The effect of betel leaves as the soak solution for Bitti (Vitex cofassus) seeds germination

Melpiany¹, B Bachtiar², S A Paembonan², and S H Larekeng³
¹The Students of the Silviculture and Tree Physiology Laboratory, Faculty of Forestry, Hasanuddin University, Makassar
²Laboratory of Silviculture and Tree Physiology, Faculty of Forestry, Hasanuddin University, Makassar
³Laboratory of Biotechnology and Tree Breeding, Faculty of Forestry, Universitas Hasanuddin, Makassar

Email: sitih5h.82@gmail.com

Abstract. Bitti (Vitex Cofassus) is one of the endemic plants in South Sulawesi. The superior quality of the bitti seeds can be achieved by performing the right treatment on the seeds. The proper seed treatment can be applied by utilizing a plant that contains a disinfectant or antiseptic, such as betel leaf. This study aimed to find out the effect of the betel leave weight used in solution and soaking duration in betel leaves solution of bitti seeds as well as to determine the optimal soaking duration in order to maintain seeds’ quality. The method used in this study was an experimental field approach with a Completely Randomized Design (CRD) factorial experiment and Tukey’s HSD posthoc test, which consisted of the weight of betel leaves and the soaking duration. The soaking duration treatments were: S1 = 12 hours, S2 = 24 hours, and S3 = 36 hours, and then the betel leave weight were: B1 = 200 g, B2 = 250 g, B3 = 300 g and B4 = 350 g each in 3 liters water. The observation variables consisted of the percentage of sprouts, germination rate, germination energy, and seed viability. Twenty four hours of soaking duration in 350 g of betel leave solution showed the highest percentages on sprouts percentage, seed viability, seed energy, and germination rate, which were 76.70%, 75%, 75%, and eight days, respectively.

1. Introduction
Bitti (Vitex cofassus) belongs to the family Verbenaceae and classified as one of the endemic plants in South Sulawesi. Bitti is categorized into Class II of wood durability, which widely used as carpentry wood, pillars of home construction, household utensils, and also construction materials [1]. Furthermore, Bitti wood is mostly utilized by the people of South Sulawesi, especially as the primary wood materials for the Pinisi boats.

Unfortunately, the higher the demand and use of bitti wood, the less the supply of the plant available, both in plantations and in natural forests. Therefore, bitti cultivation is needed to produce good quality seeds. The effort to maintain the sustainability of bitti trees is by conducting cultivation and applying appropriate management techniques [2].

Some treatments can be applied to the seeds, such as soaking and scarification [3]. Thus, the level of dormancy can be reduced, and the performance of seed germination can be increased. Seed treatment is needed to keep the bitti seeds viable during the storage process so that the seeds can be stored in a more extended period until planting time without losing their viability [4]. Proper seed
treatment utilizes disinfectant or antiseptic substances in the plants that are easily found in the environment and able to protect the seeds from fungal and bacterial infections [5].

Betel is a plant that has leaves containing phenylpropanoid and phenol compounds, which are antimicrobial and antifungal agents and inhibit the growth of bacteria. Thus, it is widely used in health. Globally, the betel leaf is commonly consumed by people as medicine. It has antibacterial and antifungal chemical content such as essential oil, and its leaves also contain tannin, sugar, and amyllum [6]. In forestry, betel is employed to prevent bitti seeds from fungal or bacterial infection until they are ready for germination or planting time [5].

The percentage of germinating seeds is essential for the success of bitti nursery in order to produce high-quality and in the same quantity of seedlings. Therefore, the study on how many betels leave weight and duration of soaking in betel leave solution is needed to obtain proper seeds germination treatment for bitti seeds.

2. Research method

2.1. Time and place
This research was conducted from October 11 to November 16, 2019, at the permanent nursery of Balai Pengelolaan Daerah Aliran Sungai (BPDAS) Jeneberang Saddang, Lembang Marinding, Mengkendek District of Tana Toraja.

2.2. Research procedure
The implementation of this research consisted of several stages:

2.2.1. Seed Procurement. The first step in this research was the procurement of the bitti seeds from The 2nd regional of Balai Perbenihan Tanaman Hutan (BPHT), Makassar, South Sulawesi. The seeds were a one-month-old bitti seeds right after selected.

