species dominance (Arifanti et al., 2022).

This study further substantiates that shaded nursery systems can enhance operational efficiency and mitigate the risk of planting failure in the field. Reduced watering requirements, decreased evaporation, and improved seedling quality can result in significant cost efficiencies in the Indonesian context, with seedling growth increasing by 24.92% at a cost of IDR 69–81 million per year. This can serve as a reference in developing seedling and rehabilitation strategies based on budget efficiency, especially in developing countries with limited resources that remain committed to NDC and FOLU Net Sink targets.

These findings contribute significantly to global agendas such as the UN Decade on Ecosystem Rehabilitation and the Global Mangrove Alliance initiative. Adaptive shade-based seeding methodologies may be included in mangrove rehabilitation guidelines protocols that are more attuned to climate fluctuations and environmental change. However, long-term success still depends on integrating these nursery techniques with appropriate site selection, landscape planning, and sustainable monitoring systems (Gatt et al., 2024). Consequently, the findings of this study enhance seedling quality and reinforce the scientific basis for resilient and sustainable mangrove regeneration globally.

Conclusion

The *B. gymnorrhiza* nursery technique using a combination of biostimulants (10 g and 30 g) and shade (45% and 80%) has several advantages over conventional nursery (without shade and without biostimulant). Shading plays a role in improving physiological and growth parameters, particularly leaf greenness index, photosynthetic efficiency, plant height, leaf number, leaf area, seedling quality, and increased nutrient availability. Although there was no significant difference between 45% and 80% shade levels, 45% shade tended to produce better seedling performance than 80% shade. Meanwhile, the effect of 10 g and 30 g biostimulant doses was only seen in the photosynthesis rate parameter, with the best value being at the 10 g dose. The interaction between shade and biostimulant showed no significant effect, presumably due to the relatively short observation period. Although the nursery costs using shade and biostimulants were higher, it resulted in better seedling performance.

Based on these findings, moderate shade (45%) combined with 10 g of biostimulant per polybag is recommended as the optimal nursery design strategy for *Bruguiera gymnorrhiza*. This treatment improves growth, physiological performance, and soil fertility, and is cost-effective for improving seedling quality, survival, and field establishment success. The study is constrained by the short experimental duration, site-specific field conditions, and the lack of posttransplantation monitoring, which may limit broader applicability and long-term inference.

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Use of Generative AI Tools Statement

During the preparation of this work the authors used the Chat GPT Pro to correct grammatical errors in the manuscript. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

Contribution of Authors

Yeny I: Conceptualization, writing—original draft, data curation, formal analysis, writing—review and editing.

Kusmana C & Dwiyanti FG: Supervision, conceptualization, writing—review and editing.

Suyadi: Conceptualization, data curation, formal analysis, writing – review and editing.

Nuroniah HS, Mindawati N & Arifanti VB: Formal analysis, writing – review and editing of chapter (The optimum shading for mangrove growth).

Siregar CA: Formal analysis, writing – review and editing of chapter (Soil fertility and environmental conditions).

Suharti S & Wahyuni T: Formal analysis, writing—review and editing of chapter (Cost of the approach of mangrove nursery).

Suliasih, Sugiharto A & Widawati S: Inventor biostimulant, writing—review and editing (Biostimulant impact).

Purnama M, Rahmila YI & Yulianti M: Data curation, formal analysis, writing—review and editing.

All authors read and approved the final draft of the manuscript and consented to its publication.

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