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Capability of Bioorganic Fertilizer *Pseudomonas* GanoEB3 for Suppressing Basal Stem Rot Disease in Oil Palm Seedlings

AW Nurul Isma[1,3], AS Idris[3], N Rosimah[1], O Hishamuddin[2], A Mohd. Puad[2]

[1]Department of Biology, Faculty of Science, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

[2]Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Science, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

[3]Ganoderma and Diseases Research for Oil Palm Unit (GANODROP), Division of Biology, Malaysian Palm Oil Board (MPOB), No. 6, Persiaran Institusi, Bandar Baru Bangi, Kajang, Selangor, Malaysia

ABSTRACT

Oil palm is one of the important crops in Malaysia. Basal Stem Rot (BSR) disease is the biggest threat for oil palm production in Malaysia caused by *Ganoderma boninense* which had been causing a huge damage for the oil palm industry in Malaysia. The objective of this study was to develop bioorganic fertilizer containing *Pseudomonas* GanoEB3 for suppressing basal stem rot disease in oil palm seedlings. Endophytic bacteria, *Pseudomonas* GanoEB3 was isolated from healthy oil palm roots and cultured on nutrient agar media. A suspension containing 10^8 CFU/mL of the bacteria cells being mixed with vermiculite powder and stored at room temperature. The vermiculite powder containing *Pseudomonas* GanoEB3 has been formulated with Bioorganic EFB and Bioorganic RS. Pathological analysis showed that Bioorganic EFB *Pseudomonas* GanoEB3 (T3) and Bioorganic RS *Pseudomonas* GanoEB3 (T4) have a good potential in inhibiting basal stem rot disease in oil palm seedlings. After eight months of experiment, oil palm seedlings treated with Bioorganic EFB *Pseudomonas* GanoEB3 and Bioorganic RS *Pseudomonas* GanoEB3 resulted the reduced percentage for following parameters; disease incidence (DI) for T3 (40%) and T4 (50%), area under the disease progress curve (AUDPC) for T3 (42 units2) and T4 (76 units2), disease severity of foliar index (DSFI) for T3 (37.5%) and T4 (45%), disease severity of bole index (DSBI) for T3 (40%) and T4 (47.5%), disease severity of root index (DSRI) for T3 (55%) and T4 (52.5%) and dead seedlings for T3 (30%) and T4 (40%) compared with control treatment (T2). It shows that both treatments have a good potential in inhibiting BSR disease in oil palm seedlings. This study revealed that Bioorganic EFB and Bioorganic RS containing *Pseudomonas* GanoEB3 are suitable as an effective biological control agent for suppressing BSR disease in oil palm seedlings.

Keywords: Basal stem rot, endophytic bacteria, *Pseudomonas* GanoEB3, bioorganic EFB *Pseudomonas* GanoEB3, Bioorganic RS *Pseudomonas* GanoEB3, pathological study

I. INTRODUCTION

Oil palm (*Elaeis guineensis*) is one of the important crops in Malaysia and it proved strengthening the economics of Malaysia. Current production of crude palm oil (CPO) of Malaysia has reached 19 million metric tons (Mmtons) which contributed 8% to the country's Gross National Income (GNI). The oil palm estates produce an estimated 80 Mmtons of dry weight biomass which includes fronds, trunks, empty fruit bunches (EFB) and other biomass fractions.

Unfortunately, the growth of oil palm is influenced by pests and diseases. The crop is exposed to pest and disease problems start from seed germination up to field

planting. Brown germ, upper stem rot and bagworm are the examples of pest and disease problems, and the most serious disease is basal stem rot (BSR). The BSR disease has caused a huge damage especially to the oil palm plantation in Malaysia for the past 50 years.

BSR disease is considered the biggest threat to sustainable palm oil production especially in South East Asia. Previous study reported that the most severe losses from BSR occurred in Malaysia and Indonesia with lower incidences was being recorded in Thailand, Africa and Papua New Guinea. Meanwhile, Lim *et al*. (1992) recorded an average of 50% yield losses from 80% of 13-year-old plantings in coastal area of Malaysia.

Controlling the pathogen is an important factor since the pathogen caused severe losses of oil palm production. BSR field control by using chemicals, such as pesticide and fungicide have not been very successful even with *in vitro* efficacy of fungicides against *G. boninense* have not been reported. This is probably attributed to the fact that the palms might already have the disease by the time the treatment was applied.

