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by Suwandi Suwandi

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GROWTH RESPONSE of *Ganoderma* sp. Mycelium Treated with Root Exudates of Herbaceous Plants

Tiara Putri Rahmadhani,[1*] Suwandi,[2] Yulia Pujiastuti [2]

1 *Student of Agroecotechnology, Faculty of Agriculture, Sriwijaya University.*

2 *Lecture of Plant Protection , Faculty of Agriculture, Sriwijaya University.*

**Corresponding author.*
E-mail address: tiaraputri@student.pps.unsri.ac.id (Odetta).
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Abstract

The purpose of the study was to study response of Ganoderma sp. that were given exudate treatment of plant roots. Ganoderma sp. is a pathogen that causes stem rot at base of oil palm. In addition to oil palm this fungus can attack hard and woody crops such as coconut, rubber, tea, cocoa etc. Isolate used is Ganoderma sp. Bio-10197 code obtained from Phytopathology laboratory SEAMEO BIOTROP Bogor. Mycelium Ganoderma sp. reproduced in malt agar media until mycelium grows over agar surface. Ganoderma mycelium was inoculated on 1x1x5 cm rubber wood pieces for 14 days until the mycelium grew over rubber sticks. The exudate used from ganyong (Canna edulis Kerr), garut (Maranta arundinacea Linn.), Ginger (Zingiber officinale Rosc.), Turmeric (Curcuma domestica Val.), Galangal (Alpinia galanga (L.) Sw.) and lidah mertua (Sansevieria trifasciata). The design used in this study was a complete randomized design (RAL) with 7 treatments consisting of 5 repeatations. The results of this study indicate that the mycelium experienced inhibition of growth, especially in the treatment of root exudate galangal shown by 60,81% percentage and tongue-in-law with a percentage of 59,58% colonization. On observation of growth characteristics of mycelium Ganoderma sp. visible zone delimited in the form of a brown mycelium pile suspected as an indication of mycelium rejection of bioactive compounds contained exudate.

Keywords: allelophatic, Ganoderma sp., herbaceous plant, and root exudates.

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1. Introduction

Herbaceous plant is one of the various types of flora in Indonesia. These plants are included in the lower plants, characterized by soft stems because they do not form wood and are generally watery. This plant can be a seasonal plant, bi-annual season or plant (Mc Naughton dan Wolf, 1992; Abdiyani, 2008).

Herbaceous plant reported to have many benefits, among others: in health care, as a medicinal plant and used as a vegetable pesticide. The seeds of the herbs are medicinal related to the secondary compounds they produce (Abdiyani, 2008).

Compounds produced by plants that can be toxic are called allelopathy. This compound can interfere with the growth of living things around it (Wiroatmojo, 1992). Situmorang (2007) reports that plants can be antagonistic or allelopatiic to the white root fungus that attacks the rubber plant. One form of release of allelopathy to the environment is in the form of root exudates (Duke, 1998; Lahoy, 2009).

Ganoderma sp. is a major cause of stem rot disease that attacks oil palm in Indonesia and Malaysia. In some plantations in Indonesia, the disease has caused crop deaths of up to 80% of the entire palm oil population and caused the decrease of oil palm products per unit area (Susanto, 2002) hingga 100%, even cause death in infected plants (Abadi,1987).

Pathogenic *Ganoderma* species in oil palm have a wide range of hosts. In natural habitats in the forest, this fungus can attack woody plants (Semangun, 2000). In addition to attacking oil palm, *Ganoderma* species can attack other plantation crops such as coconut, rubber, tea, cocoa, as well as various forest tree species such as Acacia, Populus, and Macadamia (Ariffin et al., 2000; Risanda, 2008).

The use of antagonistic plants to control plant diseases is considered as flora one of the alternative controls that have a bright prospect. This is because the use of this method does not give negative effects as well as the use of chemical pesticides. Some plants suspected of having an

antagonistic ability in inhibiting the growth of fungi include ganyong (*Canna edulis* Kerr), garut (*Maranta arundinacea* Linn.), Ginger (*Zingiber officinale* Rosc.), Turmeric (*Curcuma domestica* Val.), Galangal (*Alpinia galanga* (L.) Sw.) and lidah mertua (*Sansevieria trifasciata*) (Situmorang et al., 2007).

