INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
13–1307/2015/17–1–135–141
http://www.fspublishers.org

Full Length Article

Growth Promoting Effects of Endophytic Fungus *Phlebia* GanoEF3 on Oil Palm (*Elaeis guineensis*) Seedlings

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Abstract

Isolation from trunk and root tissues of oil palms by Malaysian Palm Oil Board (MPOB) has found several promising fungi, mainly *Hendersonia* GanoEF, *Amphinema* GanoEF2, and *Phlebia* GanoEF3. The objective of this study was to investigate the potential of *Phlebia* GanoEF3 to serve as a biological fertilizer and eventually promote the oil palm seedlings growth. Two types of organic fertilizers; Empty Fruit Bunches (EFB) powder and Real Strong Bioorganic Fertilizer (RSBF) were incorporated into the formulation to develop biofertilizers containing endophytic fungus *Phlebia* GanoEF3. Five ratios of fertilizer to fungus (10: 50, 20: 40, 30: 30, 40: 20 and 50: 10) for each formulation were prepared and *in vitro* study and the shelf life of viable cell of *Phlebia* GanoEF3 in the formulations during storage were determined. After eight months, the ratio of 30 g of EFB powder to 30 g of *Phlebia* GanoEF3 (30: 30 g) and 10 g of RSBF to 50 g of *Phlebia* GanoEF3 (10: 50 g) were found to be the suitable ratios for the *in vitro* study and application in the field. Investigation of endophytic fungus *Phlebia* GanoEF3 on the growth of oil palm seedlings in nursery trial showed that seedlings treated with EFB and RSBF organic containing *Phlebia* GanoEF3 increased the growth of the seedlings. All growth parameters measured showed significant difference in the mean values between treated and untreated seedlings. These findings showed that *Phlebia* GanoEF3 is suitable to be used as biofertilizer for oil palm seedlings. © 2015 Friends Science Publishers

Keywords: Endophytic fungus; Bioorganic *Phlebia* GanoEF3; Biofertilizer; Growth promoter

Introduction

The oil palm, *Elaeis guineensis* is an important tree crop in the tropics because of its contribution in the production of palm oil and palm kernel oil (Choo, 2012). Malaysia currently accounts for 39% of world palm oil production and 44% of world exports. If taken into account of other oils and fats produced in the country, Malaysia accounts for 12% and 27% of the world's total production and exports of oils and fats (MPOB, 2013). Oil palm in Malaysia is blessed with largely disease free except for basal stem rot (BSR) disease. According to Idris *et al*. (2004), Malaysia and Indonesia are the two countries that were suffering the most severe losses from BSR. BSR is caused by some species of *Ganoderma* from the family of Ganodermataceae. Idris *et al*. (2000a, b) had identified four species of *Ganoderma* associated with oil palm in Malaysia based on the morphological characteristics, including mycelia growth, basidiomata and basidiosphores *in vitro,* which are *G. boninense, G. zonatum, G. tornatum* and *G. miniatocinctum.*

For the last 10 years, investigation has taken a somewhat different path, with emphasis on fundamental research to form a rational basis for devising control methods. Some control over BSR in the physical form such as agronomics practices, surgery, soil mounding and sanitation in existing planting and at replanting (Idris, 2011). Chemical fertilizers and fungicides were extensively used throughout most of agricultural Asia. The heavy usage of the chemical fertilizers is now however realized to cause slow declining in productivity and the deterioration of environmental quality. In the light of these problems, any efforts to reduce its uses is necessary, hence, the use of biological control such as organic fertilizers, biofertilizers and other microbial products. The manipulation of microbials such as fungi, bacteria, actinomycetes and mychorrhiza as biocontrol agents is now being investigated to control the disease (Idris *et al*., 2010). Biological control through endophytic bacteria, actinomycetes and fungi hold potential value. An endophytic fungus is defined as an organism that lives in association with plants for most of its life cycle; this organism lives within the intercellular spaces of plants, depending on the apoplastic for the supply of nutrients. Several promising antagonist fungi, mainly *Trichoderma* (Ilias and Abdullah, 1999), *Aspergillus* (Shukla and Uniyal, 1989) and *Penicillium* (Dharmaputra *et*

To cite this paper: Anuar, E.N., R. Nulit and A.S. Idris, 2015. Growth promoting effects of endophytic fungus *Phlebia* GanoEF3 on oil palm (*Elaeis guineensis*) seedlings. *Int. J. Agric. Biol*., 17: 135–141

al., 1989) inhibiting *in vitro* growth of *G. boninense* has been reported (Ariffin *et al*., 2000). A recent isolation from trunk and root tissues of oil palms and *in vitro* screening of endophytes fungi against *G. boninese* by MPOB has brought to the finding of several promising fungi, mainly *Hendersonia* GanoEF, *Amphinema* GanoEF2, and *Phlebia* GanoEF3 (Noorhaida and Idris, 2009).

