

THE VIGOR AND VIABILITY TESTS OF BITTI (*Vitex cofassus* Reinw. ex Blume) SEEDS USING WATER IMMERSION TREATMENT IN A GREENHOUSE

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THE VIGOR AND VIABILITY TESTS OF BITTI (*Vitex cofassus* Reinw. ex Blume) SEEDS USING WATER IMMERSION TREATMENT IN A GREENHOUSE. Bitti (*Vitex cofassus* Reinw. ex Blume) is a group of plants in the Verbenaceae family. The species is classified as a promising endemic plant in South Sulawesi; however, it has received little attention and has not been used and developed optimally. The seed is a recalcitrant category, which has responses to water loss. This study aimed to determine the effect of water temperature and storage periods on the vigor and viability of Bitti seeds. The study was conducted at a greenhouse of the Faculty of Forestry, Hasanuddin University, from May to July 2022. The design of the research was a Completely Randomized Design (CRD) consisting of two factors: water immersion temperature and storage time. Each treatment consists of 30 seeds, with three replications. The results showed that a water immersion temperature of 50°C with a storage period of two weeks gave the best results in the parameters of percentage of sprouts (65,55%), germination viability (65,55%), and mean germinated seeds per day (0,49). The water immersion temperature of 90°C, which was drained for 24 hours without storage, showed the highest value for the mean germination time parameter (31,1), and the soaking temperature of 90°C drained for 24 hours with one week of storage produced gave the best value for the vigor index parameter (4,77). It is considered that germination activities on Bitti seeds require treatment with suitable water immersion. Thus, Bitti seed germination activities require water immersion at a certain temperature and storage time.

Keywords: Bitti, germination, water immersion, storage time

UJI VIGOR DAN VIABILITAS BENIH BITTI (*Vitex cofassus* Reinw. ex Blume) MENGGUNAKAN PERLAKUAN PERENDAMAN AIR DI GREENHOUSE FAKULTAS KEHUTANAN UNHAS. Bitti (*Vitex cofassus* Reinw. ex Blume) merupakan pohon yang tumbuh di daerah tropis dan termasuk dalam status kurang diperhatikan (least concern). Benih Bitti bersifat rekalsitran, yang bereaksi terhadap kehilangan air. Penelitian ini bertujuan untuk mengetahui pengaruh suhu perendaman air dan lama penyimpanan terhadap vigor dan viabilitas benih Bitti. Penelitian dilaksanakan di greenhouse Fakultas Kehutanan Universitas Hasanuddin pada bulan Mei-Juli 2022. Rancangan penelitian menggunakan Rancangan Acak Lengkap (RAL) factorial yang terdiri dari dua factor yaitu suhu perendaman air dan lama penyimpanan, menggunakan 30 benih dengan tiga pengulangan. Hasil penelitian menunjukkan bahwa suhu perendaman air 50°C dengan lama penyimpanan dua minggu memberikan hasil terbaik pada parameter persentase kecambah (65,55%), daya kecambah (65,55%) dan rata-rata benih berkecambah per hari (0,49). Adapun suhu perendaman 90°C yang ditiriskan selama 24 jam tanpa penyimpanan menunjukkan nilai tertinggi untuk parameter rata-rata waktu berkecambah 31,1 dan suhu perendaman 90°C yang ditiriskan selama 24 jam dengan penyimpanan satu minggu menghasilkan nilai terbaik pada parameter indeks vigor 4,77. Kegiatan perkecambahan pada biji Bitti memerlukan perlakuan dengan perendaman air yang sesuai. Dengan demikian, kegiatan perkecambahan biji Bitti memerlukan perendaman air pada suhu dan waktu penyimpanan tertentu.

