

Efficacy of *Hendersonia* on the growth of seedlings of oil palm (*Elaeis guineensis* Jacq.) and *Ganoderma* disease control: A field-based study using GanoEF biofertilizer at Medan, Indonesia

Abstract

The present study aimed to test the efficiency of *Hendersonia* GanoEF biofertilizer against *Ganoderma* disease in the seedlings of oil palm (OP) *Elaeis guineensis*, and was carried out from February 2020 – January 2021 in Pulo Mandi Garden Nursery, at Medan, Indonesia. Experimental set up followed a completely randomized design with four treatments. Observation results showed that the percentage of root colonization by *Hendersonia* sp. in OP roots reached an average of 70.6% in the treatment with *Ganoderma* inoculation, and an average of 69.6% in the treatment without *Ganoderma* inoculation. The GanoEF treatment provided higher growth of seedlings and faster growth of bowl diameter when the seedlings were 9 months old. Three instances of GanoEF application had high potential to inhibit the development of *Ganoderma* on OP seedlings. Endophytic fungi *Hendersonia* sp. was capable of symbiosis with OP roots with a colonization rate reaching 70.6%. Disease incidence or percentage of plants infected with *Ganoderma* in GanoEF treatment reached 20.4% which was significantly ($P < 0.05$) lower than under the control treatment (44.2%). Meanwhile, the level of severity of *Ganoderma* disease in seedlings with GanoEF treatment reached 5.23% which was significantly ($P < 0.05$) lower than the control treatment (11.1%). The efficacy of *Hendersonia* GanoEF as a biocontrol method for the OP pathogen *G. boninense* to control the basal stem rot of *E. guineensis* seedlings was well proven in this field study.

Keywords: oil palm disease, *Hendersonia*, biocontrol, basal stem rot, Indonesia

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Introduction

It is known that *Ganoderma boninense* is the cause of basal stem rot (BSR), which is a most damaging and threatening disease of oil palm (OP) (*Elaeis guineensis* Jacq.) plantations, particularly in Indonesia and Malaysia,¹ besides India, Thailand, the Philippines and Papua New Guinea.¹⁻³ In some OP plantations in Indonesia, this disease has caused 80% or more of all deaths in the populations of OP plantations, resulting in a decrease in OP production per unit area.⁴ The island of Sumatra is the most important region for OP production. The island could serve as a model for other OP-growing areas in Southeast Asia. As shown explicitly above, the area in Sumatra with a favourable environment for growing OP will shrink as a result of projected climate change. As previously projected, as the climate becomes more unfavourable, BSR by *G. boninense* will rise, posing a serious threat to Southeast Asia's sustainability on the OP crop production.⁵⁻⁸

The BSR disease has been controlled through the use of *Ganoderma* tolerant planting material, sanitation of inoculum sources during replanting, technical culture modification, as well as the use of biocontrol agents.^{9,10} Endophytic bacteria isolated from the roots of OP have previously been reported as a biological control agent for *G. boninense* disease.^{11,12} Buana et al.¹³ reported that, by using bacteria *Burkholderia* sp., the seed treatment reduced the disease incidence observed up to three months nursery trial in the OP but field trials are needed. *Trichoderma* dan *Mycorrhizae* are biological control agents that have been used for a long time to prevent *G. boninense* fungal infection in the field.¹⁴ Sukariawan et al.¹⁵ reported *Trichoderma harzianum* in various media was capable to control the *Ganoderma* disease in OP plantations. In particular, the OP empty fruit bunch and cow dung could act as *T. harzianum* media as well as a source of soil

organic matter. The fungus could stimulate the growth of OP roots. Along with the continuous exploration of natural enemy candidates for *G. boninense*, other types of fungi that are antagonistic to *G. boninense* were investigated for its efficacy as a biological control agent of *Ganoderma* disease in the OP industry. Although a number of possible biological control agents combat *G. boninense* has been identified, it has yet to be tested in the field.¹⁶ The potentials of using fungus as a biocontrol for *Ganoderma* disease has been previously indicated.¹⁷ Goh et al.¹⁸ reported the fungus *Scytalidium parasiticum* reduced disease severity and increased the vegetative growth of OP in nursery trial but field trials are needed to confirm decreased pathogenicity and disease suppression. Rebitanim et al.¹⁹ reported that GanoCare® could improve the growth and resistance of OP against *Ganoderma* disease in the nursery (8 months) and field trials (21 months). They stated that this new fertilizer technology (GanoCare®) was formulated by combining powdered empty fruit bunches with "beneficial element." However, the fungus *Hendersonia* was not mentioned in the paper.

