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Growth of Tamanu Seedlings (*Calophyllum inophyllum* L.) in flooding on Several Types of Soil

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Abstract: Tamanau (*Calophyllum inophyllum* L.), has economic value as a source of biofuel and medicine, therefore it has the potential to be developed in several wetlands in Riau, Indonesia. This study aims to examine the growth ability and morphological adaptation of tamanu to flooding conditions on mineral soil, peat soil and sand soil. This experimental research was designed using a randomized block design. The experiment consisted of flooded and non-flooded treatments on mineral soils, peat soils and sandy soils. Flooding was carried out on all types of soil with an inundation level of 2 cm above the soil surface for 30 days. The results showed that Tamanu seedlings are tolerant to flooding on mineral soils and sandy soils, but sensitive to flooding on peat soils. The morphological adaptation of tamanu seedlings to flooding in mineral soils is the formation of adventitious roots and lenticels. Tamanu seedlings when submerged in sandy soil only form pores on submerged stems.

Keywords: Flooding, Mineral soil, Peat soil, Sand soil, Tamanu (*Calophyllum inophyllum* L.).

1. Introduction

Riau Province, Indonesia is a lowland area that has quite high rainfall, lots of swamps with various types of soil including peat soil, so that the land is often inundated and flooded. Riau Province has the largest peatland in Sumatra, which is around 3.89 million hectares out of 6.49 million hectares. However, 2.31 million hectares have experienced degradation. Some of the degraded peatlands are already being used for plantations, food and horticulture [1]. Apart from that, in Riau there are also coastal areas or beaches, namely in Bengkalis, Rengat, Tembilahan, Siak, Bagan Siapi-api and Dumai. Riau also has land that still has the potential for crop cultivation, so it needs to be utilized.

Nyamplung or tamanu (*Calophyllum inophyllum* L.) is a plant species that has many economic benefits. Tamanu stems are used for commercial timber production and for medicinal purposes. Tamanu seeds can be processed into biofuels. Oil produced from tamanu seeds is used as fuel which has a higher economic value than kerosene and castor oil. The flowers are used as a mixing ingredient to scent hair oil. Nyamplung leaves can be used for the treatment of skin diseases and eye inflammation [2-7].

Tamanu has the potential to be developed in various areas in Riau with various types of soil, namely peat soil, mineral soil and sandy soil on the coast with

inundated conditions. This is because tamanu has a high tolerance for various types of soil, including sand, silt and degraded soil. Inundation of nyamplungs on mineral soils with an inundation level of 4 cm above the soil surface can survive, form lenticels at the inundation boundary and an increase in the number of leaves occurs with a damage level of 40% [8]. Tamanu can survive up to 82 and 81% on ombrogenous and topogenous peat soils in Buntai Kalimantan, respectively [9]. Tamanu can live up to more than 90% on ultisol soils [10]. For tamanu cultivation in various wetlands in Riau Province, it is necessary to test its ability to grow on peat and sand soils under flooded conditions. This study aims to examine the growth ability and morphological adaptation of Tamanu to inundation conditions using mineral soil, peat soil and sand soil as media.

2. Materials and Methods

The research was in the form of an experiment consisting of 6 treatments and soil media, namely mineral soil without flooding, mineral soil with flooding, peat soil without flooding, peat soil with flooding, sand soil without flooding, and sand soil with flooding. Mineral soils are flooded with fresh water, peat soils are flooded with peat water, and sandy soils are flooded with salt water. Each treatment with 5 replications and

so there were 30 treatment units. The study was designed using a randomized block design.

The peat soil used is a sapric type originating from Rimbo Panjang Village, Kampar, Riau at a depth of 1-20 cm. Mineral soil was obtained from Biological Garden soil, at a depth of 0 to 20 cm from the soil surface (topsoil). Sandy soil is obtained from building sand. Furthermore, the soil is homogenized, then put into 30 polybags that have been prepared. Each polybag is filled with soil $\frac{3}{4}$ part of its volume.