The use of bioorganic fertilizer (BF) at oil palm plantations is one of the methods to reduce the cost and increase the efficiency of chemical fertilizer application (Taryo-Adiwiganda *et al.*, 2006). BF has not been used commercially on oil palm plantation until 2004. In this study, two types of bioorganic were used, the bioorganic Empty Fruit Bunch (EFB) and Real Strong Bioorganic Fertilizer (RSBF). EFB is one of the by-products produced from processing of oil palm, produced in large quantities throughout the year and during the replanting process. Composting process converts the essentially organic of EFB in nature waste into humus, which is suitable for crop production. The EFB consists of moisture and solids (Thambirajah *et al*., 1995). The EFB used in this study was provided by MPOB, Bangi, Selangor. The Real Strong Bioorganic Fertilizer (RSBF) was supplied by All Cosmos Industries Sdn Bhd (Pasir Gudang, Johor, Malaysia). This bioorganic fertilizer contains 65% plant-based organic matters, 30% of chemicals and 5% Zeolite organic matters. In addition, it was formulated to allow easy and balanced plant uptakes.

Idris *et al*. (2010) and Nurrashyeda *et al*. (2011) have incorporated endophytic fungus *Hendersonia* GanoEF1 into bioorganic fertilizer formulation. Their studies found that *Hendersonia* GanoEF1 showed the ability to inhibit the growth of *G. boninense* and control the disease *in vitro* and nursery trials. Moreover, *Hendersonia* GanoEF1 also suitable as biofertilizer that enhance the growth of oil palm seedlings. Fuentes-Ramirez and Caballero-Mellado (2005) reported that the biofertilizer, which comprised of microbial inoculants or assemblages of living microorganisms benefits on crop yield and also on the plant growth. *In vitro* studies by Noorhaida and Idris (2009) showed that the pure culture of the endophytic fungus *Phlebia* GanoEF3 was capable to inhibit the growth of *G. boninense* and effectively used to control *Ganoderma* infection in oil palm seedlings. However, this particular study was undertaken without concerning the ability of this fungus to potentially act as a biocontrol agent against *Ganoderma.*

Therefore, the objective of this study was to develop *Phlebia* GanoEF3 into powder formulations by incorporating organic and inorganic fertilizer as a carrier, followed by study on the capability of *Phlebia* GanoEF3 to served as a biological fertilizer that promote the growth oil palm seedlings. *In vitro* study and the shelf life of viable cell of *Phlebia* GanoEF3 in the formulations during storage were first determined, followed by the application of *Phlebia* GanoEF3 in the nursery to evaluate their effects on the growth of oil palm seedlings.

Materials and Methods

Isolation of Endophytic Fungus *Phlebia* GanoEF3

Pure culture of endophytic fungus *Phlebia* GanoEF3 (Fig. 1) was provided by MPOB, Bangi, Selangor. The pure culture had been isolated from the oil palm roots and had been screened to evaluate its potential biocontrol activity against *G. boninense* (Idris *et al*., 2010). Fungus *Phlebia* GanoEF3 was maintained on potato dextrose agar (PDA) slants in universal bottles at 4°C until required. *Phlebia* GanoEF3 were then sub-cultured in order to mass multiply the pure culture.

Enrichment of Endophytic Fungus *Phlebia* GanoEF3

Propagules were prepared through enrichment process of the fungus by harvesting 17 day old petri plate culture. The plates were first flooded with 10 mL sterilized distilled water and the surface of the plates was then scrapped using sterilized glass hockey stick. The mycelia were transferred and propagated in liquid media consisting of potato dextrose broth (PDB) for 4 days using rotary incubator at 150 rpm at 28°C as shown in Fig. 2.