To overcome this problem, a new strategy of using biological control agents may be developed in order to find an effective and environmental friendly treatment against BSR disease. Biological control is ecologically safe and it will not pollute the environment. The approach may not necessarily be a cure for the disease but it could arrest the spread of the disease.

The suppression of plant diseases due to the action of endophytic microorganisms has been demonstrated in several pathosystems. Several mechanisms may control this suppression, either indirectly by induction of plant defense response or directly inside the plant by antibiosis and competition for nutrients. Nasyaruddin and Idris (2011) reported that *Pseudomonas* GanoEB3 pure culture has the capability in inhibiting the growth of *G. boninense in vitro*, and effective in controlling *G. boninense* infection in oil palm seedlings. Therefore, this study was conducted to study the potential of bioorganic fertilizer containing *Pseudomonas* GanoEB3 to control BSR disease in oil palm seedlings.

II. METHODS AND MATERIAL

2.1 Study Site- Pathological experiment was conducted at Ladang MPOB, Seksyen 15, Bandar Baru Bangi, Selangor, Malaysia which assigned and arranged in a Completely Randomized Design (CRD). Four-month-old oil palm seedlings (*Dura x Pisifera*) were obtained from Felcra Sungai Tekam, Pahang, Selangor have been used in this study.

2.2 Experimental Design- The experiment was carried out with four treatments and four times data taken (2, 4, 6, 8 months after treatment). Each treatment consisted of ten seedlings. Thus, the total number of seedlings used was 40 seedlings. All oil palm seedlings were grown in polybags (12x18 cm) containing a mixture of soil:sand (3:1) and watered twice daily. Pathological measurement are disease incidence (DI), area under the disease progress curve (AUDPC), disease severity of foliar, bole and root and dead seedlings as shown in Table 2.1.

Table 2.1: Experimental design of pathological analysis

		Parameter
Pathological Analysis		- Disease Incidence - Area Under the Disease Progress Curve (AUDPC) - Disease Severity Index (DSI) (foliar, bole, root) - Dead Seedlings
No of treatment = 4 No of seedling per treatment = 10	Time taken: 2 months intervals (4 times)	Total seedlings: 4x10 **40 seedlings**

Four treatments have been conducted (Table 2.2). Both Bioorganic EFB and Bioorganic RS containing *Pseudomonas* GanoEB3 were applied to the seedlings according to their treatment for every two month interval and alternate with NPK Blue Fertilizer up to eight months of experiment.

Table 2.2: Treatments of the study on the potential of Bioorganic containing *Pseudomonas* GanoEB3 to suppress basal stem rot disease

	Treatments
T1	Seedling uninoculated with *G. boninense* and untreated (Negative Control)
T2	Seedling artificially inoculated with *G. boninense* and untreated (Positive Control)
T3	Seedling artificially inoculated with *G. boninense* and treated with 30 g Bioorganic EFB *Pseudomonas* GanoEB3
T4	Seedling artificially inoculated with *G. boninense* and treated with 30 g Bioorganic RS *Pseudomonas* GanoEB3

2.3 Preparation of Rubber Wood Block (RWB)

2.3.1 Isolation of *G. boninense* from *Ganoderma* Selective Medium (GSM)

The isolation of *G. boninense* was carried out according to Ariffin and Idris (1991). The pure culture of *G. boninense* was initially isolated from infested oil palm nursery situated in Kajang, Selangor, Malaysia. Sterile *G. boninense* were excised and placed on the *Ganoderma* Selective Medium (GSM). After a week of incubation, growing hyphae around the excised tissue was transferred into new PDA plate (Figure 2.1A). *G. boninense* was then sub-cultured to mass-multiply a pure culture as shown in Figure 2.1B.