2. Materials and Method

This study used the Completely Randomized Design (RAL) method consisting of 7 treatments with 5 repeatations. The treatment are :

A: Treatment with ganyong root exudate
B: Treatment with garut root exudate
C: Treatment with ginger root exudates
D: Treatment with turmeric root exudate
E: Treatment with galangal root exudate
F: Treatment with lidah mertua root exudate
K: Control (sterile water)

2.1 Plant Test

Plant material is selected from the plant part that already has a bud. Plants are grown in polybags using soil from the experimental garden of the Agricultural Faculty of Sriwijaya University, Indralaya. Fertilization was not done during the experiment.

2.2 Root Extract Preparation

The root exudate is obtained from three tillers removed and its roots. The roots are washed by running water and then the roots are soaked for 4 hours in a glass container containing 100 ml sterile distilled water mixed with 1 cc of 100% phosphoric acid. The container is closed to avoid contamination. After 4 hours, the soaking water in the form of plant root exudate is filtered using filter paper and bacteria filter 0.20 µm (Advantec cellulose acetate disposable syringe filter) is then fed into the test tube and stored at -20 ° (freezing) condition until used (Hao et al., 2010).

2.3 Preparation of *Ganoderma* sp. Inoculum

Isolate *Ganoderma* sp. rotting pathogen of palm oil stems obtained from Phytopathology laboratory SEAMEO BIOTROP Bogor. Isolate pathogens are propagated on malt agar media. Isolate Ganoderma sp. which grows on malt media to be grown on a piece of rubber wood in a jam jar. Then incubated for 14 days ie until the mycelium meets the top surface of the rubber wood pieces.

2.4 Preparation of Rubber Wood Sticks

Pieces of 1x1x5 cm rubber wood stick soaked in water for 3 days and every 24 hours once the marinade is replaced. After the water is drained, the rubber wood pieces are wrapped and then stirred at 80° C for 24 hours. After the dioven, rubber wood pieces were sterilized using an autoclave

2.5 Root Exudates Treatment

The root exudate treatment was performed by exudate infiltration method through test soil by placement of inoculum above ground with 1 ml volume moistened with 1 ml of root exudate fluid in a 16 mm diameter reaction tube. The mouth of the test tube was closed with parafilm and incubated at 27 ° C for 7 days.

2.6 Observed Variables

2.6.1. Percentage of Colonization of *Ganoderma* sp.

The observation of the colony is done by photographing the sides of the rubberwood pieces colonized by thick mycelium after being treated with exudate. Then the colony area is calculated using ImageJ version 1.44p application (National Institutes of Health, United States). Observations were made daily until the morphology of the mycelium colony did not change after 14 days of incubation. Percentage colonization of mycelium *Ganoderma* sp. calculated using the formula:

$$\text{Percentage of colonition} = \frac{\text{Area of fungal colony}}{\text{Area of wood cutting edge}} x\ 100\%$$

2.6.2. Characteristics of *Ganoderma* sp. Mycelium Growth

Observations were made in a macroscopic way to see the response that occurred from the treatment of root exudate in the field of observation, especially to the presence of growth restriction, lysis and other abnormal colonic growth response.

2.6.7. Data Analysis

The observed data obtained were analyzed using Analysis of Variance (ANOVA) and tested further with 5% BNJ.

3. Results And Discussion

Percentage of growth colonization of mycelium *Ganoderma* sp. treated with exudate of root of ganyong plant by infiltration method through soil was not significantly different from root acute root, ginger and turmeric and controlled root exudate, but it was significantly different from the treatment of root exudate of galangal and inpatient liver (table 1). The turmeric root exudate treatment showed no significant difference with the treatment of all plant root exudate species tested, but was significantly different from the control treatment. The treatment of root exudate of galangal plant by infiltration method through soil was not significantly different from the treatment of turmeric root exudate and lidah mertua, but was significantly different from the root exudate treatment of gan-

yong, garut, ginger and control plants. Treatment with control (sterile water) was not significantly different from root exudate treatment of ganyong, arrowroot and ginger plants, but was significantly different from the treatment of turmeric root exudate, galangal and lidah mertua. Percentage of extent of growth colonization of *Ganoderma* sp. which was treated with root exudate by testing the highest soil infiltration method was in control treatment (sterile water) with mean 89,71% and percentage of growth of colonization of mycelium at *Ganoderma* sp inoculum sp. with the lowest soil infiltration method is in the treatment of root plant root exudate with mean of 59,58% (table 1).