Preparation of Bioorganic Containing Endophytic Fungus *Phlebia* GanoEF3

i). Preparation of bioorganic EFB *Phlebia* GanoEF3: Different ratios of empty fruit bunches to *Phlebia* GanoEF3 were prepared in order to determine the suitable ratio that give yield the highest value of colony forming unit per gram (CFU g^{-1}). The ratios were 10 g EFB: 50 g *Phlebia* GanoEF3, 20 g EFB: 40 g *Phlebia* GanoEF3, 30 g EFB: 30 g *Phlebia* GanoEF3, 40 g EFB: 20 g *Phlebia* GanoEF3 and 50 g EFB: 10 *Phlebia* GanoEF3 prepared and mixed well under sterile condition. Bioorganic EFB *Phlebia* GanoEF3 of different ratios were stored at room temperature of 28±2°C. The viability test was conducted according to method reported by Nurrasyheda *et al*. (2011).

ii). Preparation of bioorganic RSBF *Phlebia* GanoEF3: The preparation was similar to those in (i), where different ratios of bioorganic RSFB to *Phlebia* GanoEF3 was prepared in order to find the suitable ratio that would yield the highest value of colony forming unit per gram (CFU g^{-1}). The ratios were 10: 50 g, 20: 40 g, 30: 30 g, 40: 20 g and 50: 10 g, respectively. Bioorganic RSFB *Phlebia* GanoEF3 were mixed well under sterile condition and stored at room temperature of 28 ± 2°C. The viability test was done according to method by Nurrasyheda *et al*. (2011) with slight modification, to determine the quality of the products.

Viability Test of the Bioorganic Containing Endophytic Fungus *Phlebia* GanoEF3

According to method by Nurrashyeda *et al.* (2012), pour plate method was used to test the viability of fungus *Phlebia* GanoEF3. One gram of Bioorganic containing

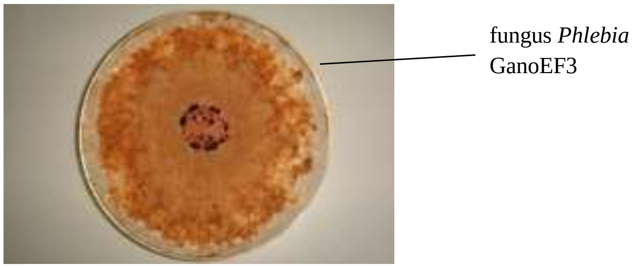

Fig. 1: Pure culture of fungus *Phlebia* GanoEF3

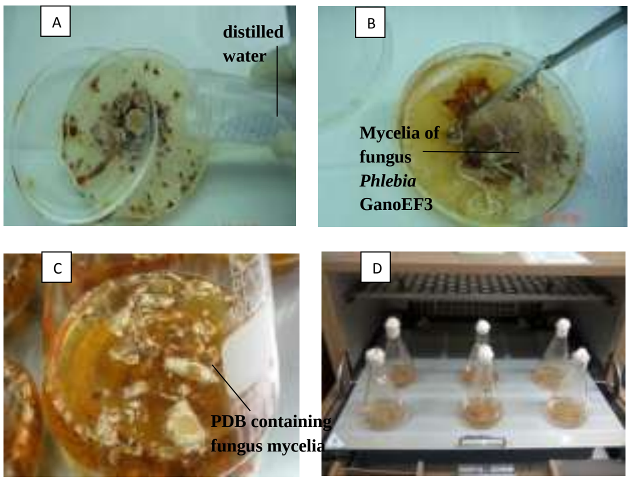

Fig. 2: (A) Distilled water was added to ease the scraping process, (B) mycelia of the fungus were scraped out (C) mycelia were transferred into flask contains PDB, and (D) flasks were shaken in incubator shaker

endophytic fungus *Phlebia* GanoEF3 (Bioorganic EFB *Phlebia* GanoEF3 and Bioorganic RSFB *Phlebia* GanoEF3 was mixed with 9 mL of sterilized distilled water and agitated using a vortex. Serial of ten-fold dilution were prepared and aliquots of 1 mL volume from the dilutions were pipetted into an empty sterile petri plate followed by the pouring of 20 mL of sterilized molten Rose Bengal Agar (RBA) over it. The plate was swirled to obtain an even mixture. Fungal colonies that appeared after 3 to 4 days of incubation at 28°C were spotted, counted and recorded and populations were reported as colony forming units per gram (CFU g^{-1}) of powder. The final value of CFU was the average of three readings.

Application of Bioorganic Containing Endophytic Fungus *Phlebia* GanoEF3 Inoculum on Oil Palm Seedlings

The experiment was conducted at Ladang 2, Fakuti Pertanian, Universiti Putra Malaysia (UPM). 162 germinated oil palm seedlings (Dura × Pisifera) age between 4 to 5 months were provided by MPOB in a Completely Randomized Design (CRD). Upon arrival, all the seedlings were pre-inoculated with Bioorganic containing endophytic fungus *Phlebia* GanoEF3, each, according to treatment. The seedlings were given two week time for adjustment and adaptation to the new environment.

