

# Effectiveness of acid-tolerant antagonist-based biofungicide in the control of basal stem rot disease caused by *ganoderma* in oil palm seedlings on peat soil

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**Abstract.** Oil palm is an important plantation commodity in Southeast Asia. As the world's largest palm oil producer, Indonesia has always been associated with global warming because most of its plantation areas are on peatlands and their management is considered not environmentally friendly. In addition to land fertility problems, a serious problem in peatland oil palm cultivation is basal stem rot disease caused by the *Ganoderma* fungus. Until now, there has been no effective method to control this disease. Biological control is an environmentally friendly alternative method and is the focus of current development, but for peatland conditions, its development is limited by the ultra-low peat pH factor. This study aims to determine the effectiveness of biofungicides based on acid-tolerant antagonists in controlling basal stem rot disease in oil palm seedlings on peat soil. The study was conducted by testing a bio-fungicide formulation in powder formulation containing 3 isolates of acid-tolerant antagonists, namely *Trichoderma viride* B3J19, *Trichoderma harzianum* E4J8, and *Trichoderma harzianum* 13EJ15. Testing was carried out on oil palm seedlings in greenhouses and fields. The results in the greenhouse showed that after six months of observation, bio-fungicides were able to reduce the intensity of the disease by 13.34% with 1 application, while 3 applications were 66.67% compared to the positive control. Meanwhile, in the field, the use of bio-fungicides with 1 application could reduce the intensity of disease by 27.29%, while 3 applications were 90.90%. These results indicate that bio fungicides in powder formula containing acid-tolerant antagonists have the potential to be used as biological control agents for oil palm basal stem rot disease caused by *Ganoderma* in peatlands.

## 1 Introduction

Oil palm (*Elaeis guineensis* Jacq.) is the world's most important and productive vegetable oil crop [1]. Since 2014, Indonesia has become the largest producer of palm oil with the largest plantation area in the world. In 2023, 44 million tons of CPO were produced from a plantati

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on area of 16,833,985 ha [2,3]. Oil palm plantations also utilize peatlands. In 2015, of the total world oil palm plantations, 3.1 million ha were peatlands. Indonesia has the largest percentage, gaining 14.58% of the total area of oil palm plantations (around 2.05 million ha) [4, 5]. In Kalimantan, there are around 730,750 ha of oil palm plantations on peatlands, of which 42.33% (309,320 ha) are in West Kalimantan (Kalbar) [5]. This condition has caused Indonesia to be associated with global warming and climate change, especially related to the unwise management of its peatlands [6]. Therefore, technological innovation is needed for environmentally friendly oil palm cultivation methods on peatlands.

Until recently, the most threatening problem for oil palm productivity on peatlands was stem rot disease caused by *Ganoderma*. On peatlands, the intensity of *Ganoderma* attacks increases and appears earlier (8 years of age). The emergence of symptoms is more intensive, with many symptoms of upper stem rot (BBA) [7]. Losses in several plantations in Indonesia reached 50% [8], even in the peatlands of West Kalimantan reaching 70%, and almost 95% of oil palm deaths are caused by *Ganoderma* attacks. Until now, there has been no effective way to control *Ganoderma* on peatlands. Several methods have been developed such as mounding, "stem operations", making isolation ditches, sanitation of plant environments, use of fungicides on stems and soil, and development of resistant plants, but the results are still varied and not satisfactory [7-12]. Losses caused by *Ganoderma* are estimated to exceed IDR 3.8 trillion annually at the national level. Assuming an attack of only 1%, national yield losses will reach 630,431 tons of CPO/year (assuming an average productivity of 3,745 tons/ha with an area of 16,833,985 ha [13].

Biological control is an alternative method of controlling *Ganoderma* which is currently the focus of development [14]. In addition to being low-cost and environmentally safe, biological methods are the most appropriate method considering that *Ganoderma* is predominantly transmitted through the roots [15]. Biological control also plays an important role in the development of environmentally friendly oil palm, as required by palm oil certification institutions such as the Indonesian Sustainable Palm Oil (ISPO) and the Roundtable on Sustainable Palm Oil (RSPO) [16,17]. However, until now biological control of basal stem rot (BSR) disease in peatlands has not been developed. Research on biological control of BPB disease has primarily been conducted in non-peatland areas, and almost all biological control agents identified originate from non-peatland environments. Additionally, there are no commercial biological fungicides specifically designed for use in peatlands.