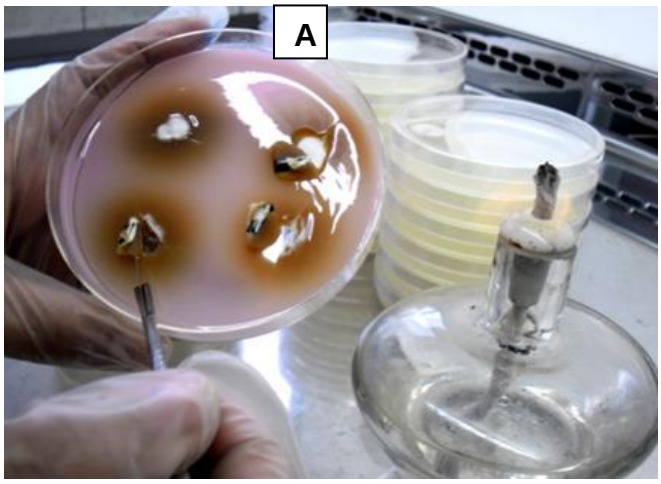

Figure 2.1 A : Isolation of G. *boninense* fruiting body from GSM into PDA plate

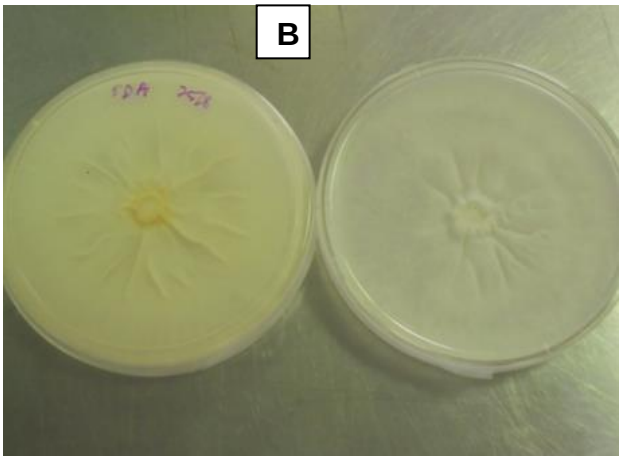

Figure 2.1 B : Pure culture of *Ganoderma boninense* after seven days incubation

2.3.2 Inoculation of *G. boninense* into Rubber Wood Block (RWB)

Rubber wood blocks (RWB) measuring (6 cm x 6 cm x 6 cm) were prepared according to Zaiton (2008) with slightly modification. RWB were washed and dried in an oven, placed in heat resistant polypropylene bags and autoclaved twice for 30 minutes at 121°C. Molten malt extract (MEA) (70 mL) was added as supplementary nutrients for *G. boninense*. The bag with RWB and molten MEA were autoclaved at 121°C for 30 minutes. After sterilization and cooling, the RWB in polypropylene bag was rotated to ensure that it was fully covered by MEA. Following that, 10 mm plugs taken from seven-day-old *Ganoderma boninense* culture were inoculated into each of the RWB. The inoculated blocks were then incubated in an incubator at 28°C±2 up to three months until fully colonized by the *G. boninense* as shown in Figure 2.2.

Figure 2.2 : Rubber wood block covered with white mycelia of *Ganoderma boninense* after three months incubation period

2.4 Pathological Disease Assessments

Disease assessment on the effect of bioorganic fertilizer *Pseudomonas* GanoEB3 to suppress *Ganoderma* disease was done acoording to quantitative assessment which are Disease Incidence (DI), Area Under the Disease Progress Curve (AUDPC), Disease Severity of foliar, bole and root index and also Dead Seedling (DS). The DI was measured according to Campbell and Madden (1990) with slight modification which is the number of visibly diseased seedlings (chlorosis and necrosis of leaves, with or without sporophore production) relative to the total number of seedlings and assessed using the following formula:

$$DI = \frac{\text{number of seedlings infected}}{\text{total number of seedlings assessed}} \times 100$$

Disease incidence reduction as compared to control treatment would indicate the effectiveness of the treatment in suppressing the disease. This kind of value was assessed by plotting the data in the form of a disease progress curve. The Area Under the Disease Progress Curve (AUDPC) can be calculated using the following formula (Campbell and Madden, 1990):

$$AUDPC = \sum_{i}^{n-1} \left(\frac{Y_i + Y_{i+1}}{2} \right) \left(t_{i+1} - t_i \right)$$

Where,
n= the number of assessment times
Y= the disease incidence
t= time of observation

The curve slope was obtained by transforming the DI data with the monomolecular model (Monit) from Campbell and Madden (1990).

Disease Severity Index (DSI) refers to the total area or volume of plants tissue that is diseased as described by Kranz (1988). Disease severity index was calculated based on the symptoms appearing on the foliage (external) (Table 2.3) according to Abdullah *et al.* (2003) with slight modification.