The development of GanoEF biofertilizer containing endophytic fungus, *Hendersonia toruloidea* GanoEF1 incorporated into inorganic and organic fertilizers was successfully produced in collaboration between Malaysian Palm Oil Board (MPOB) and All Cosmos Industries Sdn. Bhd., Pasir Gudang, Johor.²⁰ The endophytic fungus, *H. toruloidea* GanoEF1 has been found to be strongly antagonistic against *G. boninense*, which is the causal pathogen of BSR disease in the OP, under many laboratory and nursery studies.²¹⁻²⁴ According to Idris et al.,²⁰ the benefits of GanoEF biofertilizer are inclusive of the effectiveness of *Ganoderma* disease control, environmental-friendly, easy storage, and easy application in the nursery and field conditions.

Ramli et al.²² previously investigated the *in vitro* and *in vivo* antagonistic potential of *Hendersonia* sp. isolate GanoEF1 against *G. boninense*. In both bioassays, Ramli et al.²² found that *Hendersonia* sp. isolate GanoEF1 provided the best results in suppressing *G. boninense*. In an *in vitro* assay, this fungus outgrew the pathogens, and in a nursery trial, it reduced the incidence of *Ganoderma* disease. Although the use of *Hendersonia* sp. isolate GanoEF1 as a biological control agent against *Ganoderma* disease yielded promising results, more research is needed in the near future to prove its efficacy in the field.¹⁶ Recently, Tony Peng et al.²⁵ tested positively the use of *Hendersonia* GanoEF biofertilizer was proven successfully colonized in both primary and feeder roots of OP at PASFA's Bukit Kerisek (Pahang, Peninsular Malaysia). Tony Peng et al.²⁶ also observed that the GanoEF biofertilizer was more successfully colonised in both primary and feeder roots of OP after a three-time application. However, there have been limited field-based studies in Indonesia using *Hendersonia* GanoEF biofertilizer in the OP plantations.

Hendersonia sp. has been mass-produced in solid formulation under the trademark GanoEF. The efficacy of GanoEF product needs to be tested in the field against *G. boninense* isolates from Indonesia. Therefore, PT. All Cosmos Indonesia as the product formulator is collaborating with Indonesian Oil Palm Research Institute (IOPRI) to conduct field-based study in OP seedlings (1 year old) to assess the efficacy of the product on the severity of *Ganoderma* disease in the OP nursery. The objective of this study was to test the efficacy of *Hendersonia* GanoEF biofertilizer against *Ganoderma* disease in the seedlings of OP (*E. guineensis*) at Medan, Indonesia.

Materials and methods

Study location

GanoEF efficacy on OP seedlings was carried out in February 2020 to January 2021 at the Pulau Mandi OP nursery estate (02° 51'57"N; 99° 30'03"E), PT. Nusantara Plantation III, Medan, Indonesia (Figure 1). This test was carried out using IOPRI planting material for the OP varieties of D x P Simalungun.

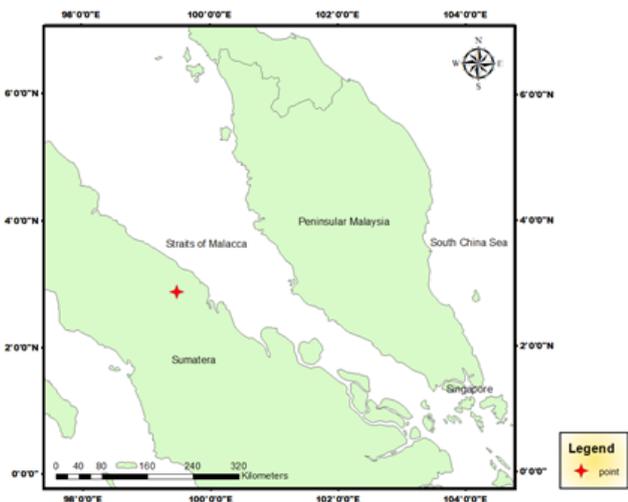


Figure 1 Field trial plot in Pulau Mandi oil palm nursery (star), PT. Nusantara Plantation III, in the present study.