2.2.2. Seed Soaking. The betel leaves solution used to soak the seeds that were not priorly treated, and the seeds were immediately soaked into a mixed solution of betel leaves and water. The four leave weight treatments were: 200 grams (62 betel leaves), 250 grams (92 betel leaves), 300 grams (122 betel leaves), and 350 grams (152 betel leaves) in every 3 liters of water. The duration of soaking consisted of three treatments: 12 hours, 24 hours, 36 hours, without soaking and soaking in water without betel leaves as the control treatment.

2.2.3. Seed sowing. After soaking the seeds, the seeds were evenly diffused across the surface of the sowbed/tub, then covered with 0.3 cm thick media (as thick as the seeds). The seeds that had been sown were immediately doused with water using a hand sprayer. The number of seeds sown in one container was 20 seeds.

2.2.4. Seed Maintenance. Seed Maintenance included watering and cleaning the seeds that might be done twice a day to keep seed moisture.

2.2.5. Seed Germination. Seed germination started to be observed when the cotyledon and plumula were raised above the soil surface. The appearance of the plumula above the soil surface indicated that the seeds had been germinated.

2.3. Data analysis
The data obtained in this research were analyzed using the ANOVA with a completely randomized design factorial experiment. The statistical model used in this research activity was:

\[ Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk} \]
Where:
\[ Y_{ijk} = \text{Observation value to the } k \text{ experimental unit obtained a combination of } ij \text{ treatment (to the } i \text{ level of factor S and to the } j \text{ level of factor B).} \]
\[ \mu = \text{Population mean (actual mean).} \]
\[ \alpha_i = \text{effect to the } i \text{ additive level from factor S.} \]
\[ B_j = \text{effect to the } j \text{ additive level from factor B.} \]
\[ (\alpha\beta)_{ij} = \text{Interactive Effect of to the } i \text{ level of factor S and to the } j \text{ level of factor B.} \]
\[ \varepsilon_{ijk} = \text{effect of error to the } k \text{ experimental unit obtained to the } k \text{ that received the } ij \text{ combination.} \]

The observation of the F test at \( \alpha = 5\% \) was used to determine the effect of every single factor and their interaction on Bitti growth. If there is a significant effect on the treatments toward the observed variable, then each level of treatment is compared using posthoc Tukey’s HSD at 1% of the error level.

3. Results and discussion

3.1. The Percentage of Germination

Figure 1 shows that the preliminary treatment of soaking the bitti seeds with 350 grams of betel leaves for 24 hours obtained the highest percentage of germination (76.67%), while the lowest one was the seed without soaking treatment (as a control treatment) (4.44%). The results of this study were similar to the report by Tika et al. (2015) [5], which soaking the Sengon seeds in different betel leave weights and durations of soaking. It was probably due to water absorption in the first process of seed germination, followed by the expansion or swelling of seed and then the softening of the seed coat. Then, bitti seeds are classified into Buni fruit that has walls with two layers. The outer layer is thin and somewhat warm, like skin, while the thick inner layer is soft and watery [7].

This water absorption is carried out by the seed coat through the imbibition and osmosis events, where the process does not require energy[3]. Absorption of water by the embryo and endosperm causes swelling of the two structures, pushing the softened seed coat until it ruptures, and performs room for the root to come out [8]. However, the seeds without the soaking treatment are impossible to absorb water so that the skins are hard to soften and broken, and eventually, the roots will be delayed to come out.
Table 1. The ANOVA of the duration of soaking and weight of betel leaves on the germination of bitti (V. cofassus) seeds

| Source of variation | Sum of squares | Degree of freedom | Mean squares | F-value | F-table 5% | F-table 1% |
|---------------------|---------------|------------------|-------------|---------|-----------|-----------|
| Duration of soaking | 4422.22       | 2                | 2211.11     | 159.2** | 3.40      | 5.61      |
| Weight              | 3335.42       | 3                | 1111.81     | 80.05** | 3.01      | 4.72      |
| Duration of soaking x weight | 1350       | 6                | 225         | 16.2**   | 2.51      | 3.67      |
| Error               | 333.33        | 24               | 13.89       |          |           |           |
| Total               | 51125         | 36               |             |         |           |           |

Note: ** Very significant effect  
* Significant Effect  
tn No effect

Table 1 depicts that among betel leave weight, duration of soaking, and the interaction between the weight of betel leave and duration of soaking had a very significant effect on the percentage of bitti seed germination. Then, the results of posthoc Tukey’s HSD test is presented in Table 2.

