Study of the effect of microbial addition in seed germination and seedling growth of Cryptocarya densiflora L.

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Abstract. Cryptocarya densiflora L. belongs to the Lauraceae family that is famous for its utilization of its leaves, stems, roots, and fruit. These species are also widely spread. The hardness of its seed coats inhibits its germination. Various efforts to accelerate the germination process by immersion in water or chemicals have done. However, the application of microbes has never been reported. Naturally, C. densiflora seeds fall to the ground directly in contact with soil microbes that might play a significant role in the germination process. This research aims to reveal the effect of inoculants’ application originated from soil microbes, Aspergillus niger, and natural microbial consortia in germination and growth of seedling C. densiflora. The experiments employed a completely randomized design using two main treatments, the inoculant formula, and the growing media. Inoculant formula factors consist of Formula 1 (A. niger), Formula 2 (microbial consortia), and Control, while the growing media consists of 3 types, A (soil and sand/1:1), B (soil, sand, and compost/1:1:1), and C (sand). All growing media were treated with 2 different treatments, sterilized (S) and non-sterilized (N). The growth stage was observed for 12 weeks after sowing with the following observed parameters: pH of the media at the end of the experiment, the percentage of sprout growth, plant height, number of leaves, root length, wet and dry weight of roots, and shoots. The results showed that the addition of microbial inoculant significantly affected the germination of C. densiflora seeds in all parameters measured, both for media A and B (sterile and non-sterile). Formula 1 gave the best results on all media, especially sterile media with parameters of plant height, number of leaves, root length, wet and dry weight of roots and shoots. The presence of microbes significantly affected germination and C. densiflora seedling growth.

Keywords: Cryptocarya densiflora, microbe, Aspergillus, consortia.

1. Introduction

The Lauraceae family has a wide distribution covering all tropical and subtropical regions. One of them is from the genus Cryptocarya. With a species diversity of more than 350 varieties, this genus is a woody plant that has a high economic value. Almost all parts of this plant can be utilized, such as for building materials as well as for medicinal ingredients[1,2]. One species of genus Cryptocarya that exists in Indonesia is Cryptocarya densiflora.

Cultivation of C. densiflora plants is usually done by collecting seedlings around the mother plant. The relatively hard seeds often become a problem in commercial propagation. The seeds are usually soaked in warm water or in a certain chemical(usually acidic) to accelerate the germination process. Soaking is done so that the hard seed coat becomes softer, allowing water to enter to accelerate germination [3]. There have been no reports yet on the use of microbes to soften seeds as an initial
process in germination. The hard seeds of *C. densiflora*, like other hard seeds, are generally composed of lignin and cellulosic materials.

Several soil microbes are indicated to be able to produce cellulase and ligninolytic enzymes. These enzymes are used by microbes to break down cellulose and lignin as food sources [5–7]. The utilization of soil microbes to support *Cryptocarya* seed germination has not yet been reported. To obtain microbes, they can be isolated from materials containing microbes [2], such as soil and plant parts that fall on the ground. One of the most common and widely distributed soil microbes is *Aspergillus niger*[8,9].

Naturally, *C. densiflora* seeds that fall to the soil will gradually encounter with soil microbes. Microbes that use food sources from the seed coat of this plant will continue to stick and develop. Eventually, these microbes will naturally help soften the skin of *C. densiflora* so that it will accelerate the dormancy period of the seeds. On the other hand, microbes also play an important role in all ecosystems, such as soil microbes which play an important role in the decomposition of organic material, nutrient cycle, and nutrients that can be absorbed by plants [10].

This study aims to determine the effect of using *A. niger* and microbial consortia from *C. densiflora* seeds in the germination and the growth of *C. densiflora* seedlings under greenhouse conditions. The results of this study are very important because microbes can be an alternative in the framework of the generative propagation of *C. densiflora* plants in the future.