Production of *Ganoderma* inoculum sources

Pure *Ganoderma* isolates were grown on rubberwood substrates with size 6x6x6cm³. Rubberwood was washed with sterile water

and each was placed in a heat-resistant polypropylene plastic (1 kg capacity) set together with a peralon neck and cotton cap. Each substrate was sterilized with an autoclave at 138 Psi at 121°C for 60 minutes. After cooling, rubberwood was inoculated with seven days old *G. boninense* isolate. The substrate was then incubated in the laboratory at room temperature for 1.5 months. Substrates were used for pathogenicity assays.

Growing media

The planting medium used is a mixture of soil and sand with a ratio of 1:1. Before being put into polybags, soil and sand were sieved beforehand to prevent the entry of remnants of roots that can be used as a source of *Ganoderma* inoculum.

GanoEF treatments

The present experimental design followed a completely randomized design consisting of four treatments (Table 1). The experiments were conducted in the open field with a sprinkler irrigation system. Between February 2020 to January 2021 at the Pulau Mandi, the monthly average temperatures ranged from 24°C to 32°C. The monthly average rainfalls were between 200mm to 450mm (annual rainfall between 1800mm and 2200mm). Each treatment was repeated 10 times where each replication consisted of 20 polybags of OP. Application GanoEF was carried out three times, namely: 50 g/ planting hole at the age of 3 seedlings months or when transplanting from pre-nursery to main nursery (at the same time) with inoculation of *G. boninense*, 50g/polybag at 6 months of age, and 50 g/polybag when the seedlings were nine months old.

Table 1 Four treatments from the present experimental design

Treatment	Description
1	GanoEF standard application (50g/polybag) with <i>G. boninense</i> inoculation,
2	GanoEF standard application (50g/polybag) without <i>G. boninense</i> inoculation,
3	without GanoEF application but with <i>G. boninense</i> inoculation (positive control)
4	without GanoEF application or the inoculation of <i>G. boninense</i> (negative control)

Plant vegetative observation

The vegetative parameters measured were plant heights, bulb diameters and the number of leaves. Plant height was calculated from the base of the stem to the highest leaf of the plant. The bulb diameter was measured at the base of the hump by using a calliper. Measurement diameter is done in two directions (North-South and East-West) and then they were averaged. The number of leaf blades counted was the leaf that had opened perfectly. The measurements of the three vegetative parameters were carried out monthly.

Observation of disease incidence and disease severity

Observations of disease incidence (DI) and disease severity (DS) were made every month until the seedlings were twelve months old (three months at pre-nursery; nine months at main nursery period) includes the visual development of the incidence of *Ganoderma* disease and disease severity by observing BSR damage at the end of the experiment (Figure 2).

The DI of *Ganoderma* is calculated by the formula²⁷ as in equation (1):

$$DI = (a/a + b) \times 100\% \quad (1)$$

Where

DI=disease incidence

a=number of infected plants

b=number of healthy plants



Figure 2 Symptoms of *Ganoderma* disease visually: (A) on stems and leaves above ground level; (B) internal symptoms in the presence of decay at the base of the split stem.

After the tested OP seedlings were twelve months old, the BSRs were dismantled to observe the symptoms of internal diseases with a system scoring as given in Table 2.

