In vitro antagonistic activity of soil microbes isolated from oil palm to *Ganoderma zonatum*

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Abstract. *Ganoderma zonatum* is the causal agent of basal stem rot of oil palm (*Elaeis guineensis* Jacq) in Riau. The research objective was to explore and select soil microbial antagonists to *Ganoderma zonatum*. Soil samples were taken from the non/rhizosphere of 27 oil palm plants in Kampar and Siak Regency, Riau Province, Indonesia, in 2018-2019. The soil microbes were grown on Potatoes Dextrose Agar for fungus and Tryptic Soy Agar for the bacterium. The antagonist was measured by a dual culture method. The colony diameter of *G. zonatum* the (dual) culture was measured to assess the inhibition potency of the potential microbial antagonists. The soil microbial collected was 138 isolates, including 58 of *Trichoderma* spp. and 80 of bacteria. The inhibition against the isolated soil microbes to *G. zonatum* growth ranged from 46-74% for *Trichoderma* spp. and 0-86% for the bacterium. Seven isolates of *Trichoderma* spp and 13 of bacterial inhibited *G. zonatum* growth >70%, have potential as biocontrol agents. Two isolates that showed the most potential as antagonists, i.e *Trichoderma harzianum* (AC2, rhizosphere) and *Burkholderia gladioli* (N1, non-rhizosphere) could be developed for further as microbial antagonists.

1. Introduction

The major constraint in oil palm (*Elaeis guineensis* Jacq.) cultivation at Riau province is Basal Stem Rot (BSR) disease caused by *Ganoderma zonatum*. The initial symptom of this disease is complicated to detect, but the visible symptom as the rotten basal stem is an advanced infection. The BSR disease in Riau province has reached 20%-30%. It significantly reduced the number of fresh fruit bunch (FFB) and causing 40% crop mortality, especially in third-generation plantations or more replanting areas. Even BSR disease can kill up to 85% of the 25 years old crops plantation population.

*Ganoderma* spp. is a soil-borne fungus. It has many forms of resting stage, for instance, resistant mycelium, basidiospores, chlamydospores, and pseudosclerotia [1]. The fungus that rots and eventually kills oil palm trees has been reported economic losses caused by this pathogen of up to 500 million USD per year [2]. Furthermore, this disease causes 80% of crop damages and mortality in many oil palm plantations in Indonesia and has decreased each infected oil palm area production [1,3].

Initially, the Basal Stem Rot disease in Indonesia is caused only by *G. boninense*. However, later on not only *G. boninense* but also *G. zonatum* was found on oil palm plantations grown in peatlands.
At the same time, three species of *Ganoderma*, namely *G. boninense*, *G. zonatum*, and *G. miniatothecium*, are associated with stem rot disease in Sarawak [5].

Biological control by using antagonist microbe against pathogen is an alternative control strategy to reduce damaging plants. Bacteria and fungus are examples of effective antagonist microbes. Antagonist microbes inhibit or parasitize pathogens by producing antibiotics or competing for nutrients [6]. Several promising biological agents, mainly *Trichoderma harzianum*, *T. viride*, *Gliocladium viride*, *Pseudomonas fluorescens*, and *Bacillus* sp. to control *Ganoderma* [1]. *Trichoderma* is an excellent biocontrol model due to its high ability to multiply, spread by itself, and easy to be isolated and cultured [7].

The objective of this research is to explore, collect and select antagonist microbes against *G. zonatum*. We focused on exploring *Trichoderma* spp. and bacteria from the rhizosphere and non-rhizosphere of the mild intensity of BSR and healthy indigenous Riau oil palm plantations.

2. Materials and methods

2.1. Exploration *Trichoderma* spp. and bacterial

Soil samples were collected from the oil palm rhizosphere and non-rhizosphere (soil around root system) from two areas of the mild intensity of BSR oil palm plantation at Cinta Damai and Pelambaian villages, Tapung Ilir Sub-district, Kampar Regency, and one healthy plantation from the Dayun village, Dayun Sub-district, Siak Regency. Three representative gardens in each village were selected, then three oil palm trees of the productive age were chosen. In the garden with the BSR disease, the soil samples were taken from the healthy oil palm around the diseased plants. One composite soil sample consisting of three plants and four soil samples per plant was taken.