Table 2.3: The signs and symptoms of foliar index were scored on a scale 0-4

Disease class	Signs and symptoms of infection
0	Healthy seedlings with green leaves without appearance of fungal mycelium on any part of plants
1	Presence of white mycelium or fruiting body on any parts of plants without necrosis or chlorosis leaves
2	Presence of white mycelium or fruiting body on any parts of plants with necrosis or chlorosis leaves (>10% <25%)
3	Presence of white mycelium or fruiting body on any parts of plants with necrosis or chlorosis leaves (>25% and <75%)
4	Presence of white mycelium or fruiting body on any parts of plants with necrosis or chlorosis leaves (>75%) or seedling dead

The disease severity of foliar symptoms was calculated using the formula from Liu *et al.* (1995) as follows:

$$DS_{foliar} = \frac{\text{Number of seedlings in the rating x rating number}}{\text{Total no. of seedlings assessed x highest rating}} \times 100$$

After eight months of inoculation with *Ganoderma boninense*, seedlings were uprooted, split longitudinally and finally visually assessed in order to study the internal symptoms according to the rating of the bole-tissue damage (Table 2.4) and also root-tissue damage (Table 2.5) produced by *G. boninense* based on the modified scale from Nur Sabrina *et al*. (2012):

Table 2.4: The signs and symptoms of bole index were scored on a scale 0-4 (Nur Sabrina *et al*., 2012)

Disease class	Signs and symptoms of infection
0	Healthy of bole tissue without appearance of fungal mycelium on any part of plants
1	Presence of white mycelium or fruiting body on any parts of plants without rotting of bole tissue
2	Presence of white mycelium or fruiting body on any parts of plants with rotting of bole tissue (>10% <25%)
3	Presence of white mycelium or fruiting

	body on any parts of plants with rotting of bole tissue (>25% <75%)
4	Presence of white mycelium or fruiting body on any parts of plants with rotting of bole tissue (>75%) or seedling dead

Table 2.5: The signs and symptoms of root index were scored on scale 0-4 (Nur Sabrina *et al*., 2012)

Disease class	Signs and symptoms of infection
0	Healthy of root tissue without appearance of fungal mycelium on any part of plants
1	Presence of white mycelium or fruiting body on any parts of plants without rotting of root tissue
2	Presence of white mycelium or fruiting body on any parts of plants with rotting of root tissue (>10% <25%)
3	Presence of white mycelium or fruiting body on any parts of plants with rotting of root tissue (>25% <75%)
4	Presence of white mycelium or fruiting body on any parts of plants with rotting of root tissue (>75%) or seedling dead

Disease severity for both internal symptoms of the bole tissue and root tissue was calculated based on the following formula derived from Liu *et al*. (1995):

$$DS_{bole/root} = \frac{\text{Number of seedlings in the rating x rating number}}{\text{Total no. of seedlings assessed x highest rating}} \times 100$$

2.5 Statistical Analysis

Statistical test was carried out using Minitab 16 statistical package (Minitab Inc.). Analysis of Variance (ANOVA, $p<0.05$) was used to determine if a statistically significance difference was observed between the treatments. Tukey multiple comparison test (at $p<0.05$) was applied to determine which means are statistically difference if the ANOVA was significant.

III. RESULT AND DISCUSSION

3.1 Disease Incidence

Disease Incidence (DI) assessment was assessed based on the appearance of foliar symptom, white button, white mycelia or fruiting body in the oil palm seedlings. In this study, disease symptom was first appeared after 4 months inoculation of *Ganoderma boninense* into oil palm seedlings for all treatments. After 4 months inoculation of *G. boninense*, T2 (positive control) showed the highest DI percentage (50%) compared with T1, T3 and T4 as shown in Figure 3.1. After 6 months inoculation of *G. boninense*, the presence of white mycelia of *G. boninense* can be observed and later it was developed into fruiting body on the 7th month inoculation.

At the end of the experiment (8 months after inoculation), T2 showed the highest DI percentage and significantly different (Tukey comparison test, $p<0.05$) 85% which is almost all the seedlings were infected with *G. boninense* and significantly higher from T1 (0%), T3 (36%) and T4 (46%). Meanwhile, T3 and T4 did not show any significant different between both treatment, but T3 showed the lower DI percentage as compared to T4 and this suggested that T3 (seedlings artificially inoculated with *G. boninense* and treated with Bioorganic EFB *Pseudomonas* GanoEB3) showed a better level of disease suppression than T4 (seedlings artificially inoculated with *G. boninense* and treated with Bioorganic RS *Pseudomonas* GanoEB3).

