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Ceriops tagal Biomass and Chlorophyll Analysis in Various Nursery Media

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Abstract

Ceriops tagal is a major mangrove species that contributes significantly to the mangrove environment. *Ceriops tagal* is a species of mangrove that supports a variety of marine organisms, including fish, shrimp, nematodes, and other biota, as well as maintaining the mangrove ecosystem's stability. A nursery is required in the recovery process. The addition of husk and husk charcoal to nursery media can enhance water, air, and plant nutrient absorption space. The objective of this study is to (1) determine how different nursery media with a mixture of husk and husk charcoal affect mud media, and (2) find which nursery media has the best influence on biomass and chlorophyll content in *C. tagal* mangroves. This study began with the propagules of *C. tagal* being planted in different composition growing media with husk and husk charcoal, and then calculating the biomass in the root, stem, leaves, and hypocotyl, as well as the chlorophyll content in the leaves of *C. tagal* organs, using an ANOVA test. Then, for the wet biomass of the root, dry biomass of the stem, root, and wet and dry biomass hypocotyl of *C. tagal*, do an honest significant difference test of 5%. According to the findings, adding husk and husk charcoal to nursery media can increase the dry biomass of the root and stem, as well as the wet and dry biomass of the hypocotyl in *C. tagal* organs. Media 5 (mud + husk charcoal in a 2:1 ratio) and media 6 (mud + husk + husk charcoal in a 1:1:1) were the best media for increasing the biomass of the *C. tagal* in each organ. Environmental factors such as the pH content of the medium influence the increase in dry biomass of the root and stem, as well as the wet and dry biomass of the hypocotyl.

Keywords*:* *biomass, Ceriops tagal, chlorophyll, nursery media*

INTRODUCTION

Ceriops tagal (family Rhizophoraceae) is a major mangrove species that plays a vital role in the ecosystem services provided by mangroves. Various marine animals such as fish, shrimp, nematodes, and others rely on the mangrove ecosystem for their survival. For these aquatic species, this ecosystem has served as a home, a location to spawn, a place to store eggs, and a food supply (Kusumahadi *et al*., 2020). Aside from capturing waves, wind storms, coast protection, and abrasion, it also has a vital physical purpose (Efriyeldi *et al*., 2019).

Ceriops tagal offers medical properties, according to Sura *et al* (2018), and can be used as a wound cleaner, for example. Furthermore, as a nutrient source, this species plays an important function in the mangrove ecosystem community. These nutrients come from the decomposition of *C. tagal* leaves, which form detritus that is consumed by aquatic creatures and provides fertility to the waters (Noor, 2006).

Ceriops *tagal* is one of the mangrove species which has a large area. According to Hanan *et al.* (2020), a mangrove ecosystem is made up of plants that grow between the sea and the land. In 2017, Indonesia's mangrove forest ecosystem covered 3,361,216 ha. There are issues in the mangrove environment that have resulted in a decrease in the area of the mangrove ecosystem, which happens every year. As a result, the rehabilitation of *C. tagal* species is necessary, and it necessitates correct cultivation to achieve positive outcomes.

Nurseries are the key factor in carrying out the rehabilitation process, aiming to protect mangroves and also the ecosystem of them (Hidayat *et al*., 2018). The selection of suitable seed types according to substrate conditions and the method of planting is an important process in rehabilitation. The selection of good seed that will be used must be done carefully to get the optimal of mangrove growth result (Kasman & Widi, 2020).

In *research* conducted by Kusuma *et al.* (2013), adding husk or husk charcoal can increase the growth of specific organs in mangroves. Adding it will increase the length of the roots, making it easier to absorb nutrients. Based on Simbolon & Setyono (2020), The husk and husk charcoal has a function, it can improve the properties of soil aggregate which will be useful for binding air and water thus the nutrients given to the media are easily absorbed through the roots.

The qualities of proper mangrove nursery media include: a media condition that is not excessively dense, and the ability to assist the creation and growth of plant roots. It can also help with adequate absorption of air, water, and nutrients, and it can be easily purchased at a minimal cost. To loosen the soil, husk and husk charcoal can be utilized as nursery medium (Dewi *et al.*, 2020).

The mangrove nursery media must have high porosity properties. This is due to the need for mangroves to get oxygen and water content (Hendriati & Novirina, 2013). The husk media and husk charcoal can bind water, does not rot easily, helps the aeration system, and as a source of inorganic substrates such as N, P, CEC for plants. The husk and husk charcoal can make the media more porous, thereby increasing the porosity and maintaining aeration of the media (Dewi *et al.*, 2020).