Tamanu seedling aged 4 months was transferred to polybags containing each mineral soil, peat soil and sand soil. After 4 weeks of age, inundation was carried out as deep as 2 cm from the surface of the polybag soil. Inundation using mineral water, peat water, and salt water. Flooding using salt water obtained from salted water using 25 g of NaCl in 1000 ml of water. The last observation was made 30 days after planting. Symptoms Growth parameters observed included increase/decrease in plant fresh weight, increase/decrease in plant height, increase/decrease in stem diameter, increase in number of leaves, increase in leaf area, percentage of live roots, formation of adventitious roots and formation of lenticels. Other parameters include the percentage of live roots, the percentage of damaged leaves, and the plant damage index. The percentage of damaged leaves was calculated based on the number of wilted and dead leaves at the beginning to the end of the observation.

Observation to determine the level of plant resistance to inundated conditions by giving a score of 0-5. Scores are given based on yellowing leaves, dropping, wilting leaves until the plant dies [11]. Scores include 0 (20-39% damaged leaves); 1 (40-59% damaged leaves); 2 (60-79% segment leaves); 3 (80-99% of leaves damaged); and 4 (100% damaged leaves). The level of resistance is determined based on the score, namely tolerant with a score of 0-1, moderately tolerant with a score of 2-3 and sensitive with a score of 4-5. Morphological parameters include the percentage of adventitious root formation and the percentage of lenticel formation. Growth parameter data were analyzed using ANOVA (Analysis of Variance) to determine the effect of treatment on the parameters tested. The results of the analysis of variance with a significant effect were further tested using the DMRT (Duncan Multi Range Test) 5% test level.

3. Results and Discussion

3.1 Environmental conditions

Changes in soil pH, air temperature, air humidity, light intensity and soil moisture at the beginning and end of the observations under inundation conditions compared to without flooding. Soil pH increases, soil moisture becomes more moist (Table 1).

There was an increase and decrease in pH after the inundation treatment. The mineral soil pH

increased from 5.8 to 6.5. Flooding in peat soils from 6.0 to 6.7, while the pH of sandy soils decreased slightly from 6.7 to 6.4. There was no increase or decrease in soil pH of the three types of soil without flooding. An increase in soil pH is due to the stagnant state of the soil becoming saturated with water so that the pH increases towards neutral [12]. The increase in pH was due to the dissolution of carbonate and bicarbonate at the start of the puddle. Soil pH also influences the decomposition of soil organic matter and processes such as mineralization, nitrification, and hydrolysis of urea [13]. The decrease in the pH of sandy soil is caused by the saturation of the water base, the lower the base saturation, the more acidic the soil or the pH decreases. Soil moisture changes in three types of soil after being flooded. The changes that occur are caused by flooding conditions causing the soil pores to become saturated with water so that the soil becomes moist.

3.2 Growth Response

Flooding significantly affected the decrease in plant wet weight, stem height, and percentage of live roots of nyamplung plants in all three types of soil after 30 days of observation. The flooding treatment reduced plant wet weight, plant height, stem diameter and percentage of live roots (Table 2).

There was a decrease in the wet weight of tamanu seedling during flooding. The highest decrease was in peat soil followed by mineral soil and sandy soil. As a result of the flooding treatment also decreased plant height and stem diameter. The decrease in plant height was highest when inundated on peat soil. In flooding on mineral soils there was a slight decrease, whereas in sandy soils there was no decrease in plant height. The highest decrease in tamanu stem diameter occurred when flooding in peat soils. Decrease in diameter is lowest in peat soils without flooding. There was an increase in tamanu diameter in mineral soils and sandy soils both in conditions of flooding and without flooding. The increase in plant diameter was highest when flooded in sandy soils, while the lowest increase occurred in sandy soils and mineral soils without flooding. The increase in diameter occurred due to morphological changes, namely the formation of lenticels and lenticel hypertrophy which changed the structure of tamanu stems. The decrease in the diameter of the tamanu stem is due to the shrinking of the stems.

From the results of this study, generally tamanu plants that experience flooding do not increase plant height, new leaves, but there is a decrease in fresh weight and reduced living roots. The decrease in tamanu wet weight was also caused by plant damage, namely wilted, yellowed, fallen leaves, shriveled and dead stems. The flooding treatment resulted in a reduction in the percentage of living roots. The highest percentage of living roots occurs in mineral soils without flooding. The lowest percentage of living roots was in submerged peat soil.