Kata kunci: Bitti, perkecambahan, perendaman air, lama penyimpanan

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I. INTRODUCTION

Bitti (*Vitex cofassus*) is a plant in the Verbenaceae family and is classified as a promising endemic plant in South Sulawesi (Melpiany et al., 2020; Muslimin et al., 2020) but has received little attention and has not been used optimally (Adrianto et al., 2015). Nonetheless, Bitti wood has considerable potential to be developed to meet the needs of local and industrial markets (Hayati et al., 2019) because it has strong durability, good texture, flexibility, and resistance to termites (Adrianto et al., 2015). Bitti wood is also included in the class II-III durable wood and strong class II-III (Kementerian LHK, 2020). This wood is used as a raw material for house construction, ship and boat manufacturing, and the furniture industry (Adrianto et al., 2015). In the Solomon Islands, Bitti is used as a raw material for making large drums, and the wood is also one of the primary commodities exported to Japan (Adrianto et al., 2015).

Given the dependence on Bitti wood, which will continue to increase, the demand for the availability of Bitti wood will also be higher in the future. This condition can seriously threaten the availability of Bitti trees in natural forests. Hence, it is necessary to reproduce this species through generative propagation using seeds. Seed of Bitti is classified as recalcitrant (Kurniawan, 2013).

Recalcitrant seeds are a significant challenge in seed storage and conservation (Gayatri et al., 2021), they have a relatively high water content (Bharuth & Naidoo, 2020; Triani, 2021) and are sensitive to water loss (Azarkovich, 2020), making it difficult to store for a long time (Lestari, 2019; Yulianti et al., 2020). This type of seed is sensitive to dry conditions, which may be caused by a lack of sufficient osmotic protection during dehydration (Chandra et al., 2021). These characteristics make it crucial to conserve this type of seed, given the declining number of native species and the associated loss of genetic diversity (Bharuth & Naidoo, 2020).

The long distance from the source of quality seeds to the destination may also cause a decrease in seed quality due to prolong shipping (Batubara et al., 2018). Therefore, seed storage is needed to support the conservation of reciprocal seeds through seed recruitment (Lestari, 2019; Rindyastuti & Siahaan, 2019). Seed storage also aims to provide or preserve planting material (Triani, 2021).

It is also crucial to provide physiological improvement and accelerate the seed germination by doing pre-treatment to get high viability and vigor (Suita, 2019). However, Bitti seed viability is naturally unsatisfactory due to its hard seed coat (Hasnat et al., 2019). The texture of the hard and thick integument makes it difficult for seeds to germinate (Priyono et al., 2021; Rumahorbo et al., 2020).

Germination is a process from seed to shoot in which the plant's metabolic processes are activated after a period of dormancy (Purwanto et al., 2019). Seed germination testing is a method to provide information about a seeds ability to grow (Hartati et al., 2019). According to Paramita et al., (2018) and Triani (2021), quality seeds are influenced by germination. One of the efforts that can be made to accelerate seed germination is to soak the seeds in water (Gea et al., 2018). Seeds will germinate more quickly and produce uniform growth if a proper treatment is applied (Putri et al., 2021).

Many works have been carried out on seed testing of forestry plants of commercial species. However, there is still limited information about *Vitex cofassus*, research on seed storage, pre-treatment, and vigor testing. The study was to determine the effect of water temperature and periods of storage on the viability and vigor of Bitti (*V. cofassus*) seed. The findings might be valuable information for other related research..

II. MATERIAL AND METHOD

A. Study Site

The Bitti seeds were obtained from mother trees in Lanna Village, Parangloe District, Gowa Regency, which is a plantation stand

with uncertified status, aged between 15 - 20 years. The study activities were conducted at a greenhouse of the Faculty of Forestry, Hasanuddin University, at coordinates of 05°07'38.38" S 119°28'49.03" E. The period of the study was 40 days from May-July 2022. The tools used in this study were machetes, hoes, shovels, sieves, sacks, soil sterilizers, thermometers, germination beds, buckets, a digital camera, SAS 9.4 Portable Software, a set of computers and stationery.

B. Methods

The seeds used were freshly harvested and stored for less than 48 hours after extraction before treatment (W0). The seeds were stored in a refrigerator for the length of storage time according to the predetermined level.