The most feasible approach is to find effective indigenous antagonists from peatlands and develop a way to utilize them to suit the characteristics of peatlands. For this purpose, in several preliminary studies, several Indigenous antagonists from peatlands have been obtained that have characteristics as acid-tolerant fungi and bacteria and are effective in controlling *Ganoderma* in vitro and greenhouse testing. The results of the preceding study showed that there were 27 isolates of acid-tolerant fungi from the genera *Gliocladium* and *Trichoderma* that had the potential to be *Ganoderma* control agents with an inhibitory power ranging from 65% - 100%. Meanwhile, from the bacterial group, 25 isolates were found to have the potential to be *Ganoderma* antagonists, although their ability decreased drastically at pH below 4. In the biological control test in the greenhouse, it was known that three isolates of antagonistic fungi (on average) can reduce the symptoms of *Ganoderma* attacks by up to 58%. However, these isolates have not been tested in the field.

Based on the ability of the isolates in these tests, research has been conducted to develop a bio-fungicide formulation to control *Ganoderma* on oil palms in peatlands. A bio-fungicide formulation in powder form containing active ingredients of 3 species of antagonistic fungi that are acid-tolerant has been developed, namely *Trichoderma viride* B3J19, *Trichoderma harzianum* E4J8, and *Trichoderma harzianum* 13EJ15. However, this formulation has not been tested in the field. This study aims to test the bio-fungicide based on acid-tolerant antagonist's effectiveness in controlling BSR disease due to *Ganoderma* on oil palm

seedlings in peat soil. This study is important to determine the potential of the bio-fungicide formulation in powder form based on acid-tolerant antagonists in controlling stem rot disease caused by *Ganoderma* in peatlands.

## **2 Materials and Methods**

The research was conducted at the experimental garden, Faculty of Agriculture, Tanjungpura University, Pontianak, West Kalimantan, Indonesia (0°3'27.875"N, 109°20'54.3"E) from September 2023 to March 2024.

### **2.1 Research design**

The effectiveness of bio-fungicide formulation in controlling BSR disease was tested on oil palm seedlings planted in peat soil as a medium. The test was conducted in 2 environmental conditions, namely semi-controlled environmental conditions in pots, and natural peat soil environmental situations. The peat soil used in this study was hemic peat. Bio-fungicides were tested on oil palm seedlings that had been inoculated with *Ganoderma* in 3 treatments, namely once application, three time applications, and no application (positive control). As a comparison, negative control treatments were made. The positive control was oil palm seedlings inoculated with *Ganoderma* and no control with bio-fungicide was carried out. The negative control was oil palm seedlings that were not inoculated with *Ganoderma* and not given bio-fungicide. The test pathogen used was a *Ganoderma* isolate from peatland that had been previously isolated, namely the *Ganoderma* G301 isolate. This isolate was isolated from the *Ganoderma* fruiting body origin from a diseased oil palm in peatlands in Kubu Raya Regency, West Kalimantan. All treatments were arranged in a completely randomized design and replicated 3 times.

### **2.2 Preparation of peat soil in pots**

Peat soil is prepared with the following steps. To obtain a homogeneous aggregate size, the soil was made friable, stirred, and sieved using a sieve with a 2 cm diameter hole. The peat soil was weighed in an air-dried condition, and 5 kg of peat soil was placed in a 30 x 30 cm polyethylene plastic (polybag). The soil was incubated in a closed plastic tarpaulin to avoid direct sunlight. Soil pH was measured at the beginning of medium preparation and the end of the study.

### **2.3 Preparation of peatland**

The land used was peat land located in a 14-year-old oil palm plantation area. Before use, the land was cleared of weeds and leaf debris, and the surface was leveled. A drain was made around the land to prevent flooding. The research location was also fenced to avoid disturbances from wild animals.

### **2.4 Preparation of *Ganoderma* inoculum**

*Ganoderma* inoculum was prepared by growing *Ganoderma* on rubber wood. The rubber wood used was cylindrical with a 5-6 cm diameter and 8 cm length. Before use, the rubber wood was soaked for 24 hours, washed clean, wrapped in a heat-resistant plastic bag, and autoclaved for 1 hour. After being left overnight, the rubber wood was autoclaved again for 1 hour. After cooling, the rubber wood was inoculated with *Ganoderma* which was grown

previously in corn-water media (1:1 ratio) for 2 weeks. The rubber wood was ready to use after 2 months or when the entire surface had been covered with *Ganoderma* mycelium.

## **2.5 Preparation of oil palm seedlings**

The oil palm seedlings used in this study were prepared in the following steps. The seedlings used were oil palm seedlings of the Sriwijaya 5 variety (DxP Sriwijaya 5, from the Sampoerna Group) which were claimed suited to peatland conditions. Oil palm seedlings were planted in a mixture of finely ground peat and mineral soil with a 1:1 volume ratio medium. The media was sterilized by steaming for 1 hour before use. After cooling, the media mixture was put into a 10 x 15 cm polybag and placed in a closed plastic tarpaulin for 1 week. The seedlings were planted by making holes in the media, placing the seedlings with the radicle position at the bottom, and covered. The seedlings were placed under 50% shading and cultivated for 2 months. After 2-3 leaves, the seedlings were ready to be used as test plants.

