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# Biocontrol of Basal Stem Rot (BSR) Disease of Oil Palm using Endophytic Fungus, Hendersonia sp.

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**Abstract**

The ability of potential antagonist, Hendersonia sp. isolate GanoEF1 to control Ganoderma boninense was investigated in this research . Five hundred endophytic fungi were isolated from roots of healthy palms standing in disease foci of the basal stem rot (BSR) disease of oil palm. These fungi were screened in vitro for their antagonistic properties towards Ganoderma boninense, the causal pathogen of BSR disease. Based on screening using dual culture and liquid culture assay, Hendersonia sp. isolate GanoEF1 proved effective in controlling the growth of G. boninense in both bioassays. In dual culture assay, isolate GanoEF1

gave a percentage inhibition of radial growth (PIRG) value against G. boninense at 89.3%, and in liquid culture assay the same isolate gave a percentage mycelial dry weight (PMDW) value of 49.8%. The study was extended to the nursery to evaluate the efficacy of Hendersonia sp. isolate GanoEF1 in controlling basal stem rot (BSR) disease incidence in oil palm seedlings artificially inoculated with G. boninense. After 6 months of treatment, BSR incidence was reduced by 37.0% to 55.2% in seedlings applied with Hendersonia sp. isolate GanoEF1 compared to untreated seedlings. The study clearly demonstrated that Hendersonia sp. isolate GanoEF1 has a promising role as a biocontrol agent of BSR of oil palm.

***Key Words*:**Oil Palm; Endophytic Fungus; Basal Stem Rot; Biological Control.

# 1 Introduction

Oil palm, Elaeis guineensis, is a highly efficient producer of vegetable oil among the oil-producing crops in the world (1). The oil palm industry in Malaysia and Indonesia plays a major role in the agricultural sector as these two countries, and they jointly produce and export 84% and 90% share of the worlds total palm oil production and export, respectively (2). As true for other crops, oil palm is susceptible to attack by plant diseases caused by fungi, bacteria, viruses, nematodes, viroids and phytoplasma. Currently, the oil palm industry is facing challenges in disease management of basal stem rot (BSR) disease caused by various species of Ganoderma. BSR is widespread, occurring in oil palm growing regions of the world and has been identified as the single major devastating disease inflicting significant losses in Southeast Asia, especially in Malaysia (3) and Indonesia (4). The disease was also reported in Africa, Papua New Guinea, Honduras, Colombia and Thailand (5). Findings had successfully identified four species of Ganoderma associated with BSR disease of oil palm; these are G. boninense, G. zonatum, G. miniatocintum and G. tornatum. Among all four, G. boninense has been identified as the most virulent and most annihilating in Malaysia and several South East Asian countries.

Based on a survey conducted by MPOB, in 2010 the incidence

of BSR disease palms in Malaysia was 3.71% with the areas affected were 59,148 ha out of the total areas of over 1.594 million ha (6). The highest areas affected with the disease in Malaysia was mainly in Johor (18,098 ha), Sabah (15,940 ha), Perak (9,869 ha) and other states less than 3,500 ha and disease free in Perlis. The economic loss caused by this pathogen is estimated about RM1.5 billion per year (7) and this disease play a major role in decreasing the oil palm yield if no control measures are implemented.

Biological control is a very common strategy which involves the use of one organism to check the growth of another organism, a pathogen that causes economic losses to crops. Advances in biotechnology have led to the significant increase in the use of microbes as biological control agent (BCA). In disease control and management of Ganoderma, development of BCA is an alternative strategy to complement existing control methods. Furthermore, this approach supports the effort towards sustainable palm oil production. These microorganisms simultaneously grow together with pathogenic fungi and produce an enzyme or organic compounds for suppression of fungal pathogens.