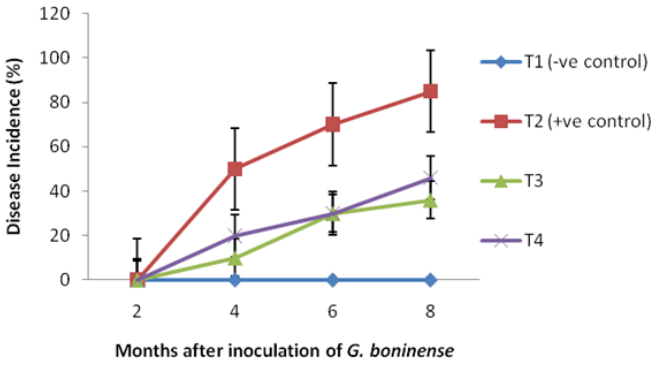

Figure 3.1: Disease Incidence (DI) of *G. boninense* inoculated oil palm seedlings based on chlorosis, necrosis of leaves, with and without production of sporophore. T1 (-ve control), T2 (seedlings inoculated with *G. boninense*), T3 (seedlings

inoculated with *G. boninense* and treated with Bioorganic EFB *Pseudomonas* GanoEB3) and T4 (seedlings inoculated with *G. boninense* and treated with Bioorganic RS *Pseudomonas* GanoEB3). Values are the means and standard error (n=10)

3.2 Area under the Disease Progress Curve (AUDPC)

Disease development was also assessed based on the Area under the Disease Progress Curve (AUDPC) and calculated using the DI, SFS or DSI value. High AUDPC value indicated that high disease symptom progress, thus showed that lower disease resistance. Seedlings inoculated with *G. boninense* and untreated (T2) showed the highest AUDPC value of 264.4 units^2 and significantly different (Tukey comparison test, $p<0.05$) compared with T1 (seedlings uninoculated and uninfected), T3 (seedlings inoculated with *G. boninense* and treated with Bioorganic EFB *Pseudomonas* GanoEB3) and T4 (seedlings inoculated with *G. boninense* and treated with Bioorganic RS *Pseudomonas* GanoEB3). T3 and T4 showed lower AUDPC value compared to T2 which are 42 units^2 and 73 units^2 respectively but no significance different amongst them as shown in Table 3.1. Lower AUDPC values indicate the effectiveness of the bioorganic *Pseudomonas* GanoEB3 in suppressing the BSR disease. Therefore, seedlings treated with Bioorganic EFB *Pseudomonas* GanoEB3 (T3) showed the most effective treatment in inhibiting the growth of *G. boninense* followed by seedlings treated with Bioorganic RS *Pseudomonas* GanoEB3 (T4).

Table 3.1: The potential of Bioorganic fertilizer containing *Pseudomonas* GanoEB3 in suppressing BSR disease in oil palm seedlings after 8 months inoculation

Treatment	AUDPC (units^2)
T1- Seedling uninoculated with *G. boninense* and untreated (Negative Control)	0[b]
T2- Seedling artificially inoculated with *G. boninense* and untreated (Positive Control)	264.4[a]
T3- Seedling artificially inoculated with *G. boninense* and treated with Bioorganic EFB *Pseudomonas* GanoEB3	42[b]
T4- Seedling artificially inoculated with *G. boninense* and treated with Bioorganic RS *Pseudomonas* GanoEB3	73[b]

Area Under the Disese Progress Curve (AUDPC) of *G. boninense* inoculated oil palm seedlings. T1 (-ve control), T2 (seedlings inoculated with *G. boninense*), T3 (seedlings inoculated with *G. boninense* and treated with Bioorganic EFB *Pseudomonas* GanoEB3) and T4 (seedlings inoculated with *G. boninense* and treated with Bioorganic RS *Pseudomonas* GanoEB3). Values are the means and standard error (n=10)

3.3 Disease Severity Index (foliar, bole and root)

Disease severity of foliar index (DSFI) (external symptoms) was calculated based on the scale from 0 to 4 as shown in Figure 3.2. The application of bioorganic fertilizer *Pseudomonas* GanoEB3 gave effects on BSR severity which the DSFI was lower as compared with seedlings inoculated and infected with *G. boninense* (T2). From the beginning of the experiment up to eight months of experiment showed that T2 significantly shown the highest DSFI value with 85.9%, followed by T4 and T3 with the value 45.5% and 38.1%, respectively as can be seen in Figure 3.3. Low severity of the foliar symptoms showed slow progress of *G. boninense* infection in oil palm and expressed partial resistance towards the disease and this study found that T3 is more effective in reducing the development of disease severity in oil palm seedlings.