## **2.6 Inoculation of oil palm seedlings with *Ganoderma***

### *2.6.1 Inoculation of seedlings in polybags with *Ganoderma*.*

Inoculation of oil palm seedlings with *Ganoderma* in polybags was carried out by the following method [18]. Rubberwood overgrown with *Ganoderma* mycelium was placed in a medium that had previously been made into a 10 cm hole. The roots of the oil palm seedlings were exposed by removing the bottom of the medium until the main roots were obtained. The seedlings were placed on the rubber wood and the main roots were ensured to be attached to the rubberwood. After that, the seedlings were covered with soil in the medium. The seedlings were left for 1 week in the shade of a shade net with a light intensity of 50% before being transferred to the research plot. After ensuring that the oil palm seedlings were growing well, the seedlings were ready to be treated as test plants for bio-fungicide efficacy.

### *2.6.2 Inoculation of oil palm seedlings in the field with *Ganoderma*.*

Oil palm seedlings Inoculation with *Ganoderma* in the field was carried out the same being in polybags. On the prepared peat land, a hole was made 10 cm deep, then rubber wood was placed in the hole and then the process was carried out as inoculating *Ganoderma* on oil palm seedlings in polybags as explained previously.

## **2.7 Efficacy of bio-fungicide formulation on oil palm seedlings**

The bio-fungicide formulation was given to oil palm seedlings with 3 types of treatments, namely single application, three time applications, no treatment (positive control), and negative control as a comparison. Each application treatment was given 1 month apart, starting from 1 week after oil palm seedling planting. Each test plant was given 30 grams of bio-fungicide for each application. The formulation was given by dusting it evenly on the soil surface around the oil palm seedlings in polybags. In this study, each gram of bio-fungicide used contained approximately  $3.16 \times 10^7$  conidia. Meanwhile, the efficacy of biofungicides on oil palm seedlings in the field was carried out in the same way as in polybags.

## 2.8 Oil palm seedling cultivation

Oil palm seedlings were allowed to grow for 24 weeks and cared for by controlling weeds around the plants. The growing medium was also always maintained in field capacity conditions by watering.

## 2.9 Disease severity observation

Disease severity was observed monthly by scoring (Table 1).

**Table 1.** Scoring criteria for disease severity in oil palm seedlings [18].

Score	Symptom
0	Healthy plants, green leaves, no fungal masses on seedlings.
1	1-3 chlorotic leaves without showing fungal masses on seedlings
2	More than 3 chlorotic leaves, necrotic leaves with or without fungal masses on seedlings, seedlings show fungal masses anywhere
3	At least 50% of leaves experience chlorosis or necrosis with or without fungal masses on seedlings
4	Dead seedlings with or without the appearance of fungal masses on seedlings

Disease intensity was calculated using the following formula [19].

$$DI = \frac{\sum_{i=1}^{k \times nk}}{Z \times N} \times 100\% \tag{1}$$

DI = Disease Intensity

nk = Number of plants with score k (k=0,1,2,3,4)

k = Score used.

Z = Highest score

N = Number of plants observed

## 3 Results and Discussion

### 3.1 Test results in pots

After 24 weeks of observation, it was found that the application of bio-fungicides containing active ingredients of acid-tolerant antagonist fungi from peatlands of West Kalimantan with 3 times applications could reduce the intensity of disease caused by *Ganoderma* to 13.89% compared to the positive control which reached 41.67% (Table 2). This means that bio-fungicides containing active ingredients of acid-tolerant antagonist fungi with 3 times applications could reduce the intensity of the disease by 66.7%. Meanwhile, control with a single application resulted in lower effectiveness and only reduced the intensity of the disease to 36.11% compared to the positive control (41.67%). Thus, bio-fungicides containing active ingredients of acid-tolerant tolerant fungi with a single application had lower effectiveness, which was only 13.34%. These results indicate that the BSR disease control by bio-fungicides based on acid-tolerant antagonists from peatlands of West Kalimantan effectiveness depends on the frequency of application. The higher the frequency of application, the greater the effectiveness of control. This result was a common phenomenon in the use of biofungicides where continuous use guarantees more successful control [20, 21]. A higher frequency of application will ensure the continuity of the number of antagonist individuals so that their function as antagonists is also more guaranteed. This is because in general the function of antagonists is also often influenced by the number of antagonists which can affect their ability

to colonize the environment [22, 23]. However, this phenomenon also depends on how the antagonist mechanism works in suppressing pathogens [24].

