The effectiveness of acid-tolerant antagonists in the control of oil palms root necrotic caused by *Ganoderma* sp. in peat soils

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Abstract. Oil palm is the main plantation commodity in Indonesia. In the last two decades, Indonesian palm oil has always been associated with global warming because most of its plantation areas are located on peatlands and the cultivation method is considered not environmentally friendly. One of the problems of oil palm cultivation on peatlands is stem rot disease caused by the fungi *Ganoderma* sp. This disease is transmitted through root contact. There is no effective way to control this disease. Biological control is an alternative method that is environmentally friendly. This study aims to determine the effectiveness of acid-tolerant antagonists in controlling oil palm root necrotic on peat soils. The study tested 5 isolates of the acid-tolerant antagonists in reducing oil palm root necrosis caused by *Ganoderma* on peat soils in experimental gardens. The results showed that the acid-tolerant antagonists were effective in controlling root necrosis in oil palm seedlings on peat soils. Its effectiveness reached 16.67%. Differences in peat soil pH and peat maturity did not affect the effectiveness of root necrotic control caused by *Ganoderma* sp. These results indicate that using acid-tolerant antagonistic fungi can help reduce *Ganoderma* sp attacks on oil palms on peat soils.

1 Introduction

Oil palm (*Elaeis guineensis* Jacq.) is an important contributor to global vegetable oil production. In the last two decades, Indonesian palm oil has always been associated with global warming because most of its plantation areas are located on peatlands and the cultivation method is considered not environmentally friendly. Indonesia, the largest palm oil-producing country in the world, has a large area of oil palm plantations in peatlands, which is 2.046 million ha, equivalent to 14.58% of the total area of Indonesian palm oil, which until 2017 was 14.03 million ha [1, 2, 3]. A serious problem of oil palm plantations in peatlands is the high incidence of basal stem rot (BSR) disease caused by *Ganoderma* sp.

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This disease is transmitted through root contact and causes palm death [4, 5]. Nowadays, it is stated that BSR disease is a very threatening disease for the sustainability of oil palm plantations on peatlands due to its increasing intensity [6].

Until today, there are no effective methods to control BSR disease in peatlands [7, 8]. Biological control is an alternative control method that is the focus of current development [9]. However, biological control of BSR disease in peatlands has not been widely reported. Tropical peatlands in the lowlands of Kalimantan have an average pH of 3.3 - 4.3 [10]. Allegedly, low soil pH causes the antagonist to be unable to develop or causes its functional ability as an antagonist to not function properly. Based on the results of preliminary research, it is known that in the peatlands of West Kalimantan there are acid-tolerant fungi and bacteria which are also capable of acting as an antagonist to Ganoderma in vitro. Some fungi are known to be able to grow at pH 2, although their functional ability as an antagonist can only be active at pH 3 – 6 [11]. Likewise, bacteria are known to be able to grow at pH 2, even though the growth rate has decreased significantly [12]. However, this ability has not been tested in the field. This research was conducted to determine the effectiveness of the use of indigenous acid-tolerant antagonists in the control of oil palm root necrotic in peat soils.

2 Materials and methods

The research was conducted at the Plant Disease Laboratory and experimental garden at the Universitas Tanjungpura, Pontianak, Indonesia (0°3’27.875” N, 109°20’54.3 E).

2.1 Experimental design

The experiment was carried out in natural peat soil, sterile peat soil, peat soil that was pH-changed, and mineral soil as a comparison. The peat soils used were hemic peat soil and sapric peat soil. A total of 3 isolates of Ganoderma-antagonistic fungi, namely isolates 13EJ15, B3J19, and E4J8 and 2 isolates of Ganoderma-antagonistic bacteria, namely isolate E4B6 and isolate E2B12 were tested in this study. Each antagonist was obtained from oil palm plantations on West Kalimantan peat land [11,12]. Each antagonist isolate was applied singly and mixed isolates. Ganoderma sp. from peatlands, namely Ganoderma G301 isolate was used as the test pathogen. As a comparison, positive and negative control treatments were used in this experiment. All treatments were arranged in factorial completely randomized design and replicated 3 times.