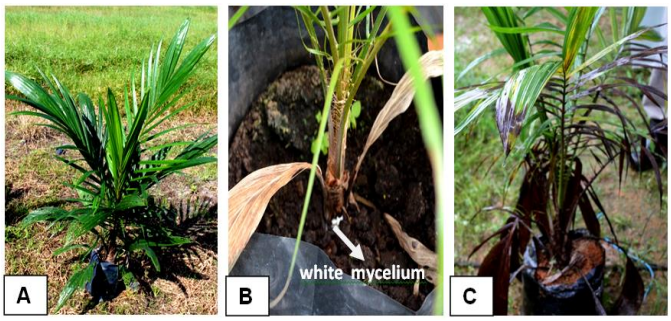

Figure 3.2 : Disease severity of foliar index (DSFI). A) Healthy seedlings with green leaves without apperance of fungal mycelium on any part of plant. B) Presence of white mycelium on any part of plant without necrosis or chlorosis leaves. C) Presence of white mycelium on any part of plant with necrosis or chlorosis leaves (>10% <25%). D) Presence of white mycelium on any part of plant with necrosis or chlorosis leaves (>25% <75%). E) Presence of fruiting body on any part of plant with necrosis or chlorosis leaves (>75%) or seedling dead

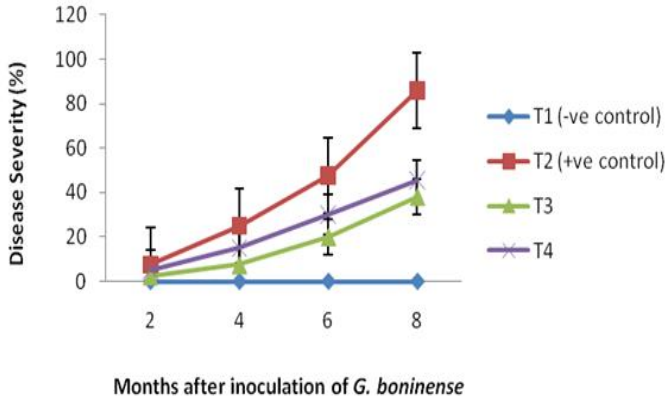

Figure 3.3: Disease Severity of Foliar Index (DSFI) of *G. boninense* inoculated oil palm seedlings for T1 (-ve control), T2 (seedlings inoculated with *G. boninense*), T3 (seedlings inoculated with *G. boninense* and treated with Bioorganic EFB *Pseudomonas* GanoEB3) and T4 (seedlings inoculated with *G. boninense* and treated with Bioorganic RS *Pseudomonas* GanoEB3). Values are the means and standard error (n=10)

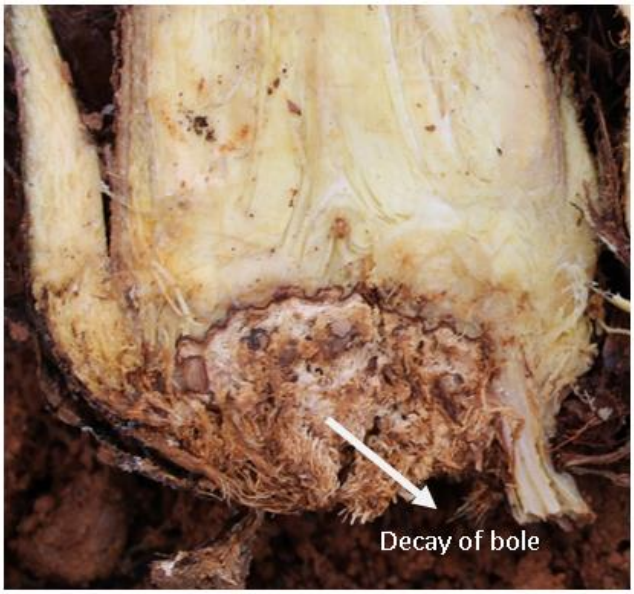

Figure 3.5 : Rotting of root tissue of seedlings challenged with *Ganoderma boninense* after eight months of experiment

Figure 3.4 : Decay of bole of inoculated seedlings with *Ganoderma boninense* after eight months of experiment