Many studies have focused on endophytes as endophytic microorganisms are to be found present and abundant and often very diverse in every plant on earth. They have been isolated from nearly all plant families growing in different climatic regions of the world (8). Endophytic microbes are microorganisms that live in the intercellular spaces of the plant for most if not all of their life cycles. Also, fungal endophytes which colonise and grow asymptomatically within healthy plant tissue, may evolve from plant pathogenic fungi and become nonpathogenic thus cause no apparent damages (9-12). As reported, most of the fungal endophytes belong to the Phylum Ascomycota and its anamorphs, and a few studies identified endophytic as members of the Phylum Basidiomycota (13-17). Colonisation of endophytes in plant tissues may in some way give significant benefits to their host. Fungal endophytes benefit plants by promoting plant growth, improving tolerance to multiple stresses, increase resistance against pathogens, protection from diseases and insects. The endophytes, in turn, will receive nutrients and shelter from the host. Studies have indicated that endophytes have the potential for biological control due to their antagonistic effect against the many plant pathogens. Rubini, Silva-Ribeiro (18) have

shown that a type of endophytic fungus, Gliocladium catenulatum exhibited antagonistic properties against Witches Broom disease caused by Crinipelis perniociosa in cacao seedlings. Nuangmek, McKenzie (19) demonstrated the fast competitive growth of endophytic fungus, Cordana sp. and the antibiotic producing endophyte, Nodulisporium sp. significantly reduced the growth Colletotrichum musae, the causal agent of the anthracnose disease of banana. The in vivo study done by Orole and Adejumo (20) revealed that endophytic fungus, Trichoderma koningii as the best endophyte by reducing the wilt-causing pathogens of maize seedlings.

Our current research focuses on fungal endophytes from oil palm for use to develop a biocontrol strategy for the oil palm plant pathogen Ganoderma boninense as a part of integrated disease control and management of BSR. Previous studies have reported on endophytic bacteria isolated from the roots of oil palm as BCA of G. boninense disease (21, 22). However, studies on endophytic fungi from the oil palm are very limited and not much was being discussed. Hence, such fungi might also be excellent candidates as BCA. As a first step towards verifying this hypothesis, we examined the efficacy of endophytic fungi isolated from roots of healthy oil palm to control Ganoderma disease in oil palm. The objective of this study was to test the antagonistic potential of Hendersonia sp. isolate GanoEF1 against G. boninense in vitro and in vivo.

# 2 Methodology/Materials

## 2.1 Ganoderma boninense culture

Ganoderma boninense isolate PER 71 pure culture plate was taken from the culture collection of GanoDROP, Malaysian Palm Oil Board (MPOB), Bangi, Malaysia. The pure culture was initially isolated from infected oil palm in Teluk Intan, Perak, Malaysia by using the Ganoderma Selective Medium (23). For the current study, this G. boninense isolate was prepared from stock culture stored at 4 C in potato dextrose agar (PDA) slant and subsequently subcultured on PDA plate when required.

## 2.2 Isolation of endophytic fungus

The endophytic fungus was isolated from roots of symptomless oil palms standing in BSR disease foci in high incidence disease areas in Teluk Intan, Perak, Malaysia by the method of (24) with some modifications. The roots were randomly sampled from 30 palms with no obvious symptoms of BSR. At each palm, five random roots with ten cm in length were taken about 1.0 m away from the palm bases at 30 to 60 cm depth. Root samples were then brought to the laboratory and rinsed under running tap water for 20 minutes to remove any adhering soil from their surfaces; they were cut into three sections of about 3 cm in length. The sections were surface sterilised by immersing them in 10% sodium hypochlorite, and subsequently in increasing concentrations of 50%, 70%, 90% and 100%. The samples were placed onto potato dextrose agar (PDA) and incubated at ambient temperature (282C). The numbers of endophytic fungi growing out of the PDA were evaluated. Hyphal tips of each morphologically different mycelium that emerged from the fragments were subcultured and transferred onto PDA media plate. Pure cultures were maintained in the refrigerator at 4C for further study.

## 2.3 Antagonistic test by dual culture technique

The antifungal activity of the endophytic fungi against G. boninense was tested on PDA plate at 28C using dual culture technique. A 6 mm disc of five-day-old culture of G. boninense (isolate PER 71) was inoculated at the periphery of the 9 cm diameter Petri plate and a 6 mm disc of the five-day-old culture of test isolates (endophytic fungus) was inoculated at the periphery but on the opposite end of the same Petri plate and incubated at ambient temperature (28 2C). Three replications were assigned to each treatment. For a positive control, G.boninense was grown on PDA without the presence of the endophytic fungus. The antagonistic potential of endophytic fungi isolates were assessed after eight days of co-incubation by measuring the percentage inhibition of radial growth (PIRG) using the following formula described by (25):

Where, R1 represents the radius of the control pathogens growth and R2 the radius of the pathogens growth towards the fungal antagonist.