Table 2. The results of Tukey’s HSD posthoc test of interaction between the duration of soaking and weight of betel leaves on the percentage of seed germination

| The Interaction of Treatment | Average percent germination | Tukey’s HSD test |
|------------------------------|-----------------------------|------------------|
|                              | 1%                          |                  |
| s1b1                         | 15                          | A                |
| s3b3                         | 15                          | A                |
| s3b2                         | 16.667                      | A                |
| s1b2                         | 21.667                      | A                |
| s1b3                         | 23.333                      | A                |
| s3b3                         | 25                          | A                |
| s2b1                         | 26.667                      | A                |
| s3b4                         | 28.33                       | A                |
| s2b2                         | 28.333                      | A                |
| s1b4                         | 33.33                       | Ab               |
| s2b3                         | 50                          | Bc               |
| s2b4                         | 75                          | C                |

Note: Different letters mean having a significant difference according to the 1% error level.

Table 2 shows that the interaction between duration of soaking and the weight of betel leaves that observed the highest percentage of germination was 12 hours of soaking duration with a weight of 350 g (75%). The soaking duration in the betel water solution was not always followed by the increasing percentage of germination, as shown in the decreasing percentage on sengon seed germination using 36 hours of soaking duration and 350 grams betel leaves.

Water intake is vital in seed germination. If the seeds lack water absorption, the seed germination will be inhibited. However, if the seeds are soaked for too long or if the process exceeds, the seed growth process will be disrupted because the seeds should absorb water or imbibition that can cause the seeds to experience the plasmolysis process due to being in the solution for too long [5] [9]. Bitti
seeds soaked in betel leaf solution can protect the seeds from damage. Moreover, the betel leaves are antiseptic and disinfectant that affects the percentage of seed germination.

3.2. Seed viability

Figure 2 depicts that soaking with 350 g of betel leaves for 24 hours had a high germination rate (75%), while the lowest germination rank was found in seeds without soaking treatment, showing the percentage of 1.67%. The seed viabilities on seeds soaked without betel leaves for 12 hours, 24 hours, and 36 hours were 8.3%, 20%, and 12%, respectively. The soaking for a longer time in the betel water solution was not always followed by the increasing percentage of seed growth, as shown on the seeds soaked for 36 hours in a solution of betel leaves. Also, soaking the seeds for too long disrupts the germination because experiencing plasmolysis [5]. The ANOVA of the seed viability is presented in Table 3.

Table 3. The ANOVA of various effects of soaking time and weight of betel leaf on bitti’s seed viability.

| Source of variation         | Sum of squares | Degree of freedom | Mean squares | F-value | F-table 5% | F-table 1% |
|-----------------------------|----------------|-------------------|--------------|---------|------------|------------|
| Duration of soaking         | 4151.39        | 2                 | 2075.69      | 80.78** | 3.40       | 5.61       |
| Weight                      | 3902.08        | 3                 | 1300.69      | 50.62** | 3.01       | 4.72       |
| Duration of soaking*weight  | 1604.16        | 6                 | 267.361      | 10.4**  | 2.51       | 3.67       |
| Error                       | 616.66         | 24                | 25.69        |         |            |            |
| Total                       | 42375          | 36                |              |         |            |            |

Note: ** Very significant effect
* Significant Effect
  * No effect

The duration of soaking, the weight of betel leaves, and the interaction between the two treatments had a very significant effect on the germination of bitti seeds (Table 3). The results of Tukey’s HSD test is in Table 4.
The results of posthoc on interactions between treatments can be seen in Table 4. It shows that soaking the seeds for 12 hours with 200 g of betel leaves was not significantly different from soaking for 24 hours with 200 g, 250 g, 300 g, and 350 g of betel leaves and for 36 hours with the same weights of betel leaves. The highest average percentage of seed viability on the interaction between treatments was in soaking for 24 hours, with 350 g of betel leaves, which was 75.

The results showed that when the bitti seeds were soaked for 24 hours with 350 g of betel leaves, the seeds finally germinated well because they could maintain their endosperm from damage caused by bacteria and fungi. Good imbibition rate causes the optimal water absorption of the seeds so that the process of seed metabolism can run well[10]. By absorbing the optimal water, the oxygen will enter into the seeds and break down their food supply that used as an energy source for the fast and simultaneous growth[11][12].