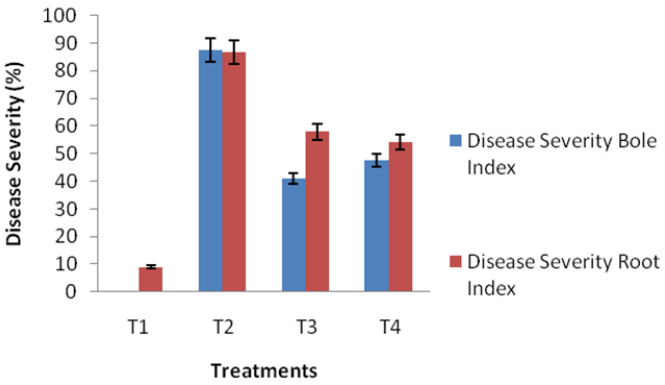

Figure 3.6 : Disease Severity of Bole and Root Index (DSBI/DSRI) of *G. boninense* inoculated oil palm seedlings

for T1 (-ve control), T2 (seedlings inoculated with *G. boninense*), T3 (seedlings inoculated with *G. boninense* and treated with Bioorganic EFB *Pseudomonas* GanoEB3) and T4 (seedlings inoculated with *G. boninense* and treated with Bioorganic RS *Pseudomonas* GanoEB3). Values are mean and standard error (n=10)

3.4 Dead Seedling

Percentage of dead seedlings for T3 and T4 showed a significantly difference (Tukey comparison test, $p<0.05$) with respect to the T2. However, the percentage of dead seedlings was not significantly difference between the treatment. Up to eight months of experiment, T2 showed the highest percentage of dead seedlings with 79% and significantly higher than T4 with 43% while T3 showed the percentage of dead seedlings with 33% as shown in Figure 3.7. T3 showed the lower percentage of dead seedlings indicate that the Bioorganic EFB *Pseudomonas* GanoEB3 has an ability to reduce the infection of *G. boninense* disease by reducing the number of dead seedling in oil palm.

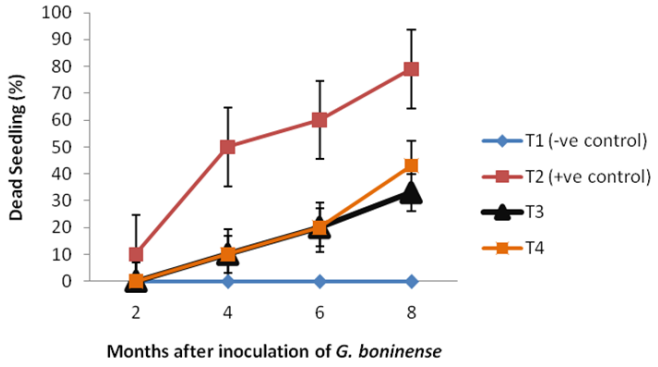

Figure 3.7 : Dead seedlings of *G. boninense* inoculated oil palm seedlings. T1 (-ve control), T2 (seedlings inoculated with *G. boninense*), T3 (seedlings inoculated with *G. boninense* and treated with Bioorganic EFB *Pseudomonas* GanoEB3) and T4 (seedlings inoculated with *G. boninense* and treated with Bioorganic RS *Pseudomonas* GanoEB3). Values are the means and standard error (n=10)

DISCUSSION

In this study, the value of DI%, AUDPC, DSFI%, DSBI%, DSRI% and also DS% of T3 and T4 is lower than T2. It suggested that both Bioorganic EFB and Bioorganic RS containing *Pseudomonas* GanoEB3 (T3 and T4) shows a good potential to suppress the growth of *Ganoderma boninense*. This study found that *Pseudomonas* GanoEB3 shown a good ability for suppressing the basal stem rot (BSR) incidence in oil palm seedlings. This can be seen through the reduction of DI% and also DS% in the oil palm seedlings treated with both bioorganic containing *Pseudomonas* GanoEB3 (T3 and T4) after inoculated with *G. boninense* up to eight month of experiment.

This finding suggested that the bacteria play an important role in inhibiting the penetration of *Ganoderma* towards the vascular system and proposed that bacteria endophytes were more concentrated in vascular system of roots (Zaiton *et al*., 2006; Sarim, 2013). Study by Dikin *et al.* (2003) reported that *Pseudomonas aeruginosa* and *Burkholderia cepacia* were a good biocontrol agents for controlling pathogen in oil palm, *Schizopyllum commune*. The ability to suppress the fungal might be due to the induction of the host defense mechanism, example the formation of barriers such as lignified cell walls (Zaiton, 2008; Ming *et al*., 2013; Roozbeh *et al*., 2013) in order to inhibit the pathogen and the production of antifungal metabolites to reduce the infection progress and thus improved the plant growth and plant vigour (Hammerschmidt and Kuc, 1995).