*Trichoderma* spp. and bacterial isolations were made at the Plant Pathology Laboratory in the Indonesian Spice and Medicinal Crops Research Institute. The isolation of *Trichoderma* spp. and bacterial used serial dilution to $10^3$ and $10^4$ plates respectively. Potatoes Dextrose Agar (PDA) added with Rose Bengal antibiotic was used to culture *Trichoderma* spp., whereas Tryptic Soy Agar (TSA) medium was used to culture bacterial. All isolates of *Trichoderma* spp. and bacteria that appeared on the four day-old cultures after incubation were isolated and transferred to PDA slant media and incubated at room temperature.

2.2. Selection *Trichoderma* spp. and bacteria isolates as an antagonist to *G. zonatum*

Selection for antagonist agent used a dual culture method on PDA medium. First, a piece of diameter 0.5 cm of agar grown with fungal mycelia was taken from the surface of the PDA medium two days before the candidate antagonists. Then the candidate antagonist was inoculated at the same Petri Dish, at a distance of five cm to the *G. zonatum*. Tree replications for each candidate antagonist and incubated at room temperature. The growth radius of *G. zonatum* toward the candidate antagonist was measured after five days of incubation at room temperature. Percentage of inhibition calculation used the formula of Fokkema [8]:

$$I = \frac{r_1 - r_2}{r_2} \times 100\%$$

The formula indicated that $I=$ percentage of inhibition; $r_1=$ the radius of the *G. zonatum* colony without the candidate antagonist (control); and $r_2=$the radius of the *G. zonatum* colony with the candidate antagonist.

3. Result and discussion

The study collected 58 isolates of *Trichoderma* spp. from the rhizosphere and non-rhizosphere of healthy Riau oil palm plants. The percentage of inhibition growth of *G. zonatum* by *Trichoderma* spp. ranged between 46% – 74%. Only one isolate has an inhibitory growth of 46%, the others were above 50% (Table 1). Based on the observation, all the isolates of *Trichoderma* grew very fast. Contact mycelial between *Trichoderma* (5 isolates) and *G. zonatum* occurred two days after incubation.
Examination under the microscope showed that there were an abnormality and lysis hyphae of *G. zonatum* from the affected zozone. Some isolates of *Trichoderma* covered the colony of *G. zonatum* after five days of incubation at room temperature. The highest percentage inhibition (74%) by *Trichoderma* spp. was shown by isolate AC2. The AC2 fungus was identified as *Trichoderma harzianum* (Figure 1). *Trichoderma* as bioagent against plant-pathogen consist of competition for nutrient and space, antibiotics, parasitism, and induction of systemic resistance inplants [9].

*Trichoderma* belonging to the order of Hypocreales and the family Hypocreaceae, commonly known as a fungal inhabitant in the rhizosphere, and has well-known as potential biological control of the soil-borne disease. *T. harzianum* and *T. viride* could significantly reduce *Ganoderma* incidence on oil palm [1]. However, *Trichoderma* could only protect the oil palm at the early stage of *Ganoderma* infection, and it cannot cure severely infected palm [10]. The application of *Trichoderma* as a single or mixture species as a Biological Control Agent (BCA) to *Ganoderma* disease, caused disease infestation slower than control (without *Trichoderma*) in palm oil seedling [11].