Table 1 Research environmental conditions

| Parameter | Mineral soil | | Peat soil | | Sand soil | |
|-------------------------------------|--------------|-----|-----------|-----|-----------|-----|
| | WF | F | WF | F | WF | F |
| pH tanah _ early research | 5.8 | 5.8 | 6.0 | 6.0 | 6.7 | 6.7 |
| pH tanah _ end of research | 5.8 | 6.5 | 6.0 | 6.7 | 6.4 | 6.7 |
| Soil moisture _ early research (%) | 27 | 27 | 30 | 32 | 26 | 28 |
| Soil moisture _ end of research (%) | 27 | 29 | 30 | 30 | 26 | 26 |

Notes : F : flooding, WF : without flooding

tamanu at various levels of flooding for 30 days of flooding, the 30 days of flooding reduced stem diameter

Table 2 Comparison of tamanu growth on several types of soil without flooding and with flooding

| Parameter | Treatment | | | | | |
|---|--------------------|-------------------|---------------------|-------------------|--------------------|--------------------|
| | Mineral soil | | Peat soil | | Sand soil | |
| | WF | F | WF | F | WF | F |
| Decrease of fresh weight (g) | -2 ^c | -18 ^{ab} | -10 ^{abc} | -24 ^a | 0 ^c | -6 ^c |
| Decrease of stem height (cm) | 0 ^b | -0.2 ^b | -1.4 ^a | -2 ^a | 0 ^b | 0 ^b |
| Decrease/increase in stem diameter (cm) | 0.06 ^{bc} | 0.18 ^c | -0.16 ^{ab} | -0.3 ^b | 0.06 ^{bc} | 0.28 ^c |
| Percentage of living roots (%) | 100 ^c | 71.2 ^b | 49.74 ^{ab} | 31.3 ^a | 98.46 ^c | 64.38 ^b |

Notes : Numbers in the same row followed by the same letter are not significantly different at the 5% level DMRT test. F : flooding, WF : without flooding

Flooding conditions cause the soil to lack O₂ thereby limiting respiration. Inhibition of the process of respiration reduces the production of ATP. Therefore, plants switch their metabolism from aerobic respiration to anaerobic through the glycolysis pathway, so that the energy produced is only 2 ATP molecules out of 36 ATP. The low energy produced will inhibit plant growth which causes no new leaves to form, a little energy is not used for growth because it is used for survival [14] [12]. Soil in a state of flooding causes the pore space to be filled with water so that the soil is saturated with water. Flooding conditions result in a decrease in root permeability which results in inhibition of water absorption by plant roots. Inhibition of water absorption causes plants to lack water resulting in cell shrinkage. Cell shrinkage causes plants to wither and dry [12, 15-18].

There was a decrease in both plant wet weight, stem height, and plant diameter on peat soil. Peat soil has a high value of cation exchange capacity but very low base saturation so that the organic matter content of peat soil is difficult for roots to absorb. Peat soil contains toxic organic acids such as phenolic acids. Phenolic acids are phytotoxic for plants which inhibit the growth and development of plant roots [19, 20]. However, in flooded sandy soil did not reduce plant height. This is due to the sandy soil structure which has coarse pores and high porosity resulting in a low water holding capacity which allows water to enter and exit more easily. Tamanu is able to grow on sandy soil in conditions of flooding or without flooding. Tamanu can grow well on sandy soil with sufficient rain.

Flooding reduces gas exchange in the soil thereby reducing the availability of O₂ for roots and inhibiting the supply of O₂ for roots [21]. Flooding also inhibits root formation causing root rot [15]. The results of this study are in accordance with Norsamsi [8] on

weight of tamanu. Penggenangan juga menurunkan pertumbuhan *Styrax pohlil* [22]. Flooding for 30 days reduced root biomass, transpiration rate, stomatal conductance and photosynthesis rate of *Distylium chinense* [23]. Flooding reduced the total dry weight, number of pods, seed size and seed yield in green beans [24]. Prolonged submergence causes *Allophylus edulis* to experience damage to the photosynthetic apparatus and death [25].

3.3 Seedling damage and seedling resistance level

Tamanu seedling showed differences in response to damage percentage, damage index and resistance level under flooding conditions in three types of soil. The percentage of plant damage is generally seen from the presence of wilted and dead leaves. Without flooding, tamanu seedlings in mineral soils and sandy soils did not suffer damage, while up to 60% damage occurred in peat soils. Tamanu seedlings under flooding conditions on sandy soil were not damaged, slightly damaged on mineral soils (20%) and on peat soils with a damage level of up to 100% (all leaves withered) (Table 3 and Figure 1). Based on the level of damage and damage index, tamanu seedlings in flooded conditions are classified as tolerant in mineral soils and sandy soils, and sensitive in peat soils. Tamanu seedlings grown without flooding on peat soils were classified as moderate tolerance, while those on mineral soils and sandy soils were classified as tolerant.