In addition, the interaction between beneficial bacteria and plants has been proven gave the positive effects on the crop health and yield (Kloepper *et al*., 1989; Sturz *et al*., 2000). Other studies by Van Loon and Bakker (2003) found that the mechanism involved in supporting the plant growth and health including increase the availability of soil nutrient, improving soil structure, inducing the plant defense mechanisms, producing antibiotics and also providing growth-stimulating substances or enzyme. Besides that, *Pseudomonas* sp. also has been classified as a Plant Growth Promoting Bacteria (PGPB) and shown to improve the plant growth. The advantages of PGPB towards the plant growth always correlated with the increase in the root morphology like lateral root length, root hair number, shoots length and yield (Van Loon and Bakker, 2003). This is generally assumed that such developmental responses is due to the interaction between bacteria and plant, thus triggered phytohormone such as auxin, cytokinins and gibberellins produced by bacteria (Persello-Cartieaux *et al*., 2003). Previous studies found that, *Pseudomonas* could produce plant hormones

including auxins and cytokinins and also volatile signals such as ethylene 2,3-butanediol and acetonin (Lambrecht *et al*., 2000; Persello-Cartieaux *et al*., 2003; Ryu *et al*., 2003).

Cytokinins compunds that is important for development of plants as they play a role as a stimulator for plant cell divisions induce seed germination, induce the biosynthesis of chlorophyll, nucleic acids and also chloroplast as early stage of leaf development (Skoog and Armstrong, 1970). Previous study shown that, most of the signs and symptoms for diseased seedlings can be manifested approximately 3 to 4 months after inoculation for 4 to 6-months-old seedlings (Sariah *et al*., 1994; Breton *et al*., 2006; Rees *et al*., 2007; Nur Ain Izzati and Abdullah, 2008). This could be verified through this study which the first symptoms such as fungal mass or initial infection symptoms can be seen after 4 months inoculation with *G. boninense*.

Studies by Bivi *et al*. (2010) found that the inhibitory effect of isolated endophytic bacteria from healthy oil palm roots on *G. boninense in vitro* showed that among 20 isolates, *Pseudomonas aeruginosa* was the only identified bacteria which showed significantly strong inhibitory on the growth of mycelia in the media culture. Zaiton *et al*. (2006) also suggested that *Burkholderia cepacia*, *Serratia marcescens* and *Pseudomonas aeruginosa* showed the antagonistic activity against *G. boninense* and introduced as a potential candidates for biological control aganist *G. boninense*.

Furthermore, studied by Azadeh and Sariah (2009) found that *Pseudomonas aeruginosa* contains high proportion of regulatory genes and many genes involved in the catabolism, efflux and transport of organic compound and also for potential chemotaxis systems. While, studied by Larry and Colin (2001) suggested that *Pseudomonas aeruginosa* PAO1 showed an ability to kill and paralyzed the nematode. This is due to the factor of hydrogen cyanide produced by this strain can kill the nematode. The potential of this strain causing opportunistic infections in humans, thus this strain has to be used with careful guidelines for agricultural purposes.

However, studies by Casler *et al*. (2002) suggested that the breeding and selection of oil palms containing more lignin making them more resistance to the *G. boninense* infection may be another suggestions for disease control and need more investigations. Meanwhile Santos *et al*. (2008) proposed that the ability of trees to express chitinase and glucanase provides a potential target for genetically resistant trees. Several types on plant chitinase have been detected from different parts of various plants which involved the defence mechanism against antifungal as studied by Liu *et al*. (2005) such as acidic iso-electric point accumulates in intercellular spaces (Metraux *et al*., 1989; Silipo *et al*., 2010) or basic iso-electric which remain in central vacuoles (Mauch and Staehelin, 1989; Collinge *et al*., 1993).

It has been demonstrated by Dikin *et al*. (2005) that the isolated endophytic bacteria are antagonistic to the pathogens *in vitro* and this study proved that the endophytic bacteria also have an ability to suppress the *G. boninense in vivo*.

IV. CONCLUSION

This finding showed that Bioorganic EFB *Pseudomonas* GanoEB3 and Bioorganic RS *Pseudomonas* GanoEB3 have a good potential in inhibiting the growth of *G. boninense*. This result proved that both Bioorganic EFB and Bioorganic RS containing *Pseudomonas* GanoEB3 has an ability to be used as a biocontrol agents for controlling BSR disease.

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VI. REFERENCES

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