Table 2 Scoring of oil palm seedlings infected with *Ganoderma* seen from the roots and dismantling plant stems

Score	Description
0	There are no necrotic symptoms on the roots and the base of the stem (healthy plant).
1	There is necrotic at the root but not at the base stem
2	There is necrotic on the roots, started to occur necrotic on the stem base < 5%
3	There is necrotic on the roots, necrotic on the stem base 5% - 25%
4	There is necrotic on the roots, necrotic on the stem base > 25%, <i>Ganoderma</i> fruiting bodies appear on stem base, necrotic to death (plant death)

The DS index was calculated by using the formula²⁷ as in equation (2):

$$DS \text{ index } (\%) = (\sum n \times V) / (N \times V) \times 100\% \quad (2)$$

Where

DS=Disease severity

n=Number of samples on certain criteria observed

v=Score value in the observed sample

N=The number of all observed samples

V=The highest score in the method which is four.

Data analysis

The data obtained were analyzed by analysis of variance using Genstat 12 program, the treatment that had a significant effect was further tested with the Duncan Multiple Range Test (DMRT) with a 95% confidence interval. In other words, different letters indicated significant differences based on the DMRT test at P<0.05.

Results and discussion

Colonization of *Hendersonia* sp. on oil palm roots

The colonizations of *Hendersonia* sp. on the roots of OP seedlings in all the treatments are presented in Table 3. The *Hendersonia* GanoEF biofertilizer experimented within the present study has the ability to colonize the roots of OPs very well. The infection percentage of *Hendersonia* sp. in OP roots reached an average of 70.6% (+4.45%) in the treatment with *Ganoderma* inoculation, and an average of 69.6% (+3.51%) in the treatment without *Ganoderma* inoculation (Table 3). Meanwhile, on treatments of positive and negative controls without GanoEF application, there was no growth detection of *Hendersonia* sp. based on rose bengal agar selective media used. This showed that the soil media used for filling polybags did not contain *Hendersonia* sp. naturally in the soils.

Table 3 Colonization of *Hendersonia* sp. on the roots of oil palm seedlings from the four treatments of the present study

Treatment	Colonization (%)
GanoEF (+) <i>Ganoderma</i>	70.6
GanoEF (-) <i>Ganoderma</i>	69.6
Control (+) <i>Ganoderma</i>	0
Control (-) <i>Ganoderma</i>	0

The ability of *Hendersonia* at OP roots against *G. boninense* infection was highly dependent on the success of its colonization on treated OP seedling roots. Theoretically, the higher percentage of roots that can be colonized, hence its ability to protect the root of the pathogen infection would also be higher.

Effect of GanoEF on oil palm seedling growth

The growths of OP seedling heights in all treatments from the present study are presented in Table 4. The results showed that the seedlings with the GanoEF application had a faster growth in the seedling height when compared to seedlings without GanoEF treatment. In general, the significant (P<0.05) difference in seedling heights between with application of GanoEF and without application of GanoEF started to be observed at 6 months after application until 9 months old during the experimental study. However, on seedlings that were both applied to GanoEF, there was no significant (P>0.05) difference in seedling heights between the *Ganoderma* inoculated seedlings and those without *Ganoderma* inoculation.

Table 4 Growth of oil palm seedling heights (cm) in all treatments seedlings from the four treatments of the present study

Treatments	1 MAP	2 MAP	3 MAP	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP	9 MAP
GanoEF (+) <i>Ganoderma</i>	30.02a	31.80a	44.85a	52.05a	65.36a	89.75a	103.74a	122.20a	134.50a
GanoEF (-) <i>Ganoderma</i>	29.93a	31.60a	42.83b	51.79a	63.07ab	85.30b	101.04ab	118.10a	135.10a
Control (+) <i>Ganoderma</i>	28.88b	30.46b	41.44b	49.12b	60.63b	80.82b	95.94b	105.70b	117.00b
Control (-) <i>Ganoderma</i>	29.10ab	30.73b	41.90b	48.81b	61.56b	81.40b	97.20b	105.80b	119.80b

Note: MAP= Month after planting to main nursery; Different letters in the same column show significant differences based on the DMRT test at P<0.05

The growths of diameter of bowls (BSR) of OP seedlings in all treatments from the present study are presented in Table 5. The diameter of the OP seedlings showed a significant ($P < 0.05$) difference

across all treatments. The significant difference ($P < 0.05$) of the diameter of the bowl was also observed when the OP was at 9 MAP or at 6 BSA.