Flooding inhibits plant growth, damage (wilted leaves, fall) and plant death. Flooding inhibits the formation of new leaves and expansion of leaves. Plant damage (wilting and falling leaves) is caused by a decrease in cell turgidity due to reduced ability of plants

to absorb water which causes plants to wilt, shrink and eventually die [15, 16, 25, 26].

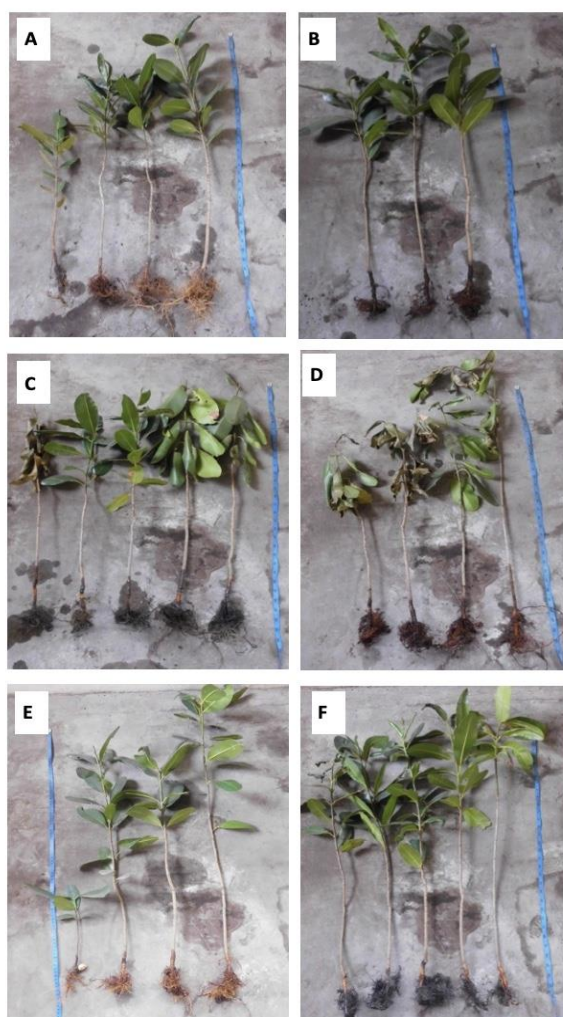


Figure 1. Morphology and damage of tamanu seedling on three types of soil and flooding: A) mineral soil without flooding, B) mineral soil with fresh water flooding, C) peat soil without flooding, D) peat soil with peat water flooding, E) soil sand without flooding, F) sandy soils with salt water flooding.

Tamanu seedling died in waterlogged peat soil with wilted, dried and fallen leaves, rotting and discolored (black) roots. These results indicate that tamanu seedling cannot grow on peat soil under flooding conditions. Peat soil is a type of soil formed from plant remains that has a very high organic content. However, it has low base saturation so that the organic matter content of peat soil is difficult for roots to absorb. In addition, the soil structure is fragile or shaped like a fibrous crust, making it difficult for plant roots to stand firm [12]. Different types of seedling show different levels of resistance to flooded conditions on peat soils. Seedlings of *Adenanthera pavonina*, *Horsfieldia crassifolia*, and *Syzygium* sp. those planted on peat land in flooded conditions died, while seedling *Dacryodes rostrata* and *Shorea balangeran* survived [15].

3.4 Morphological Adaptation

The morphological adaptation of tamanu after 30 days of flooding is the formation of lenticels and adventitious roots. Morphological adaptation occurred in tamanu seedlings which were resistant to flooding on mineral soils. Flooding after 30 days on mineral soils induced adventitious root formation (20%), and lenticels formed (20%) at 18 days after flooding (Table 4) (Figure 2).

The formation of lenticels and adventitious roots is a change in plant anatomy during flooding (1; 2; 3; 4 conditions). The formation of lenticels due to tissue enlargement at the base of the stem, due to cell division and elongation. Lenticels are gaps that facilitate gas exchange in plants, such as the entry point for O₂ and the exit point for toxic gases such as ethanol and acetaldehyde whose presence increases under conditions of flooding due to anaerobic respiration. The hypertrophic formation of lenticels is seen by swelling of the tissue at the base of the stem and is the result of cell division and enlargement. This is related to the production of auxin (IAA) and ethylene (2; 3; 18 flooding). Some plant species that are resistant to flooding form lenticels and adventitious roots (4; 5; 6; 7; 8; 9; 10; 11 adaptasi).