Mangrove biomass is made up of leaf, stem, hypocotyl, and root biomass from mangrove vegetation. Because biomass is the result of the accumulation of organic components, it can be used to assess the growth of a plant. Before it is dispersed and stored by plants, biomass in plant components goes through a fairly complex process. CO2 in the air is absorbed by plants and transformed into carbohydrates, which are subsequently dispersed to all parts of the plant through photosynthesis. The residual product from this process will be deposited in leaves, stems, twigs, flowers and fruit. The method of measuring the wet weight is directly measured after each part of the mangrove. Besides that, the dry weight measurement is carried out at the end of the observation by measuring the weight after drying in the oven at 70°C until the weight reaches a constant point (Heriyanto *et al.*, 2020).

Chlorophyll content calculation is an important parameter in plant growth since it stimulates growth and gives leaves a green hue. Plant growth and development will be affected if the nursery media is deficient in nutrients. This will have an effect on chlorophyll production and inhibit the photosynthesis process. The UV-Vis spectrophotometric method can be used for this analysis (Puspitasari, 2017).

The calculation of biomass and chlorophyll content has a relationship, namely that biomass can show the efficiency value of the physiological processes of a plant, this is because the dry biomass produced is the result of a photosynthetic process that is running well. The biomass that occurs result from the plant's ability to absorb nutrients (Suhaila & Sulhaswardi, 2013).

MATERIAL AND METHODS

The research was conducted in a mangrove at Logending Beach in Ayah Village, Kebumen Regency, at 7°43'07" S and 109°23'33" E. The Toxicology Laboratory, Plant Physiology Laboratory, and ITMEL Laboratory of Jenderal Soedirman University, Purwokerto, analyzed the biomass and chlorophyll content. The research was conducted for nine months, starting from July 2020 until March 2021.

Ceriops tagal mangrove propagules, nursery media consisting of mud, husk charcoal, and husk, aquadest, MgCO3, and 80 percent aceton were used in this study. Polybag, paranet 65 percent, bamboo, raffia ropes, soil tester, boots, aluminium foil, spectrophotometer, beaker glass, reaction tube, oven, furnace, mortar, pestle, analytic scale, scissors, tray, camera, label, and stationery were among the instruments used in this study.

Research Prosedure

The Plant Physiology Laboratory, Faculty of Biologi, Universitas Jenderal Soedirman, prepares the equipment and materials used to quantify biomass and chlorophyll content. The media was made by mixing each ingredient according to a predefined concentration ratio based on the experimental design's six-level nursery media code. Each piece of media is placed in a polybag with the proper code labeled on it.

Ceriops tagal mangrove propagules were taken in the Segara Anakan mangrove forest, Cilacap Regency. *C. tagal* mangrove propagules were taken using a random sampling technique. *C. tagal* mangrove propagules were taken with mature condition characterized with yellow neck cotyledon. *Ceriops tagal* mangrove propagules were planted in polybags containing different nursery media, namely six levels of nursery media. Polybag filled with nursery media as much as ¾ of the polybag contents. *C. tagal* mangrove propagules are attached to each nursery media with a depth of ± 6 cm placed in Ayah Mangrove Forest under the paranet 65%.

Mangrove propagules that have been planted in various nursery media are cared for under the paranet, which provides shade and keeps the propagules moist. To prevent propagule loss, the propagule storage place must also be shielded from being damaged by waves (Pramudji, 2001). Maintenance of *C. tagal* mangrove propagules planted on different nursery media was carried out for two months with field observations carried out twice, in once time for every month.

Wet weight measurements were carried out by taking the roots, stems, hypocotyl and leaves of the mangrove *C. tagal*. The part that has been taken is then weighed using analytical scales. The weight obtained is entered in the data tabulation.

Measurement of dry weight is carried out at the end of the observation by weighing the parts that have been dried in an oven at a temperature of 70°C until the dry weight is constant (Sofyan *et al*., 2014). Based on the Karenisekar & Insafitri (2020), time used in the oven for 48 hours. The weight obtained is entered into the data tabulation.