## 2.4 Antagonistic test by liquid culture assay (culture filtrate)

The endophytic fungal isolates that gave PIRG value of more than 40% were grown in 150 mL of potato dextrose broth (PDB) on a shaker at 150 rpm. After 14 days, the mycelia were harvested on Whatman No. 1 filter paper and the culture filtrate was collected. The culture filtrate was concentrated on a rotary evaporator at 45C and finally filtered by using 0.45 m and 0.20m syringe filter and stored at 5C until use. 50 mL of liquid media PDB was autoclaved in 250 mL flask at 121C at 1.05 kg/cm for 15 minutes, and allowed to cool to room temperature before a 6 mm disc of G. boninense was added to each flask. In each flask, five mL of culture filtrate of endophytic fungi was added to the flask containing G. boninense and incubated at 28C. As a control, 5 mL of sterile distilled water was added into the flask. The mycelia of G. boninense were harvested over 14 days on a muslin cloth and Whatman No.1 filter paper and oven dried at 60C. The constant weight of the oven-dried G. boninense mycelia was recorded and analysed using percentage of mycelial dry weight (PMDW) formula as described by (26):

where M1 represents the mycelial dry weight of G. boninense (control) and M2 represents the mycelial dry weight of G. boninense in the culture filtrate of the isolate. Endophyte fungal isolate GanoEF1, GanoEF2 and GanoEF3 were selected for further characterization based on their high PIRG and PMDW values against G. boninense in in vitro tests. The isolate that gave the highest value of PIRG and PMDW was selected for the nursery trial.

## 2.5 DNA extraction and identification using molecular technique

Endophytic fungi were transferred from potato dextrose agar (PDA) to potato dextrose broth (PDB) for seven days at 28oC at 150 rpm, prior to the DNA extraction to obtain enough mycelium. Mycelium was then harvested using a muslin cloth, washed with sterile water and dried on Whatman No. 1 filter paper. The mycelium was then frozen in liquid nitrogen and kept under -80oC for further analysis. Approximately, one gramme of mycelium was then ground using

mortar and pestle with the presence of liquid nitrogen until powdery appearance. DNA extraction was done using commercial DNA Genomic kit (QIAGEN, USA) with the protocol provided by the manufacturer. The concentration of DNA was determined via absorbance reading at A260/280 and A260/230, resulted in 1000 2000 ng/L with the purity more than 1.8. PCR amplification of filamentous fungi was done using fungal internal transcribed spacer (ITS) of ribosomal RNA (rRNA) gene cluster of genomic DNA using universal primers of ITS1 and ITS4 (27). The rDNA was amplified by adding 10X PCR buffer, MgCl2, dNTPs, forward and reverse primers and Taq polymerase in a total volume of 20 L. Conditions for PCR were as follow: 95oC for 10 min for denaturation, 95oC for 1 min, 55oC for 45 sec, 72o for 1 min for 35 cycles for annealing, and a final extension step at 72oC for 10 min using VapoProtect ThermalCycler (Eppendorf, German). Ten microliters of amplicons were loaded into 2% of gel agarose and subjected to electrophoresis. The purification of the PCR product to remove excess buffer, primers and nucleotides was performed using Gel Purification Kit (QIAGEN, USA). Purified PCR product was sent for sequencing (Bioneer, Korea).

## 2.6 Suppression of basal stem rot disease by Hendersonia sp. Isolate GanoEF1 under greenhouse conditions

Fresh rubber wood blocks (RWB) of 6 cm x 6 cm x 6 cm size were autoclaved for 30 minutes at 121C. Each block was put in a heat-resistant polypropylene bag and 100 mL of malt extract agar (MEA) added as a supplementary nutrient for G. boninense. The bags with RWB and molten MEA were autoclaved again for 30 minutes at 121C. After sterilisation, the RWB was rotated to ensure that they were well covered with MEA before it solidified. The RWB were then inoculated with seven days old G. boninense culture. All the blocks were incubated for two months until fully colonised by G. boninense before being used for artificial inoculation of the oil palm seedlings.