Table 3. Damage percentage, damage index, and resistance level of tamanu seedling after 30 days of treatment

| Parameter | Treatment | | | | | |
|-----------------------|--------------|----------|--------------------|-----------|-----------|----------|
| | Mineral soil | | Peat soil | | Sand soil | |
| | WF | F | WF | F | WF | F |
| Damage percentage (%) | 0 | 20 | 60 | 100 | 0 | 0 |
| Damage index | 0 | 0 | 2 | 4 | 0 | 0 |
| Resistance level | tolerant | tolerant | moderate tolerance | sensitive | tolerant | tolerant |

Notes : F : flooding, WF : without flooding

Table 4 Morphological adaptation of tamanu seedling after 30 days of flooding

| Morphological adaptation | Percentage of seedlings with morphological adaptability (%) | | |
|--------------------------|---|-----------|-----------|
| | Mineral soil | Peat soil | Sand soil |
| Lenticels | 20 | 0 | 0 |
| Adventitious roots | 20 | 0 | 0 |

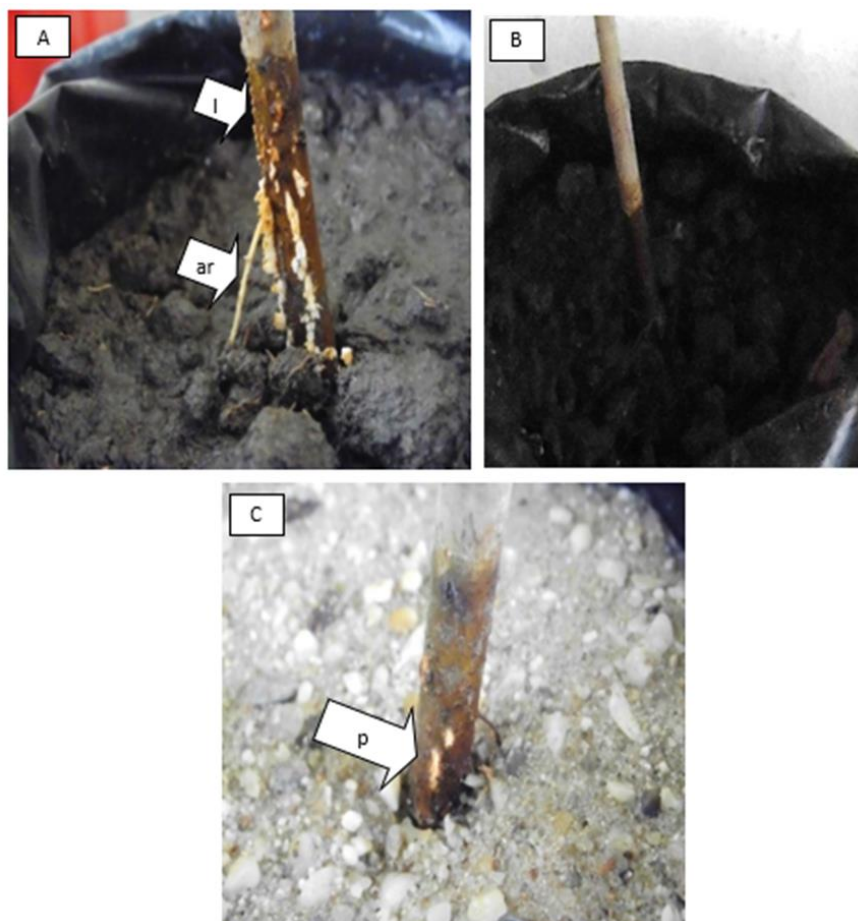


Figure 2 Morphological adaptation of tamanu seedling to flooding conditions in three types of soil: a) lenticels (l) and adventitious roots (ar) on mineral soils, b) lenticels and adventitious roots do not form on peat soils c) pore formation on sandy soils.