2. Materials and Methods

The research was conducted on a greenhouse scale by using pots with a volume of 500 grams. The experiment was designed in factorial with 5 repetitions. The treatments include 2 types of microbial formulas (Formula 1 and Formula 2) and Control (C), 3 types of growing media (A, B, and C) with both sterile and non-sterile conditions. Observation of seedling growth was carried out until the 12th week where harvesting was conducted and observed according to the parameters studied. The parameters observed include seed growth or germination, plant height, root length, wet and dry weight of the shoots, wet and dry weight of the roots, and pH.

2.1. Preparation of microbial formulas

The formulas consist of two types:

*a. Formula 1.* This formula contains *Aspergillus niger* which was a private collection. The isolate was obtained from the isolation of soil samples in the mangrove area in Suwung, Bali. The inoculants were propagated using sterile liquid media containing 1% molasses then stirred with a speed of 50 rpm/minute at room temperature for 5 days or until the population has reached $4-7 \times 10^8$ CFU/mL.

*b. Formula 2.* The formula contains microbial consortia that were obtained directly from the seeds of *C. densiflora*. A total of 5 seeds of *C. densiflora*, taken from the Bogor Botanical Gardens, were grown in sterilized 1% molasses liquid media. The media containing the seeds were stirred with a speed of 50 rpm/minute at room temperature for 5 days or until the microbial population has reached $4-7 \times 10^8$ CFU/mL.

*c. Control.* For the control group, liquid media containing 1% sterile molasses were used.

2.2. Growing Media

The growing media tested consist of 3 types, media A (a mixture of soil and sand with a ratio of 1:1), media B (a mixture of soil, sand, and compost with a ratio of 1: 1: 1), and media C (filled with sand). All growing media were given 2 treatments, non-sterile and sterile. Media sterilization was carried out by using an autoclave at 121°C with a pressure of 1 atmosphere for 15 minutes.

2.3. Growing process

*C. densiflora* seeds were soaked for 30 minutes in the liquid media of the inoculant formula 1, formula 2, and the liquid medium for control (C), respectively. The seeds were planted in pots containing 330 grams of growing media A, B, and C, both sterile and non-sterile, which 2 seeds were planted in each pot (Figure 10). Each pot was poured with an inoculant formula according to each treatment amounting to 10 ml. As the seed coat, a suitable growing medium was added to the shoot according to the treatment.
The pot was watered with sterile distilled water up to the field capacity of each growing medium. Next, the pots were placed on the experimental shelves, watered daily, and observed for their growth for up to 12 weeks.

![Image](https://example.com/image1.png)

**Figure 1.** The growing of *C. densiflora* seeds in experimental pots.

3. Results and Discussion

3.1. pH media

The results of the pH measure of the growing media at the end of the experiment are presented in Figure 2. In general, the pH value of the sterilization treatment on the growing media and the type of media treatment and the inoculant formula did not show a significant difference. The pH of the growing media ranged from 6 to 7. As for the non-sterile growing media, the acidity level ranged from 5.5 to 7, with A1 treatment significantly different from the control. Meanwhile, the treatment of non-sterile media A was lower but not significantly different.

The degree of acidity of the growing media may influence the diversity and the activity of soil microbes as well as the mineralization process that occurs in the soil. Microbial activity in producing enzymes generally occurs at a pH of 7 or slightly acidic. Likewise, the mineralization process in the soil occurs at a neutral or near-neutral pH, but the effect of pH can not stand alone. Bacteria, which are more responsible for changes in pH, require the presence of fungi [11,12].