This study was designed using a factorial Completely Randomized Design (CDR) consisting of two factors, namely: 1) water immersion factor (W) consisted of six levels i.e, (W0: No immersion (control), W1: 30°C for 24 hours, W2: 50°C for 3 hours, W3: 70°C for 1 hour, W4: 90°C for 10 minutes and W5: 90°C for 10 minutes drained for 24 hours) and 2) storage periods factor (T) consisted of four levels (T0 : 0 weeks (control), T1: 1 week, T2: 2 wweks and T3: 3 weeks). The treatments were repeated 3 times and each treatment consisted of 30 seeds. The total number of seeds used was 2.160. The linear model used is as follows:.

$$Y_{ger} = \mu + \alpha g + \beta e + (\alpha\beta)ge + \epsilon_{ger} \dots\dots\dots(1)$$

Where :

- Y_{ger} = observations in the r replication that received treatment with A factor at g level and W factor at e th level
- μ = average
- αg = influence of A factor at g level
- βe = influence of W factor at e level
- $(\alpha\beta)ge$ = interaction effect of factor A at g level and factor W at e level
- ϵ_{ger} = error component by A at g level, W factor at e level and r replication or normally distributed random influence $(0,\sigma^2)$.

C. Research Variables

The observed parameters include:

1. The percentage of sprouts calculated using the formula:

$$Germination\ Percentage\ (GP) = \frac{\Sigma sprouted\ seeds}{\Sigma planted\ seeds} \times 100 \dots(2)$$

(Surahman et al., 2012).

2. Germination viability was calculated using the formula:

$$Germination\ Viability = \frac{\Sigma normal\ seedlings}{\Sigma germinated\ seeds} \times 100 \dots(3)$$

(Husna et al., 2020).

3. Mean germination time was calculated using the formula:

$$MGT = \frac{\Sigma t . n}{\Sigma n} \dots\dots\dots(4)$$

Where :

- t = days needed to germinate,
- n = number of seeds germinated at the end of the observation (Hartmann & Kester, 1983).

4. Mean germinated seeds per day were calculated using the formula:

$$MGD = \frac{N}{t} \dots\dots\dots(5)$$

Where :

- N = total number of seeds germinated at the end of the observation,
- t = number of observation days (Hartmann & Kester, 1983).

5. Vigor index was calculated using the formula:

$$VI = \frac{A1+A2+\dots+An}{A1.T1+A1.T2+\dots+An.Tn} \times 100 \dots\dots\dots(6)$$

Where :

- A = number of seeds germinating on a given day,
- T = time corresponding to the number of seeds germinating,
- n = number of days in the final calculation (Ayuningtyas et al., 2017).

III. RESULT AND DISCUSSION

RESULTS

A. Analysis of Variance of Two Factors

The results of the seed germination analysis of Bititi can be seen in Table 1. All treatments given to Bititi seeds had a very significant

effect on both the unit parameters and their interaction.

B. Percentage of Sprouts and Germination Viability

The interaction between water immersion temperature and storage time on the parameters

of percentage of sprout and germination viability can be seen in Figure 1. It shows that the highest treatment values occur at the interaction between 50°C water immersion with two weeks of storage (W2T2).

Table 1. Recapitulation of the germination parameters of Bitti (*V. cofassus* Reinw. Ex Blume) seeds

No.	Observation Parameters	W	T	W*T	Coeff Var (%)	Pr>F
1.	Percentage of Sprouts	**	**	**	18.81	<.0001
2.	Germination Viability	**	**	**	20.07	<.0001
3.	Mean Germination Time	**	**	**	11.35	<.0001
4.	Mean Germinated Seeds per Day	**	**	**	6.80	<.0001
5.	Vigor Index	**	**	**	11.26	<.0001

Note: W = water immersion temperature, T = storage periods, Coeff. Var = coefficient of variation, ** = highly significant effect