The seedlings were divided into two parts, where 108 seedlings were used in destructive samplings to study the biomass of the seedlings, while the remaining seedlings were used in the plant growth study. For plant growth study, each treatment was replicated thrice with six seedlings per replicate, while for biomass study, each treatment replicated thrice with three seedlings per replicate. There were three treatments as shown in Table 1. For Treatment 1 (Control seedlings), the seedlings were untreated, thus served as control. The seedlings were first uprooted and transferred from the old polybag into a bigger polybag (12 cm × 14 cm). Soil mixture was then added (Fig. 3). In Treatment 2 (EFB-treated seedlings), top soil method was used to apply Bioorganic EFB *Phlebia* GanoEF3 to the seedlings. Similar to steps in the Treatment 1, seedlings were uprooted and carefully transferred into a bigger polybag (12 cm × 14 cm). The product was made sure to come in contact with the roots of the seedlings. Soil mixture was then added into the polybag as shown in Fig. 4. For Treatment 3 (RSBF-treated seedlings), all methods were similar to those in the second treatment, with the exception of the product where Bioorganic RSFB *Phlebia* GanoEF3 was used. All samplings were done four times (2, 4, 6, and 8 month after treatments), for a total of eight months. The seedlings were watered daily and supplementary fertilizer was applied every month.

Oil Palm Seedlings Growth Analysis

In order to study the oil palm growth, the effect of the fungal endophyte *Phlebia* GanoEF3 on oil palm seedlings growth was evaluated. Parameters assessed were plant height, frond production, frond length, number of leaves and stem diameter, total chlorophyll content, leaf area index and total biomass of the seedlings.

According to methods by Zaiton *et al.* (2008), plant height was measured from one cm above the soil level to the tip of the tallest leaf. Frond length was measured from the first thorn produced by the frond to the tip of the frond, while stem diameter (girth) was taken at the bole of the stem, where the circumference of the bowl is the biggest using ABS Digimatic Caliper (Mitutoyo, Japan). Frond production and number of leaves were observed and counted for new growth. Greater increases in these parameters would indicate improved growth, and a positive effect of the fungal endophyte on the plant health.

Following Roslan and Haniff (2004) methods, the total chlorophyll content was measured using Chlorophyll Meter SPAD-502 Plus (Konica Minolta, Japan), while the Leaf Area Index was measured using LI-3000C Portable Leaf Area Meter (LI-COR, USA). According to Shamala *et al.*

Table 1: Application of Bioorganic containing endophytic fungus *Phlebia* GanoEF3 formulations on oil palm seedlings

Treatment	Inoculum
1; (Control seedlings)	Seedlings untreated (Control)
2; (EFB-treated seedlings)	Seedlings treated with Bioorganic EFB *Phlebia* GanoEF3 (30 g of EFB: 30 g of fungus *Phlebia* GanoEF3)
3; (RSBF-treated seedlings)	Seedlings treated with Bioorganic RSBF *Phlebia* GanoEF3 (10 g of RSBF: 50 g of fungus *Phlebia* GanoEF3)

(2008), for biomass, the seedlings were first harvested and wash to clean off the excess soil, and then they were air dried until they were dry. The seedlings were dried in the oven of 60°C for three to four days until the dry weight are constant. The final dry weight was then measured.

Relative Growth Rate (RGR) was then determined by using the following formula provided by Hunt (1990).

$$RGR = \left[\frac{\ln W_2 - \ln W_1}{t_2 - t_1} \right]$$

Where, W = total dry weight per plant.

t = time.

W_1 and W_2 = were measured at time t_1 and t_2.

Statistical Analysis

Statistical tests were carried out using the Minitab 16 statistical package (Minitab Inc., USA). Analysis of variance (ANOVA), at $p<0.05$ was used to determine if there is a significance difference between the treatments. The Tukey multiple comparison test was applied to determine between which means the statistical differences occurred if the ANOVA was significant.