Thirty plates of Hendersonia sp. isolate GanoEF1 were subcultured on PDA and incubated at ambient temperature (282C) for seven days. The conidia were harvested by introducing ten mL of

distilled water into each plate, and by gently rubbing the colony surface with a sterile metal bacterial cell spreader. The conidia were then separated from the mycelium by pouring the suspension through a piece of sterile gauze into a sterile beaker. Conidial counts of Hendersonia sp. isolate GanoEF1 were determined by using a Neubauer haemocytometer and stock cultures of the fungus were incubated for use in the treatments.

Two trials were conducted at MPOB-UKM Research Station, Bangi, Selangor (Table 1). Each trial consisted of two treatments, replicated thrice with five seedlings per replicate. The planting materials used were three-month-old of DxP oil palm seedlings and they were arranged in a randomised complete block design (RCBD). In this study, RWB sitting technique was used to inoculate the oil palm seedlings with G. boninense. The 500 mL of Hendersonia sp. isolate GanoEF1 conidial suspension was suspended in sterile distilled water at the final concentration of 1 x 106 CFU/ml before drenching onto the seedlings. 500 mL of distilled water was used as the control treatment.

Table 1: Treatment of endophytic fungi applied to oil palm seedlings

| Treatments | Seedling description |
|---|---|
| C1 | Artificial *G. boninense* infected |
| T1 | Artificial *G. boninense* inoculation but challenged with *Hendersonia sp.* isolate GanoEF1 |

Fig.1 Block Diagram

## 2.7 Disease assessment and statistical analysis

The assessment of the effect of Hendersonia sp. isolate GanoEF1 on BSR incidence were carried out based on quantitative assessment measured as a percentage of disease incidence (DI), the severity of foliar symptom (SFS) and dead seedlings (DS) at monthly intervals. DI (%) referred to the number of seedlings visually assessed as a disease (chlorosis and necrosis of leaves, with or without production of fruiting body) described by Idris, Kushairi (28) as: (number of seedlings infected/total number of seedlings assessed) x100. SFS (%) was assessed according to Sariah and Zakaria (2000): SFS (%) = [(ax1) + (bx0.5)]/c x 100, where a the number of desiccated (browned/ wilted) leaves, b the number of yellowing leaves,

c the total number of leaves, and 1 the index for desiccated leaves and 0.5 the index for yellowing leaves. The area under the disease progress curve (AUDPC) was calculated using the formula (Shaner and Finney, 1977): AUDPC = [(yi + yi+1)/2](ti + 1 + ti) , in which n is the number of assessment times, y is the disease measurement (DI) and t is time (months) after inoculation. Disease reduction (DR) was calculated based on the value of AUDPC. Since duplicated experiments yielded similar results, the data were pooled prior to statistical analyses. All percentage data (DI, SFS and DS) were transformed by arcsine transformation and subjected to analysis of variance (ANOVA) with the means compared by the least significant difference (LSD) at P 0.05 using SAS software (SAS Institute Inc., 1995).

# 3 Results

## 3.1 Isolation of endophytic fungi from oil palm root tissues

A pure culture of endophytic fungi was observed to grow out from the surface-sterilized roots tissues of oil palms after seven days of incubation. A total of 500 culturable endophytic fungi isolates were isolated from roots of healthy oil palm at Teluk Intan, Perak, Malaysia. The separation of fungal endophytes was carried out by morphological characteristic. The pure culture of each isolate was maintained and tested against G. boninense.

## 3.2 Endophytic fungus culture and antagonistic test by dual culture test

Antifungal activities of the 500 isolates were first tested against the agent of BSR disease, G. boninense by dual culture test. This test was conducted to distinguish isolates with antagonistic potential from a large population. Out of 500 isolates tested, 456 isolates gave a percentage inhibition of radial growth (PIRG) value against G. boninense of less than 40.0% (Table 2). About 35 isolates gave a PIRG value of 40.0% to 80.0% against G. boninense. Nine isolates gave PIRG value of more than 80.0% with three of the isolates Ga-

noEF1 (89.3%), GanoEF2 (89.2%) and GanoEF3 (81.2%) showed consistently high PIRG values among all the replicates with respect to control after seven days of incubation (Figure 1).

