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Lignocellulosic Materials from the Oil Palm Empty Fruit Bunches can Promote the Growth of the Oil Palm Disease *Ganoderma boninense*: An *In Vitro* Study

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ABSTRACT

The present study aimed to investigate whether Ganoderma boninense can be grown in vitro in a medium of different lignocellulosic materials (LM) collected from the empty fruit bunches (EFB) of oil palm (Elaeis guineensis Jacq.). The mycelial growth rate of G. boninense was found at 0.50 (cm/day). A total of 55 days is needed for the full colonization of G. boninense in the polypropylene plastic bags. However, the mycelia grew well and there was no contamination during the growing stage. This showed 100% inoculation rates. This in vitro experiment indicated that the this fungus can grow in a medium of different LM released by the oil palm EFB. Therefore, this study pointed to the need of management of oil palm EFB in the plantation in order to control basal stem rot disease caused by G. boninense in the oil palm plantation. The management strategy is recommended as proper disposal of EFB in the oil palm plantation and full exploitation of LM from the EFB into value-added products. This is important to avoid disposing the oil palm EFB at the oil palm plantation because the natural decomposition of EFB can promote the growth of G. boninense.

Keywords: *Ganoderma boninense*, Oil palm wastes, Lignocellulosic materials.

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is the most important commodity in Malaysia's agricultural industry since 1957. The main residue from palm oil processing is the empty fruit bunches (EFBs), which are discarded into the environment after removal of the fresh fruits used for the extraction of palm oil [1,2]. The milling waste EFB of oil palms included lignocellulosic palm biomasses [3]. Recently, Yap and coauthors reviewed the potentials and challenges of using renewable oil palm biomass wastes in Malaysia [4]. These wastes include EFB.

According to Abdullah and Sulaiman [5], the lacking of proper management of this oil palm waste EFB will interrupt the normal growth process of the oil palm plantation because of low decomposition rate. Besides, it can also promote the increase of oil palm disease caused by *Ganoderma boninense* that are destructive to the oil palm plantation [6].

In general, Lignocellulosic Material (LM) can be produced from the oil palm industries including the EFB [5,7]. The biomass oil palm EFB is classified as lignocellulosic residues that typically contain cellulose, hemicellulose, and lignin in their cell wall that can be converted into fine chemicals. The LM refers to plant dry matters composed of

aromatic polymer (lignin) and carbohydrate polymers (cellulose and hemicellulose). The carbohydrate polymers contain different sugar monomers (six and five carbon sugars) and they are tightly bound to the aromatic polymer [8]. However, Basal Stem Rot (BSR) disease is affected by soil-borne pathogen namely *G. boninense* [6]. This pathogenic disease could be due to the improper waste management of the EFB in the oil palm plantation. In this study, EFB was focussed upon because the amount of EFBs is much greater than other residue such as the kernel shells and mesocarp fibers [1]. The oil palm EFB is the largest solid residues generated in the oil palm processing [9]. Besides, oil palm EFB is now considered one of the most important source of LM for producing chemicals and fuels and it is a potential feedstock for bio-refinery process although all the fractions obtained in pre-treatment stages are valorized [9].

Therefore, the aim of this study is to investigate whether *G. boninense* can be cultivated or grown in vitro in a medium of different LMs collected from oil palm EFB.

MATERIALS AND METHODS

The strain *G. boninense* was found and isolated from oil palm (*E. guineensis* Jacq.) at Alaf Plantation (YPJ Plantation) in Kota Tinggi, Johor, Peninsular Malaysia (Figure 1). For the experimental design, the substrate formulation consists of 66% corn stalks (ground dried corn stalks), 15% LMs, 15% wheat bran, 1% gypsum and 3% lime. The LMs in the present study were sampled from the oil palm EFB.

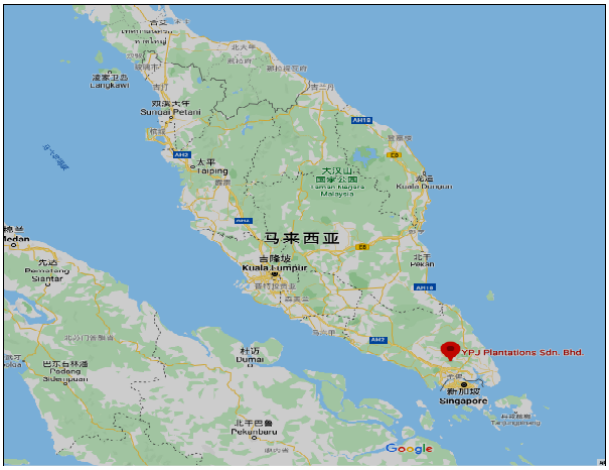

Figure 1. The collection of the strain *G. boninense* from oil palm (*E. guineensis* Jacq.) at Alaf Plantation (YPJ Plantation) in Kota Tinggi, Johor, Peninsular Malaysia

Potato Dextrose Agar (PDA) was used to culture the fungal strain in the test tubes (18 mm × 180 mm). After inoculation, they were incubated at 25°C. For the preparation of spawn, wooden stick spawn was adopted in this experiment. The polypropylene plastic bags (17 cm × 30 cm × 0.05 mm) were autoclaved at 127°C for 2 hours. Later, they were cooled to room temperature (23°C-25°C), and *G. boninense* was inoculated under aseptic conditions and it was incubated at 23°C-25°C for 20 days. After incubation, spawns that were found contaminated were discarded. Only the healthy spawns were used for the experiment.

For the preparation of substrate and inoculation, materials were weighed and mixed evenly according to substrate formulation stated above. The moisture of substrate was controlled at about 60% and pH at neutral condition. The substrates were placed into polypropylene plastic bag (17 cm × 45 cm × 0.05 mm). A total of 1000 bags of substrates were prepared and each bag contained about 0.5 kg of dry weight substrate. Filled bags were autoclaved at 127°C for 3 hours. After cooling, stick spawns (Figure 2) were inserted into the bags under aseptic conditions (Figure 3) and were incubated at room temperature. Any contaminated bags were removed once found. After the mycelia were full of bags, they were moved into the specific mushroom shed. Determination of mycelial growth rate, growth condition, duration of complete mycelium running and rate of contamination were done.

Figure 2. Photo showing the wooden stick spawn

Figure 3. Photo showing the inoculated bags

After the mycelia fully covered the substrate completely, the polypropylene plastic bags were continued for incubation for another 5-7 days, until the mycelia reach physiological maturity. Later, the complete colonized bags were moved to a growing house. The bags were arranged horizontally on racks for fruiting purpose. Two days before the bags were transferred to the growing house, formaldehyde, aerosol disinfectants were used to spray the growing house in order to kill all the pathogens and insects. The growing house was pre-wet to keep the humidity between 80%-85%, and to allow sufficient sun light and ventilation. The prevention and control of pests and diseases were strengthened. Two days after the bags were transferred to the growing house, the collar of the bag was cut off, and scattered light was given every day (avoidance of direct sunlight). Sufficient ventilation and certain concentrations of carbon dioxide were maintained during the growing stage until fully colonizing the polypropylene plastic bags (Figure 4).