Results

Viability Test of the Bioorganic Containing Endophytic Fungus *Phlebia* GanoEF3

i). Viability of bioorganic EFB *Phlebia* GanoEF3: Over the eight month storage period where the viability test was conducted for all the Bioorganic EFB *Phlebia* GanoEF3 ratios prepared, 3 ratios of EFB to fungus *Phlebia* GanoEF3 (10: 50 g, 20: 40 g and 30: 30 g) were observed to show highest number of colony forming unit per gram (CFU g^{-1}) which was 10^8 CFU g^{-1} during first month of storage, as shown in Fig. 5. The highest CFU g^{-1} for the ratios of 40: 20 g and 50: 10 g at first month of storage was only at 10^6 CFU g^{-1}. After four month into the storage period, the CFU g^{-1} for all the ratios declined, where for 10: 50 g and 20: 40 g the ratio was 10^6 CFU g^{-1}, while for 30: 30 g, 40: 20 g and 50: 10 g the viability was observed to be at 10^5 CFU g^{-1}. During final storage month, the viability for all the ratios declined to 10^4 CFU g^{-1}, except for 50: 10 g where the viability was 10^4 CFU g^{-1}.

Since there was no significant difference between the viability of 10: 50 g, 20: 40 g and 30: 30 g ratios which showed the highest CFU g^{-1} in the first month of storage, the 30: 30 g ratio was chosen to be the suitable ratio of Bioorganic EFB *Phlebia* GanoEF3 to continue with the *in vitro* study and application in the field. 30 g of empty fruit bunches to 30 g of *Phlebia* GanoEF3 in (30: 30 g) was chosen, because the preparation of formulation with that particular ratio was less time consuming. This is because; the process of harvesting and enriching the mycelia of *Phlebia* GanoEF3 requires a long amount of time to be prepared. By using 30 g ratio, the amount of mycelia *Phlebia* GanoEF3 that needed to be prepared was less than the other two ratios. Therefore, the usage of 30:30 g ratio is more practical.

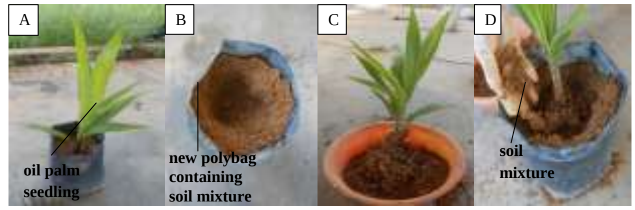

Fig. 3: (A, B, C, D) For the Control seedlings where seedlings were untreated, seedling was uprooted and transferred into new polybag

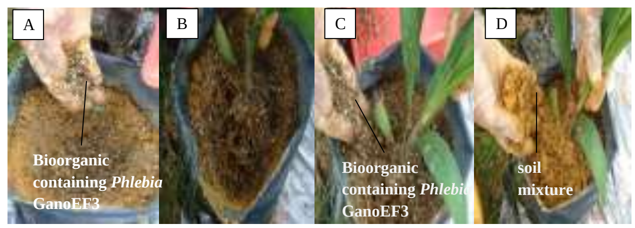

Fig. 4: For treated seedlings (EFB-treated seedlings and RSBF-treated seedlings) (A) A hole was made and the Bioorganic containing *Phlebia* GanoEF3 was applied inside and around the hole, (B, C) Bioorganic containing *Phlebia* GanoEF3 was applied on top of the roots (D) the seedling was then covered with the soil mixture

Note: Bioorganic containing *Phlebia* GanoEF3; EFB-treated seedlings: Bioorganic EFB *Phlebia* GanoEF3, RSBF-treated seedlings: Bioorganic RSBF *Phlebia* GanoEF3

ii). Viability of bioorganic RSBF *Phlebia* GanoEF3: Viability test for Bioorganic RSBF *Phlebia* GanoEF3 was also conducted over eight month storage period. The ratio of 10 g of bioorganic fertilizer to 50 g of *Phlebia* GanoEF3 (10: 50 g) gave the best result compared to the other four ratios. In the first month of the viability test (Fig. 6), the 10: 50 g ratio had the highest viability of 10^7 CFU g^{-1}. However, the viability rapidly declined to 10^6 CFU g^{-1} during the second month of storage and continue declining to 10^5 CFU g^{-1}

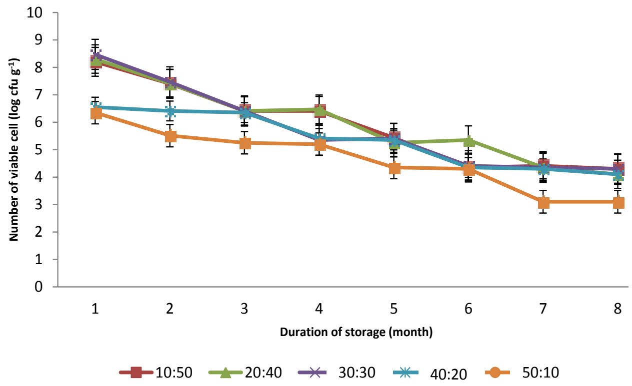

Fig. 5: Viability of Bioorganic EFB *Phlebia* GanoEF3, where the ratios of empty fruit bunches to *Phlebia* GanoEF3 are 10: 50 g, 20: 40 g, 30: 30 g, 40: 20 g and 50: 10 g