Formation of adventitious roots is caused by tamanu seedlings in flooded conditions experiencing a lack of O₂ in the soil so they have to adapt by forming adventitious roots in order to meet the O₂ needs for respiration [27]. Adventitious roots are also formed because plant roots are unable or unable to absorb water and minerals into plant roots [28]. Adventitious roots function as a place for O₂ to enter so that in stagnant conditions with water-saturated roots, plants can still take oxygen in the air. In general, the ability to produce adventitious roots is associated with increased tolerance to flooding and increased ethylene production [27-30]. In sandy soils with flooding also showed morphological changes, namely the formation of gaps in the submerged stems, the gaps in the stems were not clearly visible (Figure 2), flooding conditions did not induce adventitious or lenticel formation. Tamanu seedlings on peat soils were unable to grow and survive, the plants wilted and eventually died.

As the inundation time increases, the number of lenticels increases. Increasing the number of lenticels can increase tolerance to flooding in various species. Lenticels developing below the water table help maintain plant homeostasis during flooding by partially replacing decaying root systems, facilitating water uptake by shoots and lenticels are permeable to water [30, 31]. In addition, flooding also induces the formation of adventitious roots. In this study adventitious roots were formed in the stem where lenticels were abundant. Adventitious roots were formed in the area near the base of the stem with abundant lenticels. Adventitious root growth parallel to the water or soil surface.

Tamanu seedling on flooding on peat soil was not able to adapt and suffered 100% damage and death. Tamanu seedling experienced a continuous decrease in growth until the end of the observation which resulted in the plants becoming withered, dry and

eventually died. Seedlings of tamanu on flooding in sandy soil were able to survive even though there was no morphological adaptation of lenticels and adventitious roots, only small pores formed on the flooded stems, but the plants were not damaged. It is suspected that tamanu seedlings take a long time to form morphological adaptations because sandy soils have high porosity.

The results of this study indicate that tamanu is able to survive on sandy soil under conditions of flooding or without flooding and is not damaged. Flooding in tamanu sandy soil underwent morphological changes, namely the formation of small gaps in the submerged stems, the gaps formed were thought to induce the formation of adventitious roots if prolonged inundation was carried out. Sandy soil is a suitable growing medium for tamanu because sandy soil is a native growth medium for tamanu which is on the coast. Tamanu is able to survive and adapt to mineral soils under inundation conditions. This can be seen in mineral soils under waterlogged conditions where tamanu form adventitious roots, lenticels and lenticel hypertrophy. However, tamanu during flooding conditions suffers 20% damage. To reduce the level of damage to tamanu, a longer acclimatization should be carried out with the soil used for growth media. It is recommended to apply fertilizer containing the elements N, P, K before the flooding treatment and reduce the height of the flooding.

Tamanu has low resistance to peat soils, its growth is reduced resulting in the highest damage rate. Peat soil has a very low fertility rate, so fertilization is needed to increase the fertility of peat soil. The type of fertilizer needed is one that contains N, P, K, Ca and Mg. In addition, the content of microelements (magnesium sulfate, zinc sulfate) in peat soils can be increased by adding mineral soil.

4. Conclusion

Flooding treatment reduced the growth of tamanu seedling on mineral soil, peat soil and sandy soil, with the highest growth reduction on peat soil and the lowest growth reduction on sandy soil. Seedlings of tamanu grown on flooded sandy soils showed the lowest decrease in fresh weight, increase in stem diameter and highest percentage of living roots. Tamanu seedlings are tolerant to flooding on mineral soils and sandy soils, but sensitive to flooding on peat soils. The morphological adaptation of tamanu seedlings to flooding in mineral soils is the formation of adventitious roots and lenticels. Tamanu seedlings when submerged in sandy soil only form pores on submerged stems. It is necessary to carry out further research on the growth of tamanu seedlings on mineral soil media with shorter inundation times, longer acclimatization and lower inundation heights, while for peat soils it is recommended to apply fertilizer to increase soil fertility. In flooding in sandy soil, a longer

inundation time is needed to see how far the tamanu seeds are tolerant of inundation.

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Author Contribution Statement

Siti Fatonah- Conceptualization, Study design, Supervision, Writing – original draft; **Indah Permatasari**- Methodology, Formal Analysis, Writing – review and editing; **Dyah Iriani**- Methodology, Writing – review and editing. All the authors read and approved the final version of the manuscript.

Conflict of interest

The Authors have no conflicts of interest to declare that they are relevant to the content of this article.

Has this article screened for similarity?

Yes.

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