Table 1. Percentage of Growth Colonization of Mycelium Ganoderma sp. the Treatment of Exudate Root Testing by Soil Infiltration Method

Root exudates	Percentage of Colonitation	Characteristics of Mycelium Growth
Lidah Mertua	59,58a	Grows thin at the top, with no brown zone
Galangan	60,81a	Rather thick but uneven, there is a chocolate barrier zone
Turmeric	66,38ab	Thick evenly, there is a chocolate barrier zone
Ginger	83,22bc	Thick evenly, there is no brown zone
Ganyong	85,28bc	Thick but uneven, there is a chocolate barrier zone
Garut	86,97c	Thick evenly, there is no limiting brown zone
Control	89,71c	Evenly spread there is colonization of mycelium at the top and bottom of the rubber stick
KK	18,89	

The numbers followed by the same letter in the same column mean different is not real based on the BNJ test at the 5% level.

Characteristics Growth of Mycelium *Ganoderma* sp.

Growth mycelium *Ganoderma* sp. which colonize rubber wood rods and placed on soil moistened with sterile water is characterized by a thin misy colonization of the central part, but rather thick at the base of the woody piece in contact with the soil. The mycelium grows over a piece of wood and at the top there is a rounded mycelium bulge (Figure 1-K3). The treatment of garut exudate causes the mycelium to grow covering the entire piece of wood and thickening at the top. The treatment of garut exudate causes a thin mycelium colonization to cover the thick and thick logs on the base adjacent to the soil. Seen brown beam which is the limit of mycelium growth (Fig. 1-B2U1). At the bottom of the piece of wood in direct contact with the ground there is a brown beam that grows around the wood cut (Fig. 1-B3U5 and B1U4). Allegedly brown mycelium file is the result of the inhibitory work caused by compounds in the plant arrowroot (Situmorang *et al*., 2007).

The treatment of the galangal exudates resulted in a rather thick colonization of the mycelium in part of the wood cut but the colonization was not found on the top of the wood cut (Fig. 1-E3U3 and E3U5). The limited mycelium growth in the treatment of galangal exudates is thought to be a response to the inhibitory power of the boactive compounds contained there in. According to Salni *et al*. (2013) essential oil and water extract of galangal rhizome efficacious as anti-fungi from phenol class.

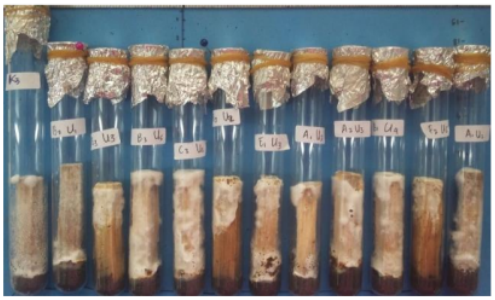

Figure 1. Characteristics of *Ganoderma* sp. in each root exudate treatment

Treatment of ginger root exudate causes mycelium to grow thick but uneven and mycelium growth up to the soil surface. There is a brown mycelium file at the bottom of the piece of wood (Figure 1-C3U1 and C2U2 Treatment of exudate ganyong causes the mycelium to grow only at the top and bottom of wood pieces with a thicker growth at the top. At the center of the mycelium wood pieces grow but very thin and almost invisible (Figure 1-A1U3). Another response to the treatment of exudate ganyong is the uniform growth of the mycelium on the wooden fragments but thickening only on certain parts and found brown beam which is the limit of mycelium growth (Fig. 1-A2U3 and A1U2).

The growth of different fungi on each replication is thought to be due to the influence of the compound contained by the root exudate of the ganyong plant. Treatment of exudate ganyong causes the mycelium to grow on the part of the wood pieces that is on the top only. The mycelium grows infinitely thin and the surface of the mycelium looks coarse (Fig. 1-F2U5). Such a mycelium growth is thought to be caused by the inhibitory power of the compound contained in the plant root exudate of the lidah mertua. According to Situmorang *et al*. (2007) compounds

contained in the plant lidah mertua is an antibiotic compound in the form of abamagenin.

4. Conclusion

The conclusion of this research is the examination with exudate infiltration method through soil, mycelium experiencing growth inhibition especially at treatment of root exudate galangal with percentage 60,81% and lidah mertua with percentage of colonization 59,58%. Ganyong root, garut and ginger root exudates result in the formation of a limiting zone in the form of a brown mycelium pile which may be a mycelium's rejection of the bioactive compound contained in exudates.

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