**Table 1.** The inhibition of *G. zonatum* colony growth by *Trichoderma* isolated from oil palm trees from Riau Province

| No | Isolate | Inhibiton (%) | Origin of Isolate |
|----|---------|---------------|-------------------|
| 1  | AA1     | 60            | Rhizosphere       |
| 2  | AC1     | 61            | Rhizosphere       |
| 3  | AF3     | 63            | Rhizosphere       |
| 4  | AC3     | 72            | Rhizosphere       |
| 5  | AB1     | 67            | Rhizosphere       |
| 6  | AD2     | 71            | Rhizosphere       |
| 7  | AD1     | 63            | Rhizosphere       |
| 8  | AG3     | 72            | Rhizosphere       |
| 9  | AG2     | 68            | Rhizosphere       |
| 10 | AF1     | 72            | Rhizosphere       |
| 11 | AF2     | 68            | Rhizosphere       |
| 12 | AC2     | 74            | Rhizosphere       |
| 13 | A1      | 58            | Rhizosphere       |
| 14 | A2      | 64            | Rhizosphere       |
| 15 | A4      | 63            | Rhizosphere       |
| 16 | A3      | 64            | Rhizosphere       |
| 17 | M4      | 63            | Rhizosphere       |
| 18 | L4      | 63            | Rhizosphere       |
| 19 | K4      | 70            | Rhizosphere       |
| 20 | K2      | 63            | Rhizosphere       |
| 21 | J3      | 62            | Non-rhizosphere   |
| 22 | J1      | 64            | Non-rhizosphere   |
| 23 | F1      | 69            | Non-rhizosphere   |
| 24 | B1      | 53            | Non-rhizosphere   |
| 25 | B2      | 63            | Non-rhizosphere   |
| 26 | M2      | 57            | Non-rhizosphere   |
|   |   |   |   |
|---|---|---|---|
| 27 | E1 | 53 | Non-rhizosphere |
| 28 | C2 | 50 | Non-rhizosphere |
| 29 | C3 | 55 | Non-rhizosphere |
| 30 | D1 | 51 | Non-rhizosphere |
| 31 | D2 | 46 | Non-rhizosphere |
| 32 | P1 | 53 | Non-rhizosphere |
| 33 | Q4 | 56 | Non-rhizosphere |
| 34 | Q2 | 66 | Non-rhizosphere |
| 35 | Q1 | 59 | Non-rhizosphere |
| 36 | E3 | 64 | Non-rhizosphere |
| 37 | N3 | 56 | Non-rhizosphere |
| 38 | N1 | 59 | Non-rhizosphere |
| 39 | O4 | 59 | Non-rhizosphere |
| 40 | O3 | 61 | Non-rhizosphere |
| 41 | O2 | 56 | Non-rhizosphere |
| 42 | N2 | 54 | Non-rhizosphere |
| 43 | D3 | 61 | Non-rhizosphere |
| 44 | B3 | 66 | Non-rhizosphere |
| 45 | C1 | 59 | Non-rhizosphere |
| 46 | N5 | 62 | Non-rhizosphere |
| 47 | Q3 | 61 | Non-rhizosphere |
| 48 | Q5 | 58 | Non-rhizosphere |
| 49 | E2 | 61 | Non-rhizosphere |
| 50 | P3 | 70 | Non-rhizosphere |
| 51 | O5 | 61 | Non-rhizosphere |
| 52 | R2 | 62 | Non-rhizosphere |
| 53 | P4 | 60 | Non-rhizosphere |
| 54 | P5 | 63 | Non-rhizosphere |
| 55 | O1 | 58 | Non-rhizosphere |
| 56 | N4 | 61 | Non-rhizosphere |
| 57 | I2 | 63 | Non-rhizosphere |
| 58 | E  | 62 | Non-rhizosphere |
The growth of bacterial differed from that of *Trichoderma*. The selected bacterial as BCA against *G. zonatum* were conducted by screening for their antagonistic activity using PDA (4 bacterial candidates against *G. zonatum*) (Figure 2). The excellent or potential bacteria that have an inhibitory effect on the growth of *G. zonatum* were chosen for further testing the dual culture method to get the value of percentage inhibition.