2.2 Peat soil preparation and oil palm seedlings

The soil was made friable and weighed in air-drying condition, and as much as 5 kg of peat soils were placed in a 30 x 40 cm polyethylene bag (polybag). Mineral soils were measured by volume to equal with the volume of peat soil in polybag in air-drying conditions due to the difference of their bulk density. Sterile peat soil was obtained by sterilization with the steaming method for 6 hours. To obtain a different peat pH, the peat soil was added with dolomite (CaMg(CO3)2) at a dose of 12.5 g/kg and 25 g/kg. Soil pH was measured at the beginning of medium preparation, 2 weeks after liming by the dolomite, and at the end of the experiment [13]. This research used commercial oil palm seeds of Marhat variety from PPKS Medan, Indonesia. Oil palm sprouts were grown in 10 x 20 cm polybags containing a mixture of peat soil and mineral soil in a 1:1 ratio. Before it was used, the medium was sterilized by steaming for 6 hours. Seedlings were cultivated for two months under 50% shade.

2.3 Preparation of inoculum and inoculation of oil palm seedlings with Ganoderma

The Ganoderma inoculum was prepared by growing the Ganoderma G301 onto 6 x 6 x 12 cm rubber woodblocks. The inoculum was ready to use after the mycelium has covered all the rubber woodblock surfaces [14]. Ganoderma inoculation of oil palm seedlings carried out
by rubber wood blocks that have been overgrown with Ganoderma mycelium were buried in
the soil in polybags 1 week before the oil palm seedlings were planted at a depth of 10 cm
[15]. The 2-month old oil palm seedling was planted on the medium, placed on a rubber
woodblock and the main root was made sure to sit on the rubber wood block, and then
backfilled. Oil palm seedling was then cultivated for 24 weeks.

2.4 Preparation and inoculation of antagonists onto peat soil medium
The antagonistic fungi and bacteria were rejuvenated. The antagonistic fungal inoculum was
prepared in a corn-water medium (1:1 ratio) for 2 weeks. The bacterial inoculum was
prepared in a bacterial suspension with a minimum density of $10^8$ cfu/ml. The antagonist
inoculum was given into the medium according to the predetermined treatment. Fungal
isolates were buried and mixed into the planting medium 3 days after Ganoderma inoculation.
Antagonistic bacteria were given in a bacterial suspension by soil drenching a day before
planting oil palm seedlings.

2.5 Observation of Root Necrotic Intensity
At the 6th month, the roots are removed by dismantling the growing medium in the pots. The
necrotic symptom on the roots was scored (Table 1) [16].

<table>
<thead>
<tr>
<th>Score</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No necrotic symptoms</td>
</tr>
<tr>
<td>1</td>
<td>$1 &lt; x &lt; 20%$ necrotic progress less than 20%</td>
</tr>
<tr>
<td>2</td>
<td>$20 \leq x &lt; 40%$ necrotic develops $20 - 40%$</td>
</tr>
<tr>
<td>3</td>
<td>$40 \leq x &lt; 60%$ necrotic develops $40 - 60%$</td>
</tr>
<tr>
<td>4</td>
<td>$x &gt; 60%$ necrotic develops more than 60%</td>
</tr>
</tbody>
</table>

$$NI = \frac{\sum (k \times nk)}{Z \times N} \times 100\%$$ (1)

NI = Necrotic intensity
nk = number of plants with a score of k (k=0,1,2,3,4)
k = Score used.
Z = Highest score
N = Number of plants observed

3 Results and discussion
Based on the observation of root necrosis, except in the treatment of B3J19 isolate and the
treatment of consortium of bacteria, the intensity of root necrosis was higher than the control
treatment. The intensity of necrosis was varied, ranging from 16.67% to 50%. The lowest
average of intensity for various treatments of hemic peat soil medium was obtained in the
treatment of B3J19 isolate, i.e. 14.59%. The highest average of intensity was obtained in the
consortium treatment of fungi and bacteria. Whereas in sapric peat soil, all the acid-tolerant
antagonist treatments resulted in a lower intensity of root necrosis than the control treatment
except for the B3J19 isolate fungus antagonist treatment. The lowest average intensity on
various treatments of sapric peat soil medium was obtained in the E2B12 isolate treatment
(25%), and the highest was in the B3J19 isolate treatment (Figure 1).

Observations on the roots after the demolition of the oil palm seedling growth medium
also demonstrated that root infection by pathogenic Ganoderma only occurred in secondary
roots. These results are different from those reported before where the infection occurred at
the primary root [18]. This is presumed to be related to differences in the aggressiveness of
the Ganoderma used in this experiment. Ganoderma was known to have different levels of aggressiveness [19, 20, 21].

Figure 1. The effect of acid-tolerant antagonists on the intensity of necrosis in oil palm seedling roots in hemic peat (top) and sapric peat (bottom).