Table 2: Number of endophytic fungi according to the percentage inhibition of radial growth (PIRG) values of G. boninense

| Total number of isolates tested | Percentage inhibition of radial growth (PIRG) values of *G. boninense* (%) | | |
|---|---|---|---|
| | < 40 | 40 – 80 | > 80 |
| 500 | 456 | 35 | 9 |

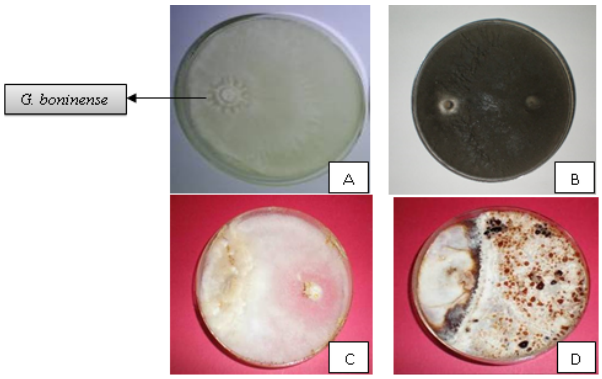


Figure 1: Dual culture assay: G. boninense in control plate (A) and G. boninense inhibited by Hendersonia sp. isolate GanoEF1 (B), Amphinema byssoides isolate GanoEF2 (C) and Phlebia radiata isolate GanoEF3 (D).

## 3.3 Antagonistic test by liquid culture assay (culture filtrate)

Liquid culture assay was conducted to detect the production of non-volatile, diffusible inhibitors produced by the endophytes either as antibiotics, enzymes or other forms of inhibitors. Forty-four isolates used in the dual culture test were tested against G. boninense using liquid culture assay based on PIRG value greater than 40%. Fourteen isolates gave a percentage of mycelial dry weight (PMDW) against G. boninense of more than 40% (Table 3). The highest PMDW value showed the ability of those fungi to be strong antagonists to control Ganoderma. Isolate GanoEF1 (Figure 2) showed

maximum percentage inhibition (49.8%) of PMDW against G. boninense with respect to control followed by GanoEF3 and GanoEF2 with 41.5% and 40.5%, respectively.

Table 3: Number of endophytic fungi according to the percentage of mycelial dry weight (PMDW) values of G. boninense

| Total number of isolates tested | Percentage of mycelial dry weight (PMDW) values of *G. boninense* (%) | | |
|---|---|---|---|
| | < 40 | 40 – 80 | > 80 |
| 44 | 30 | 14 | 0 |

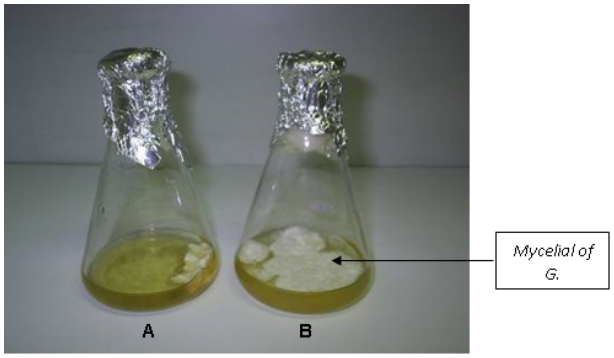


Figure 2: Liquid culture assay: G. boninense inhibited by Hendersonia sp. isolate GanoEF1 filtrate (A) and G. boninense in control filtrate (B).