The method that used is a spectrophotometric (Kamble *et al*., 2015). Leaf samples that have been taken are cut into small pieces using scissors and weighed using analytical scales until they reach a weight of 0.5 g per sample. Maceration was carried out using a mortar and pestel that have been cooled before use. Then the 0.5 g of leaves were crushed using a mortar and pestle until the leaf texture became smooth, then added 12.5 mL of 80% acetone and 0.25 g of $MgCO_3$. The leaf extract was then put into a test tube and then centrifuged at 3000 rpm for 15 minutes. The absorbance value of the leaf extract was calculated using a spectrophotometer with a wavelength of 645 nm and 663 nm. The absorbance values obtained were entered into a data tab. Chlorophyll content can be determined using a formula:

$$Chlorophyll\ a\ (\mu g/ml) = 12.7\ (A663) - 2.69(A645)$$

$$Chlorophyll\ b\ (\mu g/ml) = 22.9\ (A645) - 4.08\ (A663)$$

$$Total\ Chlorophyll\ (\mu g/ml) = 20.2\ (A645) + 8.02\ (A663)$$

Details :
A646 : absorbance values at λ646 nm of spectrophotometric results.
A663 : absorbance values at λ646 nm of spectrophotometric results.

The water content of the media was determined by taking a soil sample from each media and weighing it on analytical scales until it achieved a weight of less than 40 g. (initial weight or wet weight). Soil samples were wrapped in aluminum foil and dried at 70°C for a consistent medium weight. A formula can be used to calculate the amount of water in the media (Karenisekar & Insafitri, 2020) :

$$\%\ WC = \frac{A - B}{A}\ x\ 100\%$$

Details :
% WC: water content in value precentage,
A : wet weight of the sample,
B : dry weight of the sample.

Measurement of carbon content in media, soil samples that have been oven-dried at 70°C until their weight is constant, then burned in a 550°C furnace for 4 hours until the media has turned to ash. Using an analytical scale, the ash weight was recalculated. The formula is used to compute the weight of the ash obtained (Karenisekar & Insafitri, 2020) :

$$\%\ ash\ content = \frac{(Z - X)}{Y}\ x\ 100\%$$

Details :
X : weight of aluminium foil,
Y : dry weight of sample media,
Z : weight of sample media after been heated with furnace and weight of aluminium foil.

Measurement of carbon content in the media is carried out using a formula (Karenisekar & Insafitri, 2020):

$$C\ (gram) = (DW\ (gram) - AC\ (gram))$$

Details :
C : Carbon
DW : Dry Weight of sample media
AC : Ash Content from the result in the above equation

A soil tester was used to measure the pH of the media, which was applied directly to the media. The pH value of the media can be determined by observing the movement of the soil tester needle. In the data tabulation, the pH value of the media collected was recorded.

Analysis of the Total Nitrogen (N) content in the media is used the Kjeldahl method (Balai Penelitian Tanah, 2005). Analysis of the Phosphorous (P) content in the media used HCl 25% extraction method, while analysis of the Cation Exchange Capacity (CEC) content with the colorimetric method to test soil fertility (Balai Penelitian Tanah, 2005). A more detailed explanation regarding the analysis of the content of N, P, CEC is listed in Appendix 4.

Based on Nursin *et al* (2014), Cation Exchange Capacity (CEC) is a soil chemical property linked to soil fertility. By extracting soil samples from each media and analyzing them at the Wahana Laboratory in Semarang, the concentration of N, P, and CEC in the media was measured. The data produced from the analysis test were entered into the data tabulation after it was completed.

Data Analysis

The research was carried out experimentally with a Completely Randomized Design (CRD) with six levels of nursery media to be tested, there are:

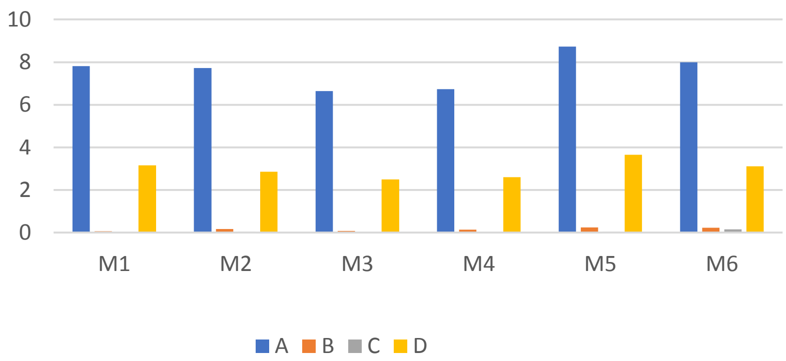
M1 : Pure mud
M2 : Mud + Husk (1:1)
M3 : Mud + Husk (2:1)
M4 : Mud + Husk charcoal (1:1)
M5 : Mud + Husk charcoal (2:1)
M6 : Mud + Husk charcoal + Husk (1:1:1)

Each media level was tested three times. The results of the root, stem, leaf, hypocotyl, and chlorophyll content measurements were evaluated using ANNOVA with a test level of 5% and 1%. Then, using the honest significant difference test (HSD) with a 5% test level, the secondary data were evaluated using descriptive analysis, and finally, conclusions were drawn.