3.3. Germination energy
The results showed that the treatment for 24 hours soaking time with 350 g betel leaves obtained the highest germination energy (Figure 3). The germination energy is the ratio between the number of germinated seeds and the seeds planted up to the peak of germination or the percentage of germinated seeds in a certain period or when the highest average of daily germination is achieved. The importance of germination energy or germination speed is based on the fact that the seeds that germinate fastly will be able to produce good seedlings and predict the percentage of seedling growth in the future [13].
Figure 3. The germination energy of bitti seeds

Table 5 presents that the weight of betel leaves and the interaction between both treatments obtained a very significant effect on the bitti seed germination energy, while soaking duration had a significant effect on bitti seed germination energy[14]. Thus, the further analysis conducted using Turkey’s HSD test, which can be seen in table 5.

Table 5. ANOVA of the soaking duration and weight of betel leaves on bitti seed germination energy

| Source of variation                  | Sum of squares | Degree of freedom | Mean squares | F-value | F-table 5% | F-table 1% |
|-------------------------------------|----------------|-------------------|--------------|---------|------------|------------|
| The duration of soaking             | 4422.22        | 2                 | 2211.11      | 159.2*  | 3.40       | 5.61       |
| Weight                              | 3335.42        | 3                 | 1111.80      | 80.05** | 3.01       | 4.72       |
| The duration of soaking*weight      | 1350           | 6                 | 225          | 16.2**  | 2.51       | 3.67       |
| Error                               | 333.33         | 24                | 13.89        |         |            |            |
| Total                               | 51125          | 36                |              |         |            |            |

Note: ** Very significant effect
* Significant Effect
in No effect

Table 6. The Tukey’s HSD test on the interaction of the treatments on germination energy.

| The interaction of the treatments | The average percentage of germination energy | Annotation 1% |
|----------------------------------|--------------------------------------------|---------------|
| s1b1                             | 18.33                                      | A             |
| s3b3                             | 28.33                                      | A             |
| s3b2                             | 25                                         | A             |
| s1b2                             | 25                                         | A             |
| s1b3                             | 28.33                                      | A             |
| s3b3                             | 28.33                                      | A             |
Soaking the seeds for 12 hours and 36 hours with 200 g to 350 g of betel leaves showed a significantly different effect with soaking for 24 hours with 350 g of betel leaves. The highest average germination energy from the interaction among both treatments was at 24 hours of soaking duration, with 350 g of betel leaves. Sutopo (2002) [15] stated that several factors influence the speed of seed germination. One of them is water. Betel leave water solution contains phenol, an antiseptic that protects seeds from fungal and bacterial infections. By protecting seeds from bacteria and fungi, the seed’s endosperm will be safe from damage during the storage process so that the seeds can germinate properly. Sutopo (2002) [15] added the seed endosperm tissue contains carbohydrates, proteins, fats, and minerals, that are needed as a raw material of energy for the embryo germination.

### 3.4. Germination Rate

The fastest germination rate was seeds soaked in 350 g of betel leaves for 24 hours (Figure 4) that started to germinate on day 8th. The soaking for 24 hours could break the seed dormancy so that the germination rate was rapid growth.

![Figure 4. The germination rate of Bitti seed.](image)

**Table 7.** ANOVA of the soaking duration and weight of betel leaves on bitti seed germination rate

| Source of variation | Sum squares of duration | Degree of freedom | Mean squares of duration | F-value | F-table 5% | F-table 1% |
|---------------------|-------------------------|-------------------|--------------------------|---------|------------|------------|
| Duration of soaking | 15.52                   | 2                 | 7.76                     | 1.04tn  | 3.40       | 5.61       |
| Weight              | 27.73                   | 3                 | 9.24                     | 1.24tn  | 3.01       | 4.72       |
| Duration *weight    | 63.16                   | 6                 | 10.52                    | 1.41tn  | 2.51       | 3.67       |
Error 178.44 24 7.43
Total 23158.91 36

Note: ** Very significant effect
* Significant Effect
tn No effect

Table 7 shows that the soaking duration, the weight of betel leaves, and the interaction between both treatments did not have a significant effect on the germination rate of bitti seed, so there was no need to do further tests.

4. Conclusion
The soaking of the seeds for 24 hours with 350 g of betel leaves showed the optimal result on bitti seed germination in terms of the seed germination percentage, the germination energy, and also the seed viability.

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