## 3.4 Characterization of endophytic fungi

The isolate GanoEF1, GanoEF2 and GanoEF3 were characterised by morphological analysis and had been further confirmed by sequencing using ribosomal gene (unpublished data). The summary of taxonomy study on these three isolates is as follows (Table 4): isolate GanoEF1 belongs mainly to Ascomycetes while isolates GanoEF2 and GanoEF3 belong mainly to Basidiomycetes. Isolates GanoEF1, GanoEF2 and GanoEF3 were identified as Hendersonia sp., Amphinema byssoides and Phlebia radiata respectively. Isolate GanoEF1 was selected for nursery trial based on its strong competitive ability against G. boninense in vitro.

Table 4: Fungi taxonomy and nomenclature

| Fungi taxonomy | Isolate code GanoEF1 | GanoEF2 | GanoEF3 |
|---|---|---|---|
| Kingdom | Fungi | Fungi | Fungi |
| Phylum | Ascomycota | Basidiomycota | Basidiomycota |
| Class | Dothideomycetes | Agaricomycetes | Agaricomycetes |
| Order | Pleosporomycetidae | Atheliales | Polyporales |
| Family | Pleosporales | Atheliaceae | Meruliaceae |
| Genus | *Hendersonia* | *Amphinema* | *Phlebia radiata* |
| Species | *unknown* | *A. byssoides* | *P. radiata* |

## 3.5 Suppression of basal stem rot disease by antagonistic Hendersonia GanoEF1 under greenhouse conditions

Generally, the BSR disease developed much more slowly in the seedlings treated with a conidial suspension of Hendersonia sp. isolate GanoEF1. A lower DI would indicate some disease suppression by the endophytes. Based on the two nursery trials, at two and four months, Hendersonia sp. conidial suspension application on seedlings recorded lower DI with no significant difference with untreated seedlings. At the end of the experiment, DI was only at an average of 33.3% and gave significant readings compared to the untreated seedlings at 100%, suggesting good disease suppression by the endophytic fungus, Hendersonia sp. isolate GanoEF1 (Figure 3).

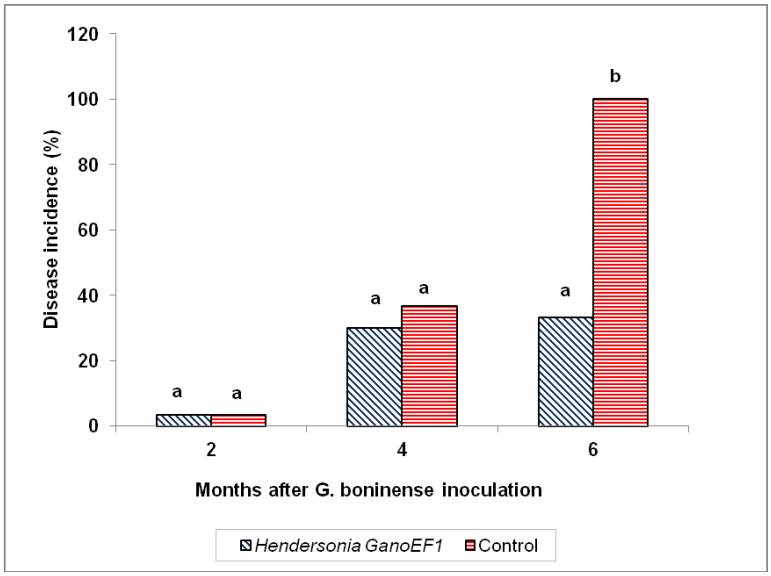


Figure 3: Average percentage of disease incidence (DI) of seedlings due to G. boninense infection at two, four and six months after treatment. Means with the same letters denote no significant difference according to t-test at $P<0.05$.

With respect to SFS, there was a significant difference between treated and untreated seedlings at four and six months after treatment. The SFS of the seedlings treated with a conidial suspension of Hendersonia sp. isolate GanoEF1 showed a significantly lower severity at an average of 11.2% and 56.7% at four and six months, respectively. As expected, the untreated seedlings recorded the highest SFS at an average of 56.7% and 93.0% at four and six months, respectively (Figure 4).

