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Detection of Ergosterol Levels in Oil Palm Seedlings (*Elaeis guineensis*) Infected with *Ganoderma Boninense* in Hydroponic Media

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Abstract*: One of the diseases that attacks oil palm (*Elaeis guineensis*) is the Basal Stem Rot (BSR) disease caused by the *Ganoderma boninense* fungus. One method of early detection of BSR disease is ergosterol detection. Ergosterol is a sterol found in fungal cells. Therefore, ergosterol can be used as a biomarker. The aim of this research was to detect ergosterol levels in oil palm seedlings infected with the fungus *G. boninense* which were cultivated in hydroponic media. The method used in the study was oil palm cultivation in hydroponic media. Oil palm plants that were 45 days old after planting in hydroponic media were then infected with *G. boninense* fungus. The inoculum source used RWB (*Rubber Wood Block*) which had been infected with *G. boninense* fungus. Furthermore, plant harvesting was carried out every day for 12 times. The research design used was a Completely Randomized Design (CRD) consisting of 2 treatments, namely control and treatment of *G. boninense* inoculum administration on hydroponic media. Each treatment was repeated 3 times. Observations were carried out in series, namely on the third day, sixth day, ninth day, and twelfth day. The observation samples to be taken were the roots, stems, and leaves. Furthermore, the harvest results were detected for ergosterol content using the UV-Vis Spectrophotometry method. The results showed that ergosterol levels in roots, stems, and leaves of oil palm seedlings (*E. guineensis*) infected by *G. boninense* fungus in hydroponic media increased significantly along with the observation days. The highest ergosterol levels were in the root organ: 53.79 mg/L; stem organ: 86.38 mg/L and in the leaf organ: 75.52 mg/L.

***Keywords*: *Elaeis guineensis*, Ergosterol, *Ganoderma boninense*, Hydroponic Media, *Rubber Wood Block*.**

1. Introduction

The development and production of oil palm faces serious challenges from attacks by pathogens and plant diseases [1]. One of the plant diseases that threatens the sustainability of oil palm production is the Basal Stem Rot (BSR) disease caused by the fungus *G. Boninense*. BSR disease is difficult to detect because its development is very slow and there is no visual difference between healthy oil palm trees and trees infected with *G. boninense*. Oil palms infected with *G. boninense* do not show external symptoms in mature oil palms until an advanced stage. As a result, BSR disease becomes more difficult to control [2]. Early detection allows treatment of infected oil palms before the disease spreads. Disease control can create ecological and economic benefits [3].

Based on this, an effective way is needed to determine the number of infections in oil palms early on. One method that can be done to detect the presence of fungus in oil palms is by quantifying ergosterol. Ergosterol is a sterol compound found in fungal cell membranes, and its concentration can be used as an indicator of fungal biomass and severity of infection. Other studies have shown that ergosterol concentration can be used as a way to estimate *G. boninense* infection in oil palm plantations and as an early detection method for BSR disease in oil palms [4].

Several studies have developed methods to detect ergosterol, namely using a UV-Vis spectrophotometer which is considered efficient in measuring the concentration of ergosterol in fungus. Thus, this method provides a rapid and accurate approach to detect Ganoderma infection in oil palm [5]. In addition, there is a study focusing on the measurement of ergosterol as an indicator of fungal biomass in plant material, using UV spectrophotometry with detection at a wavelength of 282 nm [6].

Oil palm nurseries are usually carried out using soil as a planting medium, but in this study, oil palm seedlings will be planted using hydroponic media. Hydroponics is the cultivation of plants using water containing nutrients needed by plants [7]. The advantages of hydroponic media include efficient land use and minimizing pest and disease problems, considering the large number and variety of soil microorganisms [8]. In addition, hydroponic media was chosen to reduce physical barriers between fungus and oil palms. This is the basis for choosing the use of hydroponic media in the study. The use of hydroponic media is expected to determine whether the detected ergosterol results are truly derived from the fungus *G.*

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boninense. Therefore, detection of ergosterol in oil palm plants infected with *G. boninense* is very important as an early detection effort.

2. Materials and Methods

A. Preparation of Oil Palm Seedlings

The oil palm seeds used came from the Medan Oil Palm Research Center (PPKS) with the species name *Elaies guineensis* Jacq. and No. Inter-Area Plant Health Certificate 2024.2.0700.0.K12.K.0000055. The seeds were planted in polybags containing soil media. The seedlings were maintained for 3 months. Furthermore, 3-month-old seedlings were used as research objects.