In this study, this condition was likely related to the number of conidia and the durability of the conidia of the three types of active ingredients used. Bio-fungicides that are only given once, the number of conidia available was less so that the possibility of growth and activity will also be lower than those given 3 times. The limited number of antagonist individuals will also likely cause their function as a biological control to be less effective. In addition, the dosage given was also thought to be lacking. This is because, in this study, the density of conidia in the bio-fungicide used ( $3.16 \times 10^7$ ) was also still below the recommended minimum density standard of  $10^8$  [25]. This appears to be a common phenomenon in biological control where the dose of bio-fungicide greatly affects the effectiveness of control [26, 27].

**Table 2.** Results of observations of disease intensity in oil palm seedlings in polybags

Treatment	Disease intensity average (%)					
	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Positive control	0	0	13,88	19,44	41,67	41,67
Negative control	0	0	0	0	0	0
1 x application	0	0	2,78	8,33	30,533	36,11
3 x application	0	0	0	0	11,11	13,89

### 3.2 Field Test Results

The results of the field experiment showed a similar pattern to those observed in the polybag experiment, although the disease intensity varied. In the field experiment, the administration of bio-fungicide with 3 applications also resulted in higher effectiveness compared to a single application. The disease intensity in oil palm seedlings controlled with 3 times applications of bio-fungicide was 2.78%, much lower than that controlled with only 1 application, which was 22.22%. It was also much lower than the positive control treatment in the field (30.56%) (Table 3). This means that the use of bio-fungicide with 3 applications was able to suppress the disease intensity by 90.9%, while that with only one application was only able to suppress the disease intensity by 27.29%. This also indicates that the higher the frequency of application in the field, the more effective the *Ganoderma* control will be, the same as that obtained in the pot experiment described previously. This similarity is not surprising because in general, the patterns between what occurs in simulations in the laboratory or the greenhouse and the field are usually not much different [28, 29].

The lower disease intensity in field trials (in all treatments) is likely due to the influence of several factors. These influencing factors can be viewed from two sides. First, from the pathogen side (*Ganoderma*). The natural peat soil conditions in the field are different in terms of the dynamics of the microorganism population compared to the situation in the pot [30]. With direct interaction with the environment, the microorganism population in the field will be more dynamic and more diverse (also denser) so the level of competition is also higher. In such conditions, *Ganoderma* may develop more slowly due to competitive pressure with other microorganisms, where these conditions do not occur in the medium situation in the pot, so the attack rate is also lower. This is evidenced by the intensity of basal stem rot disease in the positive control treatment in the field which is also lower. Second, from the antagonist side contained in bio-fungicides. Considering that the antagonist contained in this bio-fungicide comes from peatlands, then in natural peatland conditions, the antagonist activity will develop faster and colonize around oil palm roots due to the suitability of the environment. This can happen because peatland is its natural habitat so it does not require further adjustment [31]. Thus, the antagonist in bio-fungicide can work better in suppressing

*Ganoderma*. Third, in terms of the influence of the physical environment. The physical environment, especially the problem of water management in peatlands, greatly affects the growth of *Ganoderma* in the soil [32]. Given the fairly high rainfall in West Kalimantan, it is likely that the oil palm land that is the place of research will be flooded for quite a long time due to poorly maintained drainage conditions. Long-term flooded conditions will inhibit the growth of *Ganoderma* [33]. However, such conditions do not greatly affect the acid-tolerant antagonist contained in biofungicides. This is because the antagonist does come from that environment (indigenous).

**Table 3.** Results of observations of disease intensity in oil palm seedlings in the field

Treatment	Disease intensity average (%)					
	Month 1	Month 2	Month 1	Month 4	Month 1	Month 6
Positive control	0	0	0	2,77	13,89	30,56
Negative control	0	0	0	0	0	0
1 x application	0	0	0	0	8,33	22,22
3 x application	0	0	0	0	2,78	2,78



**Fig. 1.** Results of application of acid-tolerant antagonist-based bio-fungicides on oil palm seedlings. A, Scoring of disease severity on oil palm seedlings. B, Signs of *Ganoderma* attack in the form of the appearance of *Ganoderma* fruit bodies at the base of the oil palm seedling stem (yellow arrow). C, Severity of the disease in the bio-fungicide treatment used. D, Symptoms of leaf necrosis on oil palm seedlings due to *Ganoderma* attack.

## 4 Conclusion

This study demonstrates that the application of bio-fungicides containing acid-tolerant antagonists is relatively effective in controlling *Ganoderma*. However, its effectiveness is influenced by the frequency of application, with more frequent applications leading to greater effectiveness. The administration of bio-fungicides with a frequency of once a month for three months has been proven to be able to reduce the intensity of the disease to only 13.89% compared to the positive control which reached 41.67% in pots. While in the field, the administration of bio-fungicides with the same frequency was able to reduce the intensity of

the disease to only 2.78% compared to the positive control which reached 22.22%. Further experiments are needed, particularly regarding the application dose, as the antagonist population in the formulation was lower than the recommended level in this study.

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