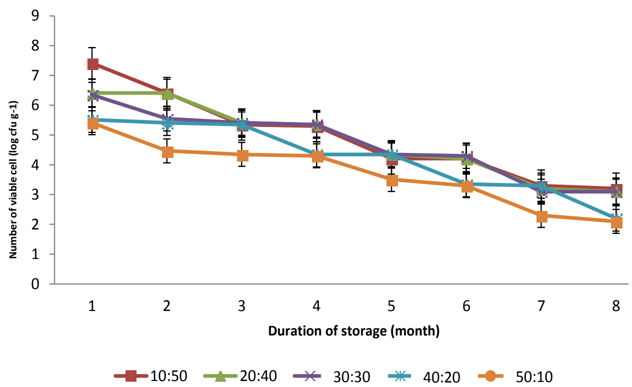

Fig. 6: Viability of Bioorganic RSBF *Phlebia* GanoEF3, where the ratios of RSBF to *Phlebia* GanoEF3 were 10: 50 g, 20: 40 g, 30: 30 g, 40: 20 g and 50: 10 g

at third and fourth month of storage. The viability stayed at 10^4 CFU g^{-1} in fifth and sixth month and declined to 10^3 CFU g^{-1} for seventh and eighth month. Based on the result obtained, Bioorganic RSBF *Phlebia* GanoEF3 with ratio of 10 g of bioorganic fertilizer to 50 g of *Phlebia* GanoEF3 (10: 50 g) was chosen to be the suitable ratio to continue with the *in vitro* study and application in the field.

In the comparison to determine the best product of Bioorganic containing *Phlebia* GanoEF3, it was found that there is no significant difference (ANOVA, $p>0.05$) between the viability of Bioorganic EFB *Phlebia* GanoEF3 (30:30 g) and Bioorganic RSBF *Phlebia* GanoEF3 (10:50 g). However, from Fig. 8, it can be concluded that Bioorganic EFB *Phlebia* GanoEF3 shows a slightly better result compared to Bioorganic RSBF *Phlebia* GanoEF3, based on the comparison of number of viable cell of *Phlebia* GanoEF3 for every month during the eight month of storage period.

Effect of Bioorganic and Endophytic Fungus *Phlebia* GanoEF3 on Oil Palm Seedlings Growth

After two months of treatment, there was no significance difference (ANOVA, $p>0.05$) between the growth of untreated and treated oil palm seedlings as shown in Table 2. However, the growth of the oil palm seedlings treated with Bioorganic EFB *Phlebia* GanoEF3 (EFB-treated seedlings) and Bioorganic RSBF *Phlebia* GanoEF3 (RSBF-treated seedlings) significantly increased (ANOVA, $p<0.05$) compared with untreated seedlings after four months of experiment. This study showed that endophytic fungus *Phlebia* GanoEF3 has a good potential to enhance the seedling plant growth. In addition, the growth of seedlings treated with both Bioorganic EFB *Phlebia* GanoEF3 and Bioorganic RSBF *Phlebia* GanoEF3 showed almost similar results. Plant growth result showed that seedlings treated with Bioorganic EFB *Phlebia* GanoEF3 and Bioorganic RSBF *Phlebia* GanoEF3 gave the highest result in plant height (103.28 and 107.50 cm), girth (57.40 and 60.06 mm), frond length (73.67 and 73.56 cm), number of leaflets (38.13 and 38.33) and number of frond (17.42 and 18.33). Physiological results also showed that both biofertilizer increased the chlorophyll content (70.43 and 69.40 mg/mL), leaf area index (4152.31 and 4355.92 cm^2) and total biomass of the seedlings (105.95 and 105.1153.97 g) respectively. Moreover, the RGR results as shown in Fig. 7 proved that Bioorganic EFB *Phlebia* GanoEF3 and Bioorganic RSBF *Phlebia* GanoEF3 showed good potential to enhance the growth of oil palm seedlings.