3.1 Effect of the peat soils on the effectiveness of acid-tolerant antagonists

Based on observations on root necrosis, acid-tolerant antagonists were only able to reduce the root necrosis symptoms by 16.67%. This result was lower than that of sapric peat soil which 52.08%. Differences in peat pH also did not affect the effectiveness of controlling root necrosis in hemic peat soil, but it did affect the effectiveness in sapric peat soil (Table 2).

Table 2. Effect of peat soils treatment on the average effectiveness of acid-tolerant antagonists.

<table>
<thead>
<tr>
<th>Growth medium</th>
<th>pH medium (pH)</th>
<th>Root necrosis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural hemic peat soil</td>
<td>4.0</td>
<td>0</td>
</tr>
<tr>
<td>Hemic peat soil + dolomite 12.5 g/kg</td>
<td>2.9 – 3.5</td>
<td>0</td>
</tr>
<tr>
<td>Hemic peat soil + dolomite 25 g/kg</td>
<td>4.1 – 5.3</td>
<td>0</td>
</tr>
<tr>
<td>Sterile hemic peat soil</td>
<td>6.4 – 6.3</td>
<td>28.12</td>
</tr>
<tr>
<td>Natural sapric peat soil</td>
<td>2.9 – 3.3</td>
<td>52.08</td>
</tr>
<tr>
<td>Sapric peat soil + dolomite 12.5 g/kg</td>
<td>2.7 – 3.4</td>
<td>0</td>
</tr>
<tr>
<td>Sapric peat soil + dolomite 25 g/kg</td>
<td>5.7 – 5.5</td>
<td>32.0</td>
</tr>
<tr>
<td>Sterile sapric peat soil</td>
<td>5.7 – 5.5</td>
<td>32.0</td>
</tr>
</tbody>
</table>
Ganoderma-antagonistic fungi isolates from peatlands have different characteristics and effectiveness. The B3J19 isolate, in vitro, has an ideal growth range at pH 4-5 [6]. However, in this control effectiveness test, it was only relatively effective on hemic peat soils. The 13EJ15 isolate, in vitro, has an ideal growth range at pH 3-6 and is relatively effective in almost all medium conditions. Meanwhile, E4J8 isolate which had an ideal growth range at pH 3-5 in vitro, was only effective on natural sapric peat. The use of a consortium of isolates also did not affect the effectiveness compared to single isolates. These results indicate that the more tolerant an isolate is to various pH levels of the growing medium, the more effective the isolate is in various peat soil environments. Based on the in vitro test results, most of the antagonistic fungi and bacteria from West Kalimantan peatlands were able to grow on a medium with a pH spectrum of 3-6 [6]. In this experiment, the addition of dolomite to hemic peat soil resulted in a pH ranging from 4.1 to 6.4. This pH range is thought to be tolerated by the antagonists, although the higher the resulting pH causes a decrease in its ability.

Meanwhile, the difference in pH in sapric peat soil which affects the control effectiveness is thought to be related to differences in the chemical characteristics of the peat soil as a result of the addition of dolomite. The decomposition rate of organic matter in sapric peat soil was higher than that of hemic peat, so the addition of the same amount of dolomite could have a relatively different impact. This could be seen at the pH level after the incubation period which tends to be higher in sapric peat soil than in hemic peat soil (Table 2). This is thought to also cause a change in chemical composition that is different from that of hemic peat soil. The addition of dolomite lime donates Ca²⁺ (or Ca²⁺ and Mg²⁺) alkaline cations into the soil with the result that it could replace the position of H⁺ and or Al³⁺ ions contained in the soil absorption complex. A decrease in the concentration of H⁺ ions increases OH⁻ ion which causes an increase in soil pH and an increase in soil saturation [22]. However, an increase in pH that was too high causes the decomposition process to be too fast [23]. A pH level that was close to neutral would damage the natural properties of the peat so that it can change the chemical reactions and the availability of nutrients in the soil [24]. Increasing pH in peat has been shown to increase the activity of phenol oxidase enzymes, as well as to increase the abundance of actinobacteria and fungi [25]. Phenolic acid was allelopathic for plants [26]. These phenolic acids have the effect of inhibiting plant root development and nutrient supply in the soil [27, 28]. Generally, the impact is also accumulative [29].

4 Conclusion
The acid-tolerant antagonists tested in this study were effective in controlling Ganoderma in oil palm seedlings on peat soil. The effectiveness in reducing root necrosis is 16.67%. The difference in the pH of the peat soil in this study did not affect the effectiveness. Further studies are needed to determine environmental factors in peat soil other than pH that affect the effectiveness of control by acid-tolerant antagonists.

References
7. CG Fee. The Planter. 87. 1022. 325-339 (2011)