3.2. Germination

The observation results of the percentage of seed or sprout growth (Figure 3) showed that all treatments present significantly different results compared to the control. The percentage of seed growth that reaches 100% occurred in the non-sterile media for treatment A1, A2, B1, and B2, as well as the sterile media for treatment A1 and A2. Even though the growth percentage only reached between 75 - 80% in both sterilized and non-sterile growing media, the conditions in the growing medium C with the inoculant formula statistically showed a significant difference when compared to the control which only had a growth percentage of 60 - 65%. These results indicate that the application of the *A. niger* inoculant formula and consortia formula affects the ability of *C. densiflora* sprout seeds in all the media tested. This effect ranged from 33.3% for the sterile media and 25% for the non-sterile media. These results also prove that both formulas 1 and 2 are good for helping the germination process of *C. densiflora* seeds on the sterile and non-sterile medium A (soil and sand) and medium B (soil, sand, and compost) with the non-sterile treatment.
The observation result of the plant height parameter (Figure 4) showed that the highest seedling growth was obtained from the A1 treatment in the sterile growing medium (22 cm). Meanwhile, in the non-sterile growing media, it was obtained from the A2 treatment, which was 18.5 cm. In sum, the A1 and A2 treatment results both in the sterile and non-sterile growing media were significantly different from those of the control. For the treatments in medium B, only the B1 treatment with a seedling height of 20 cm for the sterile media treatment and 18.2 cm for the non-sterile media treatment showed a significant difference when compared to the control. The control seedling heights for the sterile and non-sterile media were 15 cm and 14.3 cm. In the medium C treatment, only A. niger treatment with the non-sterile condition showed a statistically significant difference when compared to the control, with the plant heights of 16 cm and 14 cm.
Figure 4. Effect of inoculants and growing media on the seedling height of *C. densiflora* at the age of 12 weeks.

These results indicate that the *A. niger* inoculant formula provides the best result on the sterile media A and B, and media C in the non-sterile condition. The growth of *C. densiflora* seedling treated with *A. niger* inoculant formula was able to grow well when compared to the control, which was 57.2% higher in medium A and 33.3% higher in medium B and 14.3% higher in medium C. The condition of the 12-week-old plant seedling is displayed in Figure 5.

Figure 5. Conditions of *C. densiflora* seedling at the age of 12 weeks, sterile media/S (top), non-sterile media/N (bottom).

3.4 Number of Leaves
The observation of the number of leaves during the 12 weeks seedling age period showed that the *A. niger* formula was highly significant in the sterile media A and B when compared to the controls (AC and BC). In the non-sterile media treatment, only A1, A2, B1, and C1 treatments showed a different number of leaves, namely 5 leaves each, compared to the control treatment with only 4 leaves. This condition reveals that the treatment of the *A. niger* formula showed the best effect on media A and B in sterile conditions, reaching 75%. Meanwhile, the best effect for media C in the non-sterile media was 25% (Figure 6).
Figure 6. The effects of inoculants and the types of growing media on the number of leaves of *C. densiflora* seeds at the age of 12 weeks.

3.5. Root length
The results of root length are presented in Figures 7 and 8. The longest roots were obtained by giving the *A. niger* formula treatment on the sterile medium (A1), namely 25.5 cm, and non-sterile medium (C1), namely 17.5 cm. This value was significantly different when compared to the control, which is 15.25 cm or 67.3% for AC and 11.5 cm or 52.2% for CC. This condition explains that the *A. niger* formula has the best effect on the root length growth of *C. densiflora* seedling on the sterilized medium A and the non-sterile medium C.

Figure 7. The effects of inoculants and growing media on the root length of *C. densiflora* seedling at the age of 12 weeks.

Figure 8. The root conditions of *C. densiflora* seedling at the age of 12 weeks, sterile media/S (left), and non-sterile/N (right).
3.6. Wet and dry weights
The inoculant formula had a significant effect on the sterile media A and B, but it was not different for medium C. A. niger formula in medium A (a mixture of soil and sand) showed the highest value for wet weight, namely 0.77 grams or 103% when compared to the control. Meanwhile, for the dry weight of the roots, it was 0.13 grams or 117% when compared to the control. These results displayed a significant difference statistically. Furthermore, this condition did not occur in the medium C (sand) where the wet and dry weights of the seedling roots did not show a significant difference. The results of the observations are shown in Figure 9.