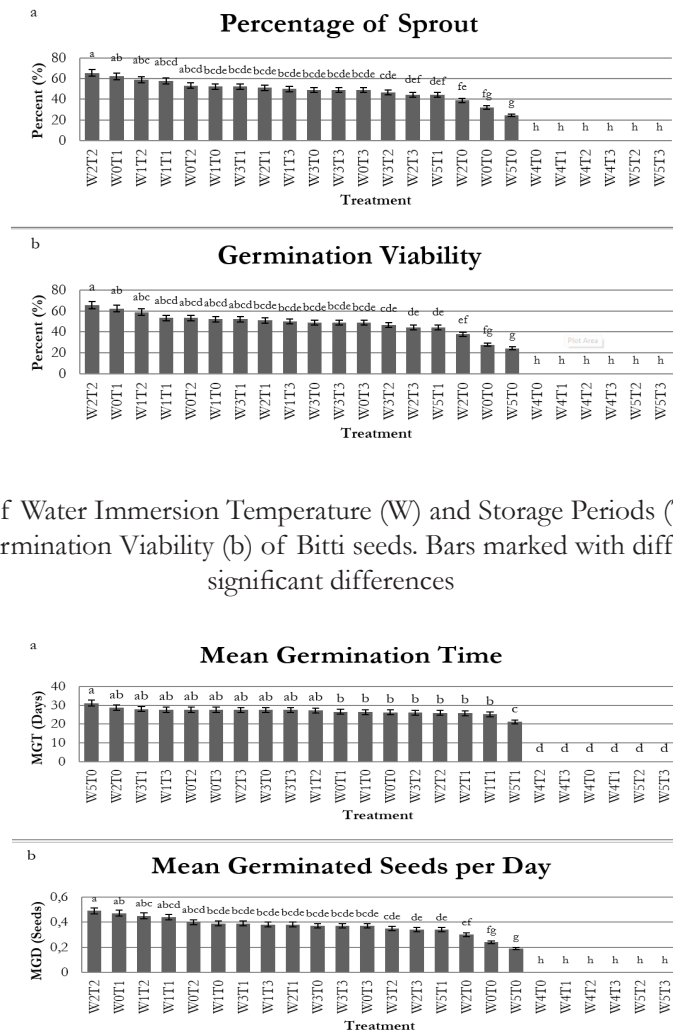


Figure 1. The effect of Water Immersion Temperature (W) and Storage Periods (T) on the Percentage of Sprout (a) and Germination Viability (b) of Bitti seeds. Bars marked with different letters indicate significant differences

Figure 2. Mean Germination Time (a) and Mean Germination Per Day (b) of Bitti seeds after 40 days of planting, after treatment of water immersion temperature and storage periods. Bars marked with different letters indicate significant differences

C. Mean Germination Time and Mean Germinated Seeds per Day

The highest value of mean germination time is the treatment of temperature at 90°C, drained for 24 hours, with no storage (W5T0), meanwhile, the mean temperature for germinating seeds per day is 30°C with a storage period of two weeks (W2T2).

D. Vigor Index

The results of the interaction effect of water immersion temperature and storage length on the vigor index variables can be seen in Figure 3. The seeds soaking in 90°C water, drained for 24 hours, and one week storage (W5T1) gave the best results in Bitti vigor index for 40 days observation.

DISSCUSSION

As stated in the book of Vademekum (Kementerian LHK, 2020) Bitti seeds can be soaked in hot water (90°C) for 10 minutes and then drained for 24 hours. In this study, the interaction treatment of a temperature of 90°C, drained for 24 hours, and a storage period of one week succeeded in making Bitti seeds germinate on the sixth day. However, the percentage of sprouts was only 6,67, and in the second and third weeks of storage, no shoots emerged in this treatment.

Lower germination rates can occur when the treatment of seeds is too excessive beyond the capacity that the seed can accept. The likely reason is that the immersion temperature of

90°C made the seed coat more permeable, allowing an excessive water intake that exceeded their capacity and resulted in a lack of oxygen, ultimately leading to the seeds demise (Alghofar et al., 2017). It means that the initial temperature of the immersion water should be conditioned due to the possible damage to the embryo (Kusuma et al., 2019).