Table 5 Growth of diameter of bowls (or BSR) (cm) of oil palm seedlings in all treatments seedlings from the four treatments of the present study

Treatments	1 MAP	2 MAP	3 MAP	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP	9 MAP
GanoEF (+) <i>Ganoderma</i>	10.43a	13.07a	20.51a	28.39a	37.69a	52.11a	59.28a	67.22a	75.46ab
GanoEF (-) <i>Ganoderma</i>	10.46a	12.59a	19.48b	27.50ab	35.75a	50.36a	60.38a	68.12a	76.60a
Control (+) <i>Ganoderma</i>	10.42a	11.72a	18.65c	27.04b	33.18b	44.66b	54.22b	62.83b	70.86c
Control (-) <i>Ganoderma</i>	9.35a	11.76a	17.09d	23.95c	32.97b	47.06b	55.41b	63.12b	72.25bc

Note: MAP= Month after planting to main nursery; Different letters in the same column show significant differences based on the DMRT test at $P < 0.05$

The growths of numbers of leaves of OP seedlings in all treatments from the present study are presented in Table 6. In general, there was

no significant ($P > 0.05$) difference across all treatments in the numbers of leaves from 1 to 9 MAP.

Table 6 Growth of number of leaves of oil palm seedlings from the four treatments of the present study

Treatments	1 MAP	2 MAP	3 MAP	4 MAP	5 MAP	6 MAP	7 MAP	8 MAP	9 MAP
GanoEF (+) <i>Ganoderma</i>	5.00a	6.07a	8.03a	9.34a	10.45a	13.00a	14.28a	14.25a	14.86a
GanoEF (-) <i>Ganoderma</i>	5.10a	6.10a	8.13a	8.07b	9.92a	12.49a	13.69ab	14.03a	15.12a
Control (+) <i>Ganoderma</i>	5.00a	6.03a	7.98a	9.21a	10.45a	12.37a	13.53ab	14.55a	15.01a
Control (-) <i>Ganoderma</i>	4.90a	6.00a	7.94a	9.00a	10.10a	12.32a	13.38b	14.30a	15.12a

Significant differences found in vegetative parameters such as height plants and the diameter of the OP seedlings indicated the important role of the GanoEF biofertilizer that stimulated the growth of OP seedlings. Based on the description, this product also contains organic and inorganic fertilizers which of course can affect the development OP seedlings.²⁰

Incidence of disease (ID) and disease severity (DS) of *Ganoderma* disease

The values of ID and DS of *Ganoderma* disease in all treatments from the present study are presented in Table 7. The ID describes the number or percentage of sample plants which was infected by *Ganoderma*. In general, the ID values in GanoEF treatment were lower than the positive control and negative control treatments. The ID in the treatment GanoEF was 20.4% significantly ($P < 0.05$) lower than the treatment positive control (44.2%).

Table 7 Incidence of disease (ID) and disease severity (DS) of *Ganoderma* from the four treatments of the present study

Treatments	ID	DS
GanoEF (+) <i>Ganoderma</i>	20.4b	5.23b
GanoEF (-) <i>Ganoderma</i>	0.00c	0.00c
Control (+) <i>Ganoderma</i>	44.2a	11.05a
Control (-) <i>Ganoderma</i>	0.00c	0.00c

Note: Different letters in the same column show significant differences based on the DMRT test at $P < 0.05$

The ID in the treatments can only be observed at the end of the experiment by observing symptoms of necrosis that occurred in the BSRs of OP seedlings. While in the main nursery, the visual symptoms of *Ganoderma* infection on the crown of seedlings was difficult to observe, in both in GanoEF treatment as well as in the positive control. According to Susanto,²⁸ the *Ganoderma* disease does not often cause visual symptoms in plants hosts such as OP, and coconuts.