Table 1. The Honest Significant Difference Test of *Ceriops tagal* Organs

Treatment	Wet Biomass Hypocotyl (g)	Dry Biomass Root (g)	Dry Biomass Stem (g)	Dry Biomass Hypocotyl (g)
M1	7.82(ab)	0.07(a)	0.03 (ab)	3.16 (ab)
M2	7.73(ab)	0.18 (bc)	0.04 (ab)	2.86 (a)
M3	6.65 (a)	0.08(a)	0.03(a)	2.50 (a)
M4	6.74 (a)	0.14 (ab)	0.03 (ab)	2.60 (a)
M5	8.73 (b)	0.25(c)	0.04 (ab)	3.65 (b)
M6	7.99(ab)	0.23(c)	0.16 (b)	3.11 (ab)

Details: Unequal letters (a, ab, bc and c) in the column indicate the real increase the wet biomass of hypocotyl and dry biomass root, stem, and hypocotyl of at each organ of *C. tagal*; (M1) Pure Mud, (M2) Mud + Husk (1:1), (M3) Mud + Husk (2:1), (M4) Mud + Husk charcoal (1:1), (M5) Mud + Husk charcoal (2:1), (M6) Mud + Husk + Husk charcoal (1:1:1).



Graphic 1 The The Honest Significant Difference Test of *Ceriops tagal* Organs
Details: A (Wet Biomass of Hypocotyl), B (Dry Biomass of Root), C (Dry Biomass of Stem), D (Dry Biomass of Hypocotyl); (M1) Pure Mud, (M2) Mud + Husk (1:1), (M3) Mud + Husk (2:1), (M4) Mud + Husk charcoal (1:1), (M5) Mud + Husk charcoal (2:1), (M6) Mud + Husk + Husk charcoal (1:1:1).

RESULT AND DISCUSSION

The hypocotyl, roots, and stems of the biomass content in each organ have a high significant value, according to the findings of this study. This reveals that a novel media composition with a blend of husk and husk charcoal can promote the growth of C. tagal organs when compared to mud-only medium. According to the report of Hendriati & Noviriana (2013), the media in which mangroves grow must have a certain level of porosity, allowing for the growth of mangroves, water intake, and respiration. Husk media and husk charcoal have been shown to improve nutrient and oxygen absorption by *C. tagal* mangroves.

Treatment of diverse media compositions in the leaf organ had little effect on increasing biomass in wet and dry biomass in C. tagal leaf organs. This is due to a physiological process that occurs in mangroves naturally, according to Widyasari (2010), the more leaves a tree has, the more sunlight it can receive for photosynthesis, but when the leaves do photosynthesis, carbohydrates are absorbed and transferred to other sections of the tree.

As a result, the biomass content of the non-photosynthetic section will be higher than that of the photosynthetic leaves. Meanwhile, study of chlorophyll content in *C. tagal* leaves revealed that media treatment with various compositions had no effect on chlorophyll content increase in *C. tagal* leaves. This can be caused by mangroves being planted for two months in media with varying compositions that are not ideal, resulting in insufficient chlorophyll content in the leaves. According to the Adip *et al* (2014), the taller the tree, the easier it is for the leaves to absorb sunlight, causing the chlorophyll content in the leaves to increaseBased on table 1 and graphic 1, the best media for raising the biomass of wet and dry biomass of hypocotyl, as well as dry biomass of root, was media with a composition of mud + husk charcoal in a ratio (2:1) or media 5. The best media for raising the biomass of dry biomass of root and stem of C. tagal organs was media 6 with a composition of mud + husk charcoal + husk in a ratio of 1:1. According to Sofyan *et al* (2014), this was attributable to the media structure with the inclusion of husk charcoal, which had a larger

Table 2. Result of Environmental Factors Measurement Analyses of *Ceriops tagal* in Different Nurseries Media

M	WC (%)	CC (%)	pH	N (%)	P (%)	CEC(me/100g)
M1	16.89	9.42	5	0.3	0.039	0.26
M2	40.32	18.02	5	0.34	0.043	0.25
M3	23.75	14.13	5.5	0.26	0.044	0.31
M4	41.13	14.21	5	0.32	0.045	0.22
M5	25.17	9.02	6	0.29	0.041	0.28
M6	32.57	13.66	5.5	0.25	0.038	0.36

Details : (M1) Pure Mud, (M2) Mud + Husk (1:1), (M3) Mud + Husk (2:1), (M4) Mud + Husk charcoal (1:1), (M5) Mud + Husk charcoal (2:1), (M6) Mud + Husk + Husk charcoal (1:1:1); M (media); WC (Water Content); CC (Carbon Content); N (Nitrogen); P (Phosphor); CEC (Cation Exchange Capacity)

carbon content and superior ability of husk charcoal in media drainage, as compared to husk and 100% mud treatment.