B. Preparation of Hydroponic Planting Media and Rubber Wood Block (RWB)

Hydroponic planting media uses an aquarium measuring 40 cm x 15 cm x 50 cm filled with 5 L of hydroponic solution. The hydroponic media used is AB Mix nutrients containing micro and macro nutrients. AB Mix stock solution is made by dissolving all Nutrients A and B each with 500 ml of distilled water in a separate place. Preparation of hydroponic solution: to make 1 L of hydroponic solution consists of 990 ml of aquades, 5 ml of Stock A, and 5 ml of Stock B. Each aquarium is filled with 5 L of hydroponic solution. To support the growth of fungi, the outer surface of the aquarium is coated with carbon paper. This treatment is to protect the fungi from being exposed to direct sunlight. RWB was prepared as many as 40 pieces. RWB is a carrier medium for *G. boninense* inoculum. The RWB was obtained from the Marihat Unit of the Palm Oil Research Center, North Sumatra.

C. Ganoderma Inoculation on Oil Palm Seedlings

Rubber Wood Block as a source of *G. boninense* inoculum, 10 pieces were placed in an aquarium containing hydroponic solution. Each aquarium contains 4 oil palm seedlings that have previously been adapted to hydroponic media for one week. After the application of RWB on hydroponic media, plant growth was observed. Plant growth was observed through the morphology of the roots of oil palm seedlings to determine any indications of *G. boninense* fungal infection.

D. Preparation of Observation Sample Extracts

The observation samples used were roots, stems, and leaves. Observations were made on the third day, sixth day, ninth day, and twelfth day. Plant samples for observation were cleaned with water and dried using an oven. The dried samples were pulverized using liquid nitrogen. A sample of 100 mg was put into a test tube and 4 ml of 10% KOH in methanol was added. The sample was vortexed for 10 seconds and the test tube was placed in a water bath at 60ºC for 90 minutes. After heat treatment and the addition of 1 ml of distilled water and 2 ml of n-hexane, the tube was vortexed again for 30 seconds and centrifuged for 15 minutes at 3000 rpm, then the top layer or phase was discarded. The remaining solution was re-extracted with 2 ml of n-hexane. The combined hexane fractions were evaporated overnight in a water bath at 45ºC. The precipitate was dissolved in 2 ml of methanol by heating at 40ºC for 15 min, then filtered through a 0.2 mm PTFE syringe filter and submitted to ergosterol analysis using a UV-Vis Spectrophotometer [9].

E. Ergosterol Analysis Using a UV-Vis Spectrophotometer

Before detecting ergosterol in the sample solution, it is necessary to make a standard ergosterol solution that functions as a comparison standard that will be dissolved with methanol at a certain concentration. The standard ergosterol standard is weighed as much as 50 mg and dissolved in 100 ml of methanol, shaken until homogeneous, until a standard concentration of ergosterol of 500 ppm in methanol is obtained. Next, the 500-ppm solution was re-diluted until solutions with concentrations of 0.2 ppm, 0.4 ppm, 0.6, ppm 0.8 ppm, 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm were obtained. Then all solutions were measured at a wavelength of 282 nm with methanol as a blank. The sample to be tested as much as 1 ml was inserted into the UV-Vis spectrophotometry cuvette and the absorption spectrum formed at a wavelength of 282 nm was observed. In quantitative testing, the absorbance of the identified test analyte is then calculated for its concentration based on the regression equation obtained in determining the standard curve.

F. Data Analysis

The research design used was a Completely Randomized Design (CRD) consisting of 2 treatments, namely control and treatment of *G. boninense* inoculum administration on hydroponic media. Each treatment was repeated 3 times. Observations were carried out in series, namely on the third day, sixth day, ninth day, and twelfth day. The observation samples to be taken were the roots, stems, and leaves. The data obtained will be analyzed descriptively quantitatively because the aim is to determine the ergosterol levels of the research samples, namely roots, stems, and leaves on the third day, sixth day, ninth day, and twelfth day after inoculation.