Discussion

The usage of endophytes fungi, *Phlebia* GanoEF3 as biofertilizer is being study in order to enhance the growth of oil palm. The formulation is critical and an important step in order to develop fertilizer containing microorganisms. The formulation of biocontrol agents is a technique to preserve and deliver antagonists to targets and to improve their activities (Burges and Jones, 1998). The effectiveness of using endophytic biocontrol agent in the field or greenhouse is largely determined by the shelf life and delivery techniques of the formulation (Stephens and Rask, 2000; Khavazi *et al.*, 2007; Albareda *et al.*, 2008). Therefore, we have developed the ideal formulation of bioorganic fertilizers, which are EFB and RSBF incorporated with endophytic fungi, then, the capability of these formulation as growth enhancer were studied. This findings showed that 30 g of EFB powder to 30 g of *Phlebia* GanoEF3 (30: 30 g) and 10 g of RSBF to 50 g of *Phlebia* GanoEF3 (10: 50 g) were found to be the ideal ratios due to highest viability result given. Both ratios were chosen to be applied in the field because the preparation of both formulation require less time. The fertilizers were then applied in the field for eight months period. The results found both ratios increased the growth of oil palm seedling.

Table 2: Frond Production, Frond Length, Plant Height, Number of Leaflet, Stem Diameter, Total Chlorophyll, Leaf Area Index and Total Biomass of untreated seedling and treated seedlings after 2 months and 8 months treatment. Data are mean and standard deviation (n=6, n=3)

Treatment	Number of Frond		Frond Length (cm)		Plant Height (cm)		Number of Leaflet		Stem Diameter (mm)		Total Chlorophyll (mg/m^2)		Leaf Area Index (cm$^{2)}$		Total Biomass (g)	
Month	2	8	2	8	2	8	2	8	2	8	2	8	2	8	2	8
T1	8.33 ± 1.49^{a}	14.11 ± 1.28^{b}	27.94 ± 4.25^{a}	63.17 ± 7.16^{b}	77.44 ± 9.61^{a}	93.78 ± 8.74^{b}	19.67 ± 3.38^{a}	36.23 ± 3.42^{b}	29.52 ± 6.22^{a}	46.83 ± 6.25^{b}	38.82 ± 4.01^{a}	59.13 ± 5.02^{b}	1440.3 ± 469.8^{a}	3403.25 ± 684.3^{b}	33.97^{a} ± 9.69	79.79 ± 18.11^{b}
T2	8.17 ± 1.04^{a}	17.42 ± 1.13^{a}	32.72 ± 6.12^{a}	73.67 ± 6.52^{a}	78.33 ± 13.16^{a}	103.28 ± 13.26^{a}	22.78 ± 3.83^{a}	38.13 ± 4.07^{a}	28.82^{a} ± 3.37	57.40 ± 3.37^{a}	36.62 ± 5.88^{a}	70.43 ± 5.94^{a}	1309.8 ± 306.9^{a}	4152.31 ± 469.5^{a}	33.06 ± 9.94^{a}	105.95 ± 16.28^{a}
T3	8.22 ± 1.17^{a}	18.23 ± 0.94^{a}	32.83 ± 7.57^{a}	73.56 ± 7.81^{a}	79.33 ± 13.41^{a}	107.50 ± 14.24^{a}	22.78 ± 4.66^{a}	38.33 ± 4.07^{a}	30.06 ± 3.51^{a}	60.06 ± 4.38^{a}	39.63 ± 4.99^{a}	69.60 ± 6.65^{a}	1379.5 ± 357.3^{a}	4355.92 ± 540.1^{a}	33.73 ± 8.20^{a}	105.11 ± 13.85^{a}

Note: Means that do not share a letter are significantly different at $p \leq 0.05$ using Tukey's method.
T1 (Control seedlings): Seedlings untreated (control)
T2 (EFB-treated seedlings): Seedlings treated with Bioorganic EFB *Phlebia* GanoEF3 (30 g of EFB: 30 g of fungus *Phlebia* GanoEF3)
T3 (RSBF-treated seedlings): Seedlings treated with Bioorganic RSBF *Phlebia* GanoEF3 (10 g of RSBF: 50 g of fungus *Phlebia* GanoEF3)