Different conditions occurred in the treatments where the growing media were not sterilized. The inoculant formula derived from the consortia of C. densiflora seeds on medium A (a mixture of soil and sand) showed significantly different values for both the highest wet and dry weight of the roots, namely 0.74 grams or 106% and 0.13 grams or 160% when compared with the controls (NAC) which were only 0.36 grams and 0.05 grams (Figure 10). The above conditions indicate that the inoculant formula derived from the consortia of C. densiflora seeds has a more significant impact on the root growth in the non-sterile media, while the pure inoculant formula will have a more visible role in the sterile growing medium.

![Figure 9](image1)

**Figure 9.** The effects of inoculant and sterile media on the wet weight and dry weight of the roots of *C. densiflora* seedlings at the age of 12 weeks.

![Figure 10](image2)

**Figure 10.** The effect of inoculant and non-sterile growing media on the wet weight and dry weight of the seedling roots of *C. densiflora* at the age of 12 weeks.
3.7. Wet and dry weights of the shoot of plants

For the sterile media, the application of A. niger and consortia formulas derived from seeds showed significantly different results for both wet weight and dry weight of upper plant seedlings when compared to controls, except for medium C where only A. niger formula gave different results (see Figure 11). The highest weight was shown in the treatment of A. niger formula on medium A, 2.25 grams or 101% when compared to the control and 0.5 grams or 118% for the dry weight of treatment in medium B. This condition indicates that the application of the A. niger formula has a significant effect on both wet and dry weights of C. densiflora seedlings grown in the sterile media A and B.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure11.png}
\caption{Effect of inoculant and sterile media on the wet weight and dry weight of the shoots of C. densiflora seedling at the age of 12 weeks.}
\end{figure}

The results of non-sterile media for wet weight and dry weight of the shoot are presented in Figure 12. The A. niger formula gave the best results for the wet weight of the shoot in medium B, which was 1.87 grams or 70% when compared to the control. On the other hand, the consortia inoculant formula derived from seeds showed the best results in medium A, both on wet weight and dry weight, namely 1.96 grams and 0.61 grams or 67% or 166% when compared to controls respectively (Figure 12). These results showed that the consortium formula provides the best results for both wet weight and dry weight of the shoot of C. densiflora seedling grown in the non-sterile media mixed with soil and sand.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure12.png}
\caption{The effects of inoculant and non-sterile growing media on the wet weight and the dry weight of the shoot of C. densiflora seedling at the age of 12 weeks.}
\end{figure}

Generally, the formulas containing microbes in seedling media can accelerate the germination of seeds (Figures 3 and 4), as well as the growth of seedling C. densiflora (Figures 4, 6, 7, 9 - 12). Seedling growth until the age of 12 weeks from the measured parameters, namely plant height, number of leaves, root length, wet and dry weight of both the top and root plants, showed that the microbial formula was able to significantly increase the seedling growth (Figure 6). These results prove that microbes, in this
case, fungi, both in single and contortive forms, hold an important role in the germination process and seedling growth. It is also closely related to the function of microbes in the ecosystem. In all ecosystems, soil microbes play an important part in the decomposition of organic matter, nutrient cycle, and nutrients that can be absorbed by plants [10].

Microbe has an important role in all ecosystems [1,10,13,14]. The interactions that occur between various microbes greatly determine the changes in the ecosystem function [13]. Changes in ecosystem function are made possible by changes that occur in the components making up the ecosystem. Microbes as one of the constituent components of an ecosystem have different relationships and responses to the same or different environments. Microbes have a specific relationship with the host as well as the surrounding environment [15]. One of the roles of microbes in the ecosystem is as a producer of enzymes. The presence of enzymes allows the breakdown of organic materials in the environment. The enzyme produced by A. niger is expected to be able to soften the hard skin of C. densiflora which leads to the germination process occurring more quickly. Aspergillus is also known as the endophytic genus which has a significant role in plant health. This type of fungi does not have a specific host, but it has high adaptability and a good ability to survive [16]. Microbes such as A. niger are capable of producing ligninolytic and cellulase enzymes[5,6]. The presence of these enzymes enables the seed coat of C. densiflora, which is composed of lignin and cellulose, to be softer.