3. Results and Discussion

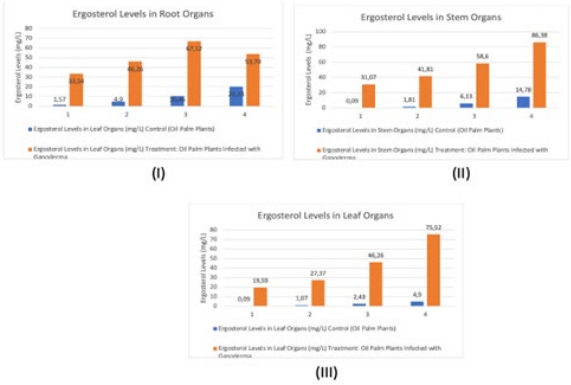

Fig. 1. Ergosterol levels of oil palm plants infected with Ganoderma boninense: (I) Root organs; (II) Stem organs and (III) Leaf organs

The oil palm plant samples to be detected for ergosterol levels are the root, stem and leaf organs. Sampling for ergosterol level analysis was carried out on the 3th day, 6th day,

9th day, and 12th day after RWB application. Ergosterol detection was used to comprehensively describe the distribution of Ganoderma infection. The ergosterol levels of oil palm plants infected with Ganoderma are shown in Figure 1.

Based on Figure 1, the results of the study showed an increase in ergosterol levels in all plant organs over time in the inoculum treatment compared to the control treatment. On the third day, the ergosterol levels in plant organs (roots, stems and leaves) inoculated with *G. boninense* were higher than the control. This shows that physical penetration by fungi on the surface of oil palm roots has been successful and has begun to infect the root tissue and continues to the leaves. The increase in ergosterol levels in oil palm plant organs reflects the proliferation of fungi in the root tissue to the leaves which is increasingly intensive. Ergosterol levels continue to increase along with the development and spread of fungi in plant tissue [10]. However, in the roots there was a decrease in ergosterol levels on the 12th day in the inoculum treatment compared to the ninth day. This decrease can be caused by several factors related to the dynamics of *G. boninense* infection in oil palm. The decrease in ergosterol levels can reflect natural variability in the dynamics of fungal populations. Fungal infections may not occur linearly and fluctuations in the number of fungi or their metabolic activity can cause variations in ergosterol levels [11]. In all plant organs, the results showed that both treatments (administration of *G. boninense* inoculum and control) gave significant ergosterol levels. The ergosterol levels in oil palm without *G. boninense* inoculum were seen to be much smaller, but the absorbance value was still detected on the UV-Vis Spectrophotometer. This value likely indicates the levels of ergosterol in other fungi besides *G. boninense*. It is suspected that the nutrient solution in the hydroponic system can be a food source for pathogenic fungi other than *G. boninense*. High nutrient concentrations can encourage the growth of unwanted organisms [12]. High levels of ergosterol in oil palm plant organs infected with *G. boninense* illustrate the level of infection that occurs. *G. boninense* produces ergosterol as a major component in its cell membrane. Therefore, increased levels of ergosterol indicate an increase in the number of fungal cells in plant tissue. The infection process of *G. boninense* fungus on oil palm roots begins with contact between Ganoderma inoculum and plant roots, which allows for basal stem rot (BSR) infection. Once inside, the pathogen begins to spread through the plant tissue, producing toxins and enzymes that damage plant cells. The fungus *G. boninense* is able to produce enzymes that degrade root cell walls, breaking down structural polysaccharides such as cellulose and hemicellulose. These enzymes are pectinase, cellulase, and ligninase [13]. Next, the *G. boninense* fungus spreads from the roots to the stems through the xylem vessels, which are channels for transporting water and nutrients in the plant. The fungal mycelium can move through the xylem, utilizing the flow of water and nutrients to reach the upper part of the plant. This fungus infects by decomposing the cell walls around the xylem which then disrupts the transport of water and nutrients. In addition, fungal infections can spread to the stem tissue through the cortex tissue. Fungal hyphae spread through the intercellular spaces and cortex cells. Hyphae continue to grow and branch to expand the area of infection to the leaves. Although mycelium does not grow directly on the leaves, damage to the nutrient and water transport system in the roots and stems will also affect the condition of the leaves. Infection of the vascular tissue causes the transportation system in oil palms to be disrupted, so that the leaves do not get enough water and nutrients needed for photosynthesis and growth.

4. Conclusion

Ergosterol levels in roots, stems, and leaves of oil palm seedlings (*E. guineensis*) infected by *G. boninense* fungus in hydroponic media increased significantly along with the observation days. The highest ergosterol levels were in the root organ: 53.79 mg/L; stem organ: 86.38 mg/L and in the leaf organ: 75.52 mg/L.

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