In the GanoEF treatment, the DS reached 5.23%, which was significantly ($P < 0.05$) lower when compared to positive control treatment (11.1%). The DS describes the level of damage in the form of necrotic symptoms that occur in the BSRs of OP seedlings (Figure 3). This study showed evidently that GanoEF could inhibit and developed antagonistic effects on *Ganoderma* growth in OP seedlings. Earlier, Turner²⁹ observed that examinations of oil-palm estates in Malaya revealed that the frequency of *Ganoderma* infection of OPs was highest on newer plantings on previously borne coconut palms.



Figure 3 Necrotic root that occurs in oil palm seedlings by inoculation of *Ganoderma*. It can be seen that the necrotic tissue has started at the base of the BSR.

The current DI and DS values were lower than those reported by Edy et al.,³⁰ who found that *Ganoderma* from forest and rubber trees had lesser pathogenicity against OP seedlings. They reported that DI and DS values were 64.3% and 26.4%, respectively, at the OP plantation in Bukit Dua when compared to rubber plantation (DI=68.45; DS=34.8%) and Forest (DI=87.0%; DS=46.1%), while DI and DS values were 52.7% and 22.9%, respectively, at the OP plantation in Harapan rainforest when compared to rubber plantation (DI=60.8; DS=32.1%) and Forest (DI=83.0; DS=64.3%).

Therefore, based on the above results, it can be concluded that *Hendersonia* GanoEF1 had inhibited the development or rate of *G. boninense* infection at Nusantara Plantation in Medan. The use of *H. toruloidea* GanoEF for the reduction of *Ganoderma* infection in the OP plantations have been proven effective by Idris et al.,²⁰ Ramli et al.²² and Kamarudin et al.³¹ Hence, *Hendersonia* GanoEF1 is a biotechnological fertilizer product that can promote soil fertility and prevent *Ganoderma* incidence in OP plantation.²⁰

Munthe and Dahang³² reported 24–28% of root colonization of *H. toruloidea* GanoEF1, based on the roots of OP collected from two locations in Riau Province, Indonesia. The one-time application of GanoEF1 was made by putting 750g per plant of *Hendersonia* GanoEF1 before establishing. The root samples were taken at 12 MAP. Based on one-time application basis of GanoEF, Tony Peng et al.²⁵ reported that *H. toruloidea* colonization percentages in primary and feeder roots of immature OP were 19.3–21.6%, and 37.5–42.5%, respectively, at OP plantation at PASFA's Bukit Kerisek (Pahang, Peninsular Malaysia). According to Tony Peng et al.,²⁶ *Hendersonia* GanoEF1 colonisation in both primary and feeder roots was 40% in the treated palms. This convincingly demonstrated that *Ganoderma* disease could be effectively managed in an OP plantation. They suggested using *Hendersonia* GanoEF1 within the immature OP root for a three-time GanoEF biofertilizer application.

The regulation of thiamine biosynthesis genes in OP seedlings (seven-month-old) in response to root colonisation by endophytic *Hendersonia toruloidea* was studied by Kamarudin et al.³¹ They provided the first evidence of endophytic colonisation enhancing thiamine production in OP seedlings. Thiamine, often known as vitamin B1, is an essential cofactor in many metabolic pathways in all living species, including glycolysis, the pentose phosphate pathway, and the tricarboxylic acid cycle.³³ This knowledge could help to understand the efficacy of *Hendersonia* on the growth of seedlings of OP and *Ganoderma* disease control.

Conclusion

In the main nursery, the *Hendersonia* GanoEF were applied three times with a dose of 50g/polybag at the main nursery stage to produce levels of *Hendersonia* colonization on roots reached an average of 70.6% in treatment with *Ganoderma* inoculation and 69.63% in the treatment without *Ganoderma* inoculation. In plants treated with GanoEF, levels of significantly lower incidence and severity of *Ganoderma* disease compared to control plants. In addition, the GanoEF application can also accelerate the vegetative growth of OP seedlings from the age of 6 months after transplanting. Further field-based studies by using GanoEF in the long term are recommended and necessary to understand its efficacy against *Ganoderma* after 5, 10, 15 and 20 years of application, and the yield of OP.

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Conflicts of interest

Authors declare that there is no conflict of interest.

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