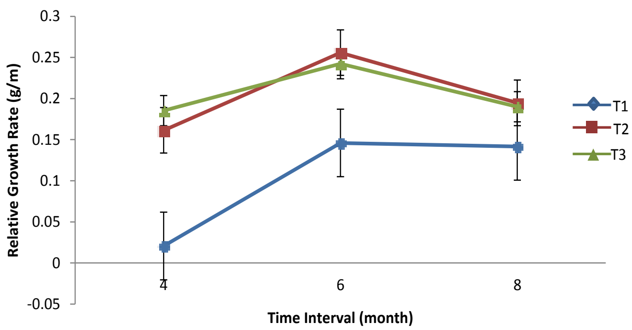

Fig. 7: Relative Growth Rate (RGR) of oil palm seedlings according to treatment
Note: T1: Control seedlings, T2: Seedlings treated with Bioorganic EFB *Phlebia* GanoEF3, T3: Seedlings treated with Bioorganic RSBF *Phlebia* GanoEF3

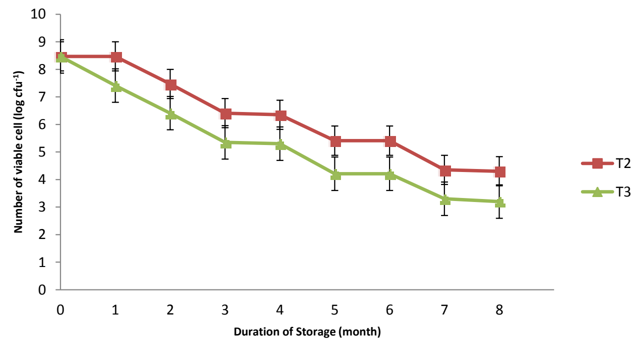

Fig. 8: Comparison of viability of products; Bioorganic EFB *Phlebia* GanoEF3 (30:30 g) and Bioorganic RSBF *Phlebia* GanoEF3 (10:50 g)
Note: T2: Seedlings treated with Bioorganic EFB *Phlebia* GanoEF3, T3: Seedlings treated with Bioorganic RSBF *Phlebia* GanoEF3

Beneficial endophytic microorganisms comprise especially fungi and bacteria that colonize internal plant tissues without causing visible harm to their hosts (Petrini, 1991). Introducing endophytic bacteria as biocontrol agents, which able to penetrate and become systemically disseminated in the host plant presents an ecological niche. In addition, all plants in natural ecosystems appear to be symbiotic with fungal endophytes. Endophytes are considered plant mutualisms, which receive nutrition and protection from the host plant while the host plant may benefit from enhanced competitive ability and increased resistance to herbivores, pathogens and various abiotic stresses. This highly diverse group of fungi can have profound impacts on plant communities through increasing fitness, increasing biomass and decreasing water consumption, or decreasing fitness by altering resource allocation (Saikkonen *et al.*, 1998). Endophytes were also found residing entirely within plant tissues and may grow within roots, stems and/or leaves. On the other hand, EFB and RSBF also increased the plant growth, because they contains high value nutrient. EFB contain a high proportion of cellulosic matter; the bunch consists of 70% moisture and 30% solids (Thambirajah *et al*., 1995). The high content of moisture of EFB might have change the pH of the product thus making the environment to be less suitable for fungus *Phlebia* GanoEF3. The Real Strong Bioorganic Fertilizer (RSBF) contains 65% plant-based organic matters, 30% of chemicals and 5% Zeolite organic matters. It was formulated to allow easy and balanced plant uptakes. Therefore, in this study, we found that the combination of endophytic fungus and organic fertilizer had improved the growth of oil palm. Kwizera *et al*. (2013) reported in their study that *Nicotiana tabacum* treated with microorganism with addition of bioorganic fertilizer significantly increased the plant height, girth, biomass and the chlorophyll content. Moreover, the growth of oil palm seedlings treated with EFB had shown increment. Roe and Cornforth (2000) reported that the availability of nutrients could improve the capacity of soil to hold water. According to Noraini (2007), the application of EFB compost and arbuscular mycorrhizal significantly increased the growth of maize, which the inoculated plant were able to absorb more nutrients than the un-inoculated plant.

Conclusion

Endophytic fungus *Phlebia* GanoEF3 incorporated with EFB and RSBF is capable to promote the growth of oil palm seedlings. Therefore, the use of endophytic fungus *Phlebia* GanoEF3 as a biofertilizer should be recommended as an alternative of chemical fertilizer for oil palm cultivation.

Acknowledgement

The author wish to thank Universiti Putra Malaysia (UPM) for providing the nursery site and facilities to make this study possible. In addition, the author would like to thank Malaysian Palm Oil Board (MPOB) for all the provided supplies and assistance.

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(Received 11 November 2013; Accepted 07 March 2014)