According to Kurniaty (2010), Bitti seeds can still germinate after three weeks of storage. In the study, the pre-treatment of soaking in water at 90°C without draining for 24 hours showed no signs of germination. However, seeds were still found to germinate when they were treated with a temperature of 90°C, drained for 24 hours, and those seeds that had a one-week storage treatment. The treatment without immersion (control) and three weeks of storage, a temperature of 30°C without storage, and a temperature of 50°C with two weeks of storage, caused the emergence of early sprouts on the 11th day. From this description, the results obtained in this study were not in line with the results obtained by Kurniawan (2013) that Bitti seeds can germinate on the 29th day after sowing, with a percentage of sprouts of 64,5%. These differences were caused by differences in immersion with different water temperatures to provide further germination responses (Asy'ah et al., 2019). The difference in immersion temperature, which gives different results, is also possibly due to the ability of each seed to respond to the given temperature shock, as reported by Rumahorbo et al. (2020),

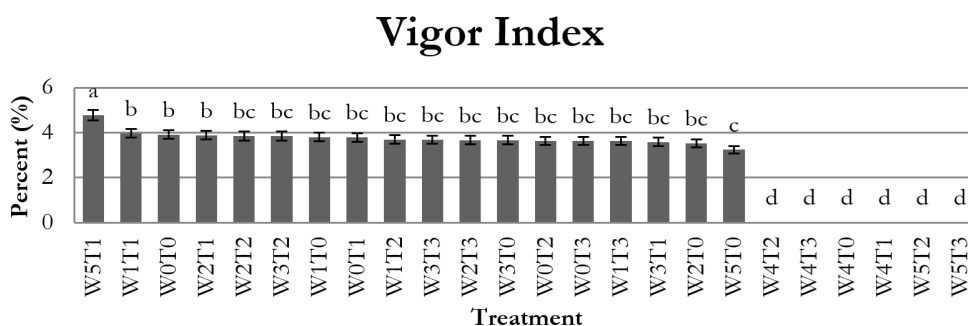


Figure 3. Vigor Index of Bitti seed after 40 days of planting, after treatment of water immersion temperature and storage periods. Bars marked with different letters indicate significant differences

that soaking seeds using hot water can speed up imbibition. This means that differences in temperature will also result in the ability of each seed to imbibe differently.

According to Lemmens et al. (1995), the percentage of early germination of Bitti seeds was between 0-20%, but pre-treatment by soaking in hot water (70°C) had caused the percentage of germination to increase to 70%, and the seeds began to germinate between 10-40 days. But in this study, the temperature of 70°C was only able to give a percentage of sprouts of 49,16%, and germination began on the 13th day for treatments without storage and one week of storage, while treatment of two and three weeks of storage resulted germination on the 16th day.

The research conducted by Melpiany et al. (2020) suggested that seeds without soaking treatment are unlikely to absorb water because their skins are difficult to soften and crack, resulting in delayed roots coming out. However, in this study, the control treatment (without immersion) also significantly affected all parameters. Even so, treatment with hot water immersion was still very effective at the germination level (Koobonye et al., 2018; Mamani et al., 2018). This can occur due to environmental factors, as reported by Asy'ah et al. (2019), that temperature, light, and humidity also help the germination process. In this research, the sunlight received by Bitti sprouts was full, and the humidity of the sprouting media was maintained. The water immersion treatment helps imbibition and activation of maltase, lipase, and protease enzymes (Kurniawan, 2018) and metabolic reaction processes (Rori et al., 2018).