The study collected eighty bacteria isolates from the rhizosphere and non-rhizosphere of healthy oil palm (Table 2) included twenty-three bacteria isolates from the rhizosphere, and only one isolate can inhibit the growth of *G. zonatum* about 78%, while the others did not. Fifty-seven isolates of bacteria were isolated and collected from the non-rhizosphere of a healthy oil palm. Twelve isolates inhibited the growth of *G. zonatum* ranged between 78–86%, while the others did not. All antagonist bacteria displayed a zone inhibition. The wide of zone inhibition were variation depends on the bacteria isolated. Bacterium isolate N1 showed the highest percentage inhibition to *G. zonatum*, 86%, and we identified N1 as *Burkholderia gladioli* (Figure 3). The further observation under a microscope, the hyphae tips were abnormal or lysis compare to control. *B. gladioli* strain BBB-01 produced volatile compounds such as hydrogen cyanide that can suppress the growth of *Magnaporthe oryzae* (dual culture on PDA) [12]. The observation using a scanning electron microscope (SEM) confirmed *B. gladioli* strain BBB-01 that the mycelia of *M. oryzae* showed fragmented, while the control hyphae were intact [12]. That result indicated that *B. gladioli* BBB-01 not only slowed down the fungal growth, also killed the fungal-pathogen. *B. gladioli* produce extracellular hydrolytic enzymes such as chitinase, protease, cellulase, amylase, and glucanase affecting fungal growth and killing it.
A wide range of antagonistic, gram-positive, and gram-negative genera such as \textit{Bacillus}, \textit{Burkholderia}, \textit{Enterobacter}, and \textit{Pseudomonas} could inhibit the colony growth of \textit{G. boninense} in the laboratory and also success to control the fungus in the field trials [13]. Genus \textit{Burkholderia} is a potential biocontrol agent in agriculture. Several strains of \textit{Burkholderia} are antagonistic against phytopathogenic fungi, besides plant growth-promoting rhizobacteria [14]. The genus \textit{Burkholderia} is common as soil inhabitants, distributed in soil pH 9-12, and potential as a biological control agent in agriculture [15].