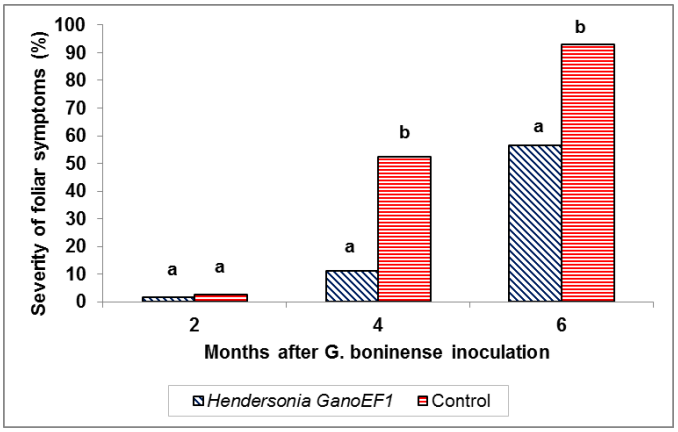


Figure 4: Average percentage of the severity of foliar symptoms (SFS) of seedlings due to G. boninense infection at two, four and six months after treatment. Means with the same letters denote no significant difference according to t-test at P<0.05.

Percentage of dead seedlings at two months was 0% for both treated and untreated seedlings and at four months, the percentage of dead seedlings in untreated treatment increased to an average of 10.0%. As expected, the untreated seedlings showed a higher percentage of dead seedlings (83.3%) and significantly different with the treated seedlings at an average of 36.7% at six months after challenge with G. boninense (Figure 5).

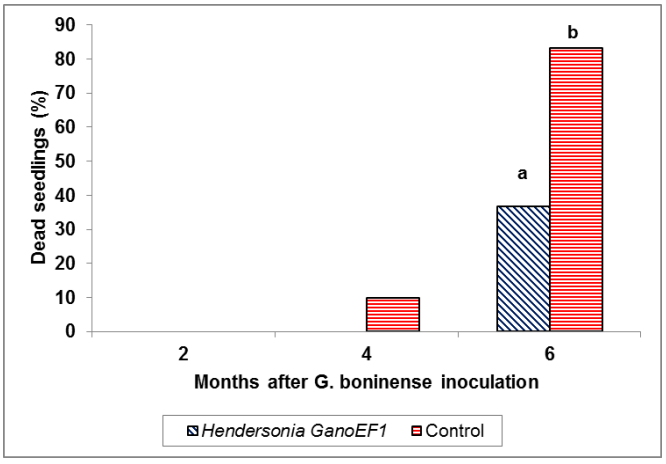

Figure 5: Average percentage of dead seedlings due to G. boninense infection at two, four and six months after treatment. Means with the same letters denote no significant difference according to t-test at $P<0.05$.

The disease development was also evaluated by the AUDPC (Table 5). The ability of this isolate to reduce BSR loss was expressed as the percentage of DR derived from the values of AUDPC. The AUDPC values suggest the amount of disease developed in each treatment. The treatment with the lowest AUDPC value indicates that the effectiveness of the biocontrol agent in reducing the disease. Seedlings treated with a conidial suspension of Hendersonia sp. isolate GanoEF1 gave the lowest AUDPC of 113.3 and 86.7 in Trials 1 and 2, respectively, compared to the untreated seedlings with AUDPC of 180.0 and 193.3 in Trials 1 and 2, respectively. At six months after inoculation, BSR incidence was reduced by 37.0% to 55.2% in the treated seedlings.

Table 5: Effect of conidial suspension of Hendersonia sp. isolate GanoEF1 on basal stem rot (BSR) disease development in oil palm seedlings at six months after treatment

| Treatments | Trial 1 | | Trial 2 | |
|---|---|---|---|---|
| | AUDPC# | DR## (%) | AUDPC# | DR## (%) |
| Seedlings untreated with conidial suspension of *Hendersonia sp.* isolate GanoEF1 and inoculated with *G. boninense* (as control, C1) | 180.0 | - | 193.3 | - |
| Seedlings treated with conidial suspension of *Hendersonia sp.* isolate GanoEF1 and inoculated with *G. boninense* (T1) | 113.3 | 37.0 | 86.7 | 55.2 |

Note: # Area under disease progress curve (AUDPC).## Disease reduction (DR). Average disease reduction (DR) = 46.1%.