The addition of organic matter to the mangrove nursery media in the form of husk charcoal will increase the dry biomass in each organ at the same time in the wet and dry biomass of hypocotyl and dry biomass stem organs of *C. tagal*. The modified media contains organic matter particles that allow the pore space to serve as a source of water, air, and penetration space. More pore space causes root system growth and makes it simpler for roots to absorb water and nutrients, which can lead to an increase in hypocotyl moist biomass (Hanafiah, 2007). The addition of husk charcoal and husk creates an area that the roots can penetrate thus that the absorption of nutrients by the roots is easier and more abundant Thus the organs in other parts such as stem and hypocotyl will experience good growth (Kusuma *et al*., 2013).

The pH of the two media, namely on media 5 with a pH of 6 and on medium 6 with a pH of 5.5, supports the following: both pH values are the most stable pH values compared to other nursery media (Table 2). The availability of dissolved oxygen required for mangroves to carry out their breakdown process is influenced by pH. The bacteria that carry out the decomposition process will reduce if the dissolved oxygen level is low, and the availability of nutrients required by mangroves will drop (Sari *et al*., 2016)Table 2 shows the value of environmental factors' influence on each media. The carbon content of each media varied between 9.02 percent and 18.02 percent, with the carbon content of *C. tagal* mangroves falling into the medium category. This has an impact on the biomass of *C. tagal*. The carbon value is related to the biomass value of *C. tagal*; the greater the biomass value, the greater the carbon value. The value of the carbon content of organic matter is 47% of the total biomass (Badan Standarisasi Nasional, 2011).

However, on the value of water content in the media, the total of the media ranged between 16.89% and 41.13%. The highest value of water content found in media 4 which has a composition in the form of mud and husk charcoal with a ratio of 1: 1 with a value of 41%. The addition of husk charcoal is able to bind water thus it can facilitate the *C. tagal* to absorb nutrients for increasing the amount of biomass in *C. tagal* organs. This provide evidence that the media containing husk charcoal can give a significant value of biomass content in *C. tagal* organs (Naimnule, 2016).

The determination of the pH value greatly affects the biochemical processes in the media, if the pH has decreased, there will be a decrease in dissolved oxygen while the need for oxygen use is very much needed. The optimal tolerance relationship is around 7 - 8.5. However, the pH in the mangrove nursery media with a value of 5 - 6 is classified as low but in the mangrove the pH still in the tolerance range (Wantasen, 2013).

It is used to determine the nutrient content of each N, P, and CEC substrate when calculating Nitrogen, Phosphorous, and Cation Electron Capacity from diverse nursery media. The element N is necessary for the production of amino acids, nucleic acids, and proteins, all of which are essential for plant metabolism. Element P is a type of organic material that can be obtained from artificial fertilizers or soil minerals. Based on Fajar *et al* (2013), the value of the N content of each medium is included in the moderate group because the value is in the interval 0.21 - 0.50%. Each media is included in the moderate group in the P element because it is 20-40%. The CEC level for each medium was classified as moderate because it was in the 20-40% interval. According to Asnindar *et al* (2019), CEC (Capacity Exchange Cation) is the amount of positive charge of cations absorbed by soil colloids at a specific soil pH. Medium CEC levels in the *C. tagal* nursery media are caused by the pH content which tends to be less than optimal so that it will interfere with the soil cation exchange process which will inhibit the process of supplying nutrients used as a measure of the fertility of a plant. But overall, measurements of the media show a fairly good fertility value because the levels are at moderate intervals. Based on Kurniawan *et al* (2017), if there is a lack of nutrients, it will affect the growth of a plant.

CONCLUSION

This study conclude that changing the nursery media can alter hypocotyl wet biomass, root dry biomass, stem dry biomass, and hypocotyl dry biomass in *C.tagal* mangroves. The optimum media for enhancing *C. tagal* growth are media composed of mud + husk charcoal in a 2:1 ratio (medium 5) and media composed of mud + husk + husk charcoal in a ratio (1:1:1) (medium 6).

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