\begin{table}[h]
\centering
\caption{The inhibition of \textit{G. zonatum} colony growth by soil bacteria isolated from oil palm trees from Riau Province}
\begin{tabular}{lll}
\hline
No & Isolate & Inhibition (%) & Origin of Isolate \\
\hline
1 & AG1 & 0 & Rhizosphere \\
2 & AG2 & 0 & Rhizosphere \\
3 & AG3 & 0 & Rhizosphere \\
4 & AG4 & 0 & Rhizosphere \\
5 & AF1 & 0 & Rhizosphere \\
6 & AF2 & 0 & Rhizosphere \\
7 & AF3 & 0 & Rhizosphere \\
8 & AD1 & 0 & Rhizosphere \\
9 & AD2 & 0 & Rhizosphere \\
10 & AD3 & 0 & Rhizosphere \\
11 & AC1 & 0 & Rhizosphere \\
12 & AC2 & 0 & Rhizosphere \\
13 & AB1 & 0 & Rhizosphere \\
14 & AB2 & 0 & Rhizosphere \\
15 & AB3 & 0 & Rhizosphere \\
16 & AA1 & 0 & Rhizosphere \\
17 & AA2 & 0 & Rhizosphere \\
18 & AA3 & 0 & Rhizosphere \\
19 & AE1 & 0 & Rhizosphere \\
20 & AE2 & 0 & Rhizosphere \\
21 & A1 & 0 & Rhizosphere \\
22 & A3 & 0 & Rhizosphere \\
23 & A4 & 78 & Rhizosphere \\
24 & Q4 & 0 & Non-rhizosphere \\
25 & Q3 & 0 & Non-rhizosphere \\
26 & Q2 & 0 & Non-rhizosphere \\
27 & Q1 & 0 & Non-rhizosphere \\
28 & P4 & 0 & Non-rhizosphere \\
29 & P3 & 0 & Non-rhizosphere \\
30 & P2 & 0 & Non-rhizosphere \\
31 & P1 & 0 & Non-rhizosphere \\
\hline
\end{tabular}
\end{table}
|   |   |   | Non-rhizosphere |
|---|---|---|-----------------|
| 32 | O4 | 0 | Non-rhizosphere |
| 33 | O3 | 0 | Non-rhizosphere |
| 34 | O2 | 0 | Non-rhizosphere |
| 35 | O1 | 0 | Non-rhizosphere |
| 36 | N4 | 84 | Non-rhizosphere |
| 37 | N3 | 0 | Non-rhizosphere |
| 38 | N2 | 0 | Non-rhizosphere |
| 39 | N1 | 86 | Non-rhizosphere |
| 40 | M4 | 83 | Non-rhizosphere |
| 41 | M3 | 0 | Non-rhizosphere |
| 42 | M2 | 0 | Non-rhizosphere |
| 43 | M1 | 81 | Non-rhizosphere |
| 44 | L4 | 0 | Non-rhizosphere |
| 45 | L3 | 0 | Non-rhizosphere |
| 46 | L2 | 0 | Non-rhizosphere |
| 47 | L1 | 0 | Non-rhizosphere |
| 48 | K4 | 0 | Non-rhizosphere |
| 49 | K3 | 0 | Non-rhizosphere |
| 50 | K2 | 0 | Non-rhizosphere |
| 51 | K1 | 0 | Non-rhizosphere |
| 52 | J1 | 84 | Non-rhizosphere |
| 53 | J2 | 83 | Non-rhizosphere |
| 54 | J3 | 0 | Non-rhizosphere |
| 55 | J4 | 0 | Non-rhizosphere |
| 56 | K2 | 0 | Non-rhizosphere |
| 57 | B3 | 0 | Non-rhizosphere |
| 58 | B2 | 0 | Non-rhizosphere |
| 59 | B1 | 0 | Non-rhizosphere |
| 60 | C2 | 0 | Non-rhizosphere |
| 61 | C1 | 0 | Non-rhizosphere |
| 62 | D1 | 0 | Non-rhizosphere |
| 63 | D2 | 78 | Non-rhizosphere |
| 64 | E4 | 84 | Non-rhizosphere |
| 65 | E5 | 0 | Non-rhizosphere |
| 66 | E1 | 83 | Non-rhizosphere |
| 67 | E3 | 0 | Non-rhizosphere |
| 68 | F1 | 80 | Non-rhizosphere |
| 69 | F2 | 0 | Non-rhizosphere |
| 70 | F3 | 0 | Non-rhizosphere |
| 71 | G2 | 0 | Non-rhizosphere |
| 72 | G1 | 0 | Non-rhizosphere |
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|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 73 | H3 | 82 | Non-rhizosphere |
| 74 | H2 | 85 | Non-rhizosphere |
| 75 | H1 | 0  | Non-rhizosphere  |
| 76 | H4 | 0  | Non-rhizosphere  |
| 77 | I3 | 0  | Non-rhizosphere  |
| 78 | I4 | 0  | Non-rhizosphere  |
| 79 | I1 | 0  | Non-rhizosphere  |
| 80 | I2 | 0  | Non-rhizosphere  |

Note: Inhibition 0 mean: the colony of *G. zonatum* grown covered the bacteria colony (no antagonism)

Figure 3. Dual culture between *G. zonatum* and N1 bacteria isolate, after five days incubation. Upper side (A and B), the bottom side (Cand D). The colony of *G. zonatum* in control plate (A and D).

4. Conclusion
The microbial antagonist against *Ganoderma zonatum* collected and isolated from rhizosphere and non-rhizosphere of healthy oil palm plantation in Riau included 58 isolates of *Trichoderma* spp. and 13 isolates of bacteria. Amongst those isolates, AC2 isolate of *Trichoderma* (*T. harzianum*) from rhizosphere and one bacterium isolate N1 (*B. gladioli*) from non-rhizosphere showed the highest ability inhibition, i.e., 74% and 86%, respectively. Therefore, both isolates are the best candidate as potential bioagents for *G. zonatum* control.

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