The sterilization treatment of the growing medium has shown different results. The microbial response is highly dependent on the soil type. Microbial behavior that is related to the availability of nutrients will form a specific microecology in the root area, root surface, and even root tissue [8]. The existence of microbes in the soil is beneficial, especially concerning the mineralization of organic matter into nutrients. Several groups of fungi produce growth hormones that are useful for plants [17].

The application of A. niger inoculants can support the growth of sprouts and seedlings of C. densiflora. In addition, the use of compost can assist the vegetative growth phase. Meanwhile, the enzyme produced by the inoculant A. niger is thought to function in controlling plant growth at the germination level which is in line with the biomass recovery [8].

4. Conclusion

A. niger inoculant formulae and microbial consortia can hasten the germination of C. densiflora seedlings. The germination rate of C. densiflora seeds in sterile and non-sterile medium made of soil and sand is greatly affected by the inoculant formula of A. niger. The non-sterile media of soil, sand, and compost are affected by the microbial consortia inoculant formula produced from seeds. The A. niger inoculant formula has a substantial effect on seedling development, plant height, the number of leaves, and root length in the sterile medium of soil and sand or soil, sand, and compost. It also has an impact on the wet and dry weight characteristics of roots in both sterile and non-sterile medium. The sterile media has a greater impact on the inoculant formula for seedlings in terms of both wet and dry weight of the shoots.

References

[1] Aislabie J, Deslippe JR 2013. In Dymond JR ed. Ecosystem services in New Zealand Manaaki Whenua Press, Lincoln, New Zealand.
[2] Braga RM, Dourado MN, Araújo WL 2016. Braz J Microbiol 47(Suppl 1) 86-98
[3] Sharma KK, Singh US, Sharma P, Kumar A, Sharma L. 2015 J. of Appl. and Natural Science 7(1) 521-539
[4] El-ghonomy DHI, Ali TH, Moharam ME. 2014 Antonie Van Leeuwenhoek 106(5) 853-64
[5] Pingili M, Marla SR, Raparla R, Vanga S. 2017 Int.J.Curr.Microbiol.App.Sci. 6(12) 2200-2206
[6] Alberto M, Vali EC, Ayala M, Luis J, Mallol F 2019 Biotechnology Research and Innovation 3 (1) 177-186
[7] Rahmansyah M, Sugiharto A, Juhaeti T. 2017 Pros Sem Nas Masy Biodiv Indon 3(3) 426-432
[8] Bilıkay I S, Karakoç Ş, Aksöz N. 2010 Turk J Biol 34 (2010) 313-318
[9] Stromberg M R, Steenwerth KL, Jackson L E, Caldero F J, Scow KM. 2003 Soil Biology &
Biochemistry 35(2003) 489–500
[10] Martin G, Guggiari M, Bravo D, Zopfi J, Cailleau G, Aragno M, Job D, Verrecchia E, Junier P 2012 Environ Microbiol. 14(11) 2960-70
[11] Cho, SJ., Kim, MH. & Lee, YO. 2016 J Ecology Environ 40(10)
[12] Mcguire K L, Treseder K K 2010 Soil Biology & Biochemistry 42(2010) 529-535
[13] Liao H, Huang F, Li D, Kang L, Chen B, Zhou T and Shaolin P 2018 Plant and Soil 430 1-2
[14] Selosse M, Baudoin E, Vandenkoornhuyse P. 2004 Comptes Rendus Biologies 327(7) 639–48
[15] Zhang H, Tang Y, Ruan C, Bai X. 2016 Records of Natural Products 10(1) 1-16
[16] Whitelaw M. A 2000 Advances in Agronomy 69 99-151