# 4 Discussion

Previous studies have shown that the use of microbes can help limit Ganoderma damage in oil palm. The biological properties of several antagonistic fungi, mainly Aspergillus (29), Penicillium (30) and Trichoderma (31-34) as well as endophytic bacteria (35) and

Streptomyces sp. (36) have been studied and proven to be antagonistic against G. boninense. Shamala (37) reported on endophytic Trichoderma exhibiting a potential to inhibit G. boninense growth through in vitro assessment. Endophytic bacteria, Burkholderia sp. isolated from the roots of oil palm tree in North Sumatera, Indonesia also showed an inhibition activity against G. boninense growth in vitro (38). Our results support these findings by showing that endophytic fungi isolated from the healthy roots of oil palm also restrict in vitro growth of the causal agent of BSR disease, G. boninense. In vitro results may not necessarily indicate on what is really occurring in the plant systems. However, in vitro studies are useful for identifying probable candidates that exhibit biocontrol properties from a large population. The promising candidates identified in the in vitro studies were further corroborated in the nursery trials. Overall, these results strongly suggest that the fungal endophyte species associated with oil palm play an essential role in the resistance of their hosts to pathogen damage and those endophytes can potentially be used as effective BCA.

The results of our study indicate that the use of endophytic fungus, Hendersonia sp. isolate GanoEF1 isolated from the oil palm roots can control Ganoderma disease. To the best of our knowledge, this is the first report on the isolation of Hendersonia sp. from oil palm to control BSR. The Hendersonia sp. isolate GanoEF1 showed excellent antagonistic properties against G. boninense in both in vitro assays. The lower percentage of DI, SFS and DS from the seedlings treated with this isolate over the control treatment suggested that the inoculated seedlings with endophytic fungus had built some form of tolerance to the physical damage by G. boninense. This study also found that isolate Hendersonia sp. was able to significantly suppress Ganoderma disease based on its DR at the average of 46.1%. Overall, disease development was faster in the untreated seedlings compared to the seedlings treated with Hendersonia sp. isolate GanoEF1.

In this study, disease reduction in the seedlings treated with Hendersonia sp. isolate GanoEF1 after challenge with G. boninense suggested that the endophytic fungus could play a role by inhibiting the penetration of Ganoderma fungus into the vascular systems via the roots of oil palm. Gao, Dai (39) characterised the progressive endophytic colonisation by fungal endophytes in the in-

tercellular or intracellular may establish the endophytic niche in the host tissues which they will gain a source of nutrition provided from plant fragment. At the same time, the endophytic fungus will protect the plant by rapid colonisation therefore limiting the available substrates for the pathogen to grow (40, 41). Bacterial and fungal endophytic microorganisms may contribute in inhibiting penetration by Ganoderma and its movement to the vascular system as they mostly occur in the vascular systems adjacent to the phloem and xylem vessels. Observation in the histological study by Zaiton, Sariah (21) supported that endophytic bacteria being more concentrated in vascular systems of the roots taken from symptomless palms. Fungal endophytes reduce the activity of plant pathogens by several mechanisms, including antibiosis, mycoparasitism, induction of plant resistance and promotion of plant growth and physiology. Moreover, some endophytic fungi stimulate the plant secondary metabolites production such as phytoalexins as a means of induced defence mechanisms in plants. Research conducted by Buana, Wahyudi (38) revealed that endophytic bacteria, Burkholderia B212 has a potential in producing antifungal agent such as pyrrolnitrin for the mechanism of biological control of plant pathogen. Further investigation of bioactive compounds produced by Hendersonia sp. isolate GanoEF1 or the mechanism involved in controlling the Ganoderma disease is important to be evaluated since the strain is a potential source for exploring novel bioactive metabolites to reduce the disease-causing activity by the pathogenic Ganoderma fungus.

# 5 Conclusion

Hendersonia sp. isolate GanoEF1 gave the best results in controlling G. boninense in both bioassays. This fungus overgrew the pathogens in the in vitro assay and reduced the incidence of Ganoderma disease in the nursery study. Although encouraging results were obtained in using Hendersonia sp. isolate GanoEF1 as BCA against Ganoderma disease, further studies must be conducted in the very near future to confirm their effectiveness in the field.

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