Lestari (2019) and Yadav et al. (2018) stated that the increase in storage periods would cause the rate of germination or seed germination to decrease. This research also found that even with high temperature water immersion treatments, when the storage period was three weeks, the germination did not exceed 50%. Aji et al. (2020) reported that this could happen because of the maturity level of the

seeds and the presence of obstacles from the seeds themselves. In this study Bitti seeds used were seeds that had a good and even level of maturity. However, storage periods, especially for recalcitrant seeds, is a key factor in the success and quality of seed germination (Bisht et al., 2021). Long-stored recalcitrant seeds will experience a lack of moisture content during the embryogenesis stage and cell membranes will become damaged (Gayatri et al., 2021; Triani, 2021).

In line with the seed vigor value, Figure 3 shows that the treatment of one week of storage has a higher value than those seeds with no storage. Vitis et al. (2020) reported that long storage resulted in a decrease in seed vigor. The occurrence of a sequence mismatch could be caused when the seeds were taken for germination; the seeds were not selected based on specific standards but were taken randomly, so it was suspected that many seeds from the first week had better properties than seeds that were germinated without storage and vice versa. The effect of other factors in this research might have occurred when differences in immersion temperature were employed.

Appropriate treatment interactions are critical to assessing seed and maintaining high seed viability and vigor (Vitis et al., 2020). Quality and viability are indicators of seeds ability to germinate, produce regular seeds, and describe their germination power (Keti et al., 2022). Seed viability is also described through the maximum growth potential (Wulandari & Farzana, 2020). Meanwhile, high seed vigor is characterized by its long storage resistance, resistance to pest attacks, fast and even growth, and the ability to produce normal mature plants (Keti et al., 2022). The level of seed vigor will reflect the growth strength and growth of sprouts. In other words, the higher the vigor, the better the germination strength (Putri et al., 2021). In addition, seeds that grow quickly and simultaneously can better deal with sub-optimum field conditions, which is part of the value of good seed vigor (Asy'ah et al., 2019).

One of the aims of seed assessment is to determine the quality level of the germinated seeds by looking at the ability of the seeds to produce sprouts that grow typically or abnormally. Abnormal sprouts are seeds with embryos that have died, and the endosperm is no longer intact due to decay, so the dormant seeds will not break and germinate (Aji et al., 2020). In other cases, especially the seeds that have been treated with 90°C immersion without draining, mold was found in the sand sprout media. It is certainly an indication that the seed can no longer grow. Fungi can cause seeds to rot and become damaged and eventually the seeds will die (Widarawati et al., 2022).

The percentage of sprouts and germination viability are the influential parameters in determining normal and abnormal sprouts. In Figures 1a and 1b, there were no significant differences in the yield parameters of percentage of sprout and germination viability. It is because, during the observation activities, only a few abnormal sprouts were found in each treatment. Based on the data found, odd sprouts were only seen in W1T1 (four abnormal sprouts), W0T0 (four abnormal sprouts), and W2T0 (one abnormal sprout) treatment. The abnormal sprouts did not reach 10% of the total germinated seeds. The cases of abnormal sprouts that were found during the observations were relatively uniform; namely, at the beginning of the appearance of the ovule, they looked normal as usual, but when they entered the age of a few days, there was a fungus infestation around the sprouts. The conditions of moist and closed media cause poor germination and eventually die.

The endosperm of the seed contains high carbohydrates and glucose that provide a good ingredient for fungi growth surrounding the seed. This condition makes the embryo unable to germinate due to the unavailability of energy (Putri et al., 2021). It is also stated that one of the factors that influences the occurrence of abnormal sprouts is the planting medium is too dense and moist. This causes the radicle to bend and the primary root does not grow

well. Seed maturity is an essential factor in the germination process. The immature seeds do not have sufficient food reserves, and the imperfect seed structure may inhibit the germination process (Gea et al., 2018) that causing incomplete growth

IV. CONCLUSION

It is concluded that the best treatment to increase the percentage of sprouts, germination viability, and mean germination of seeds per day was the immersion temperature of 50°C after being stored for two weeks (W2T2) under refrigerator storage conditions. The high temperature treatment of 90°C is not recommended because it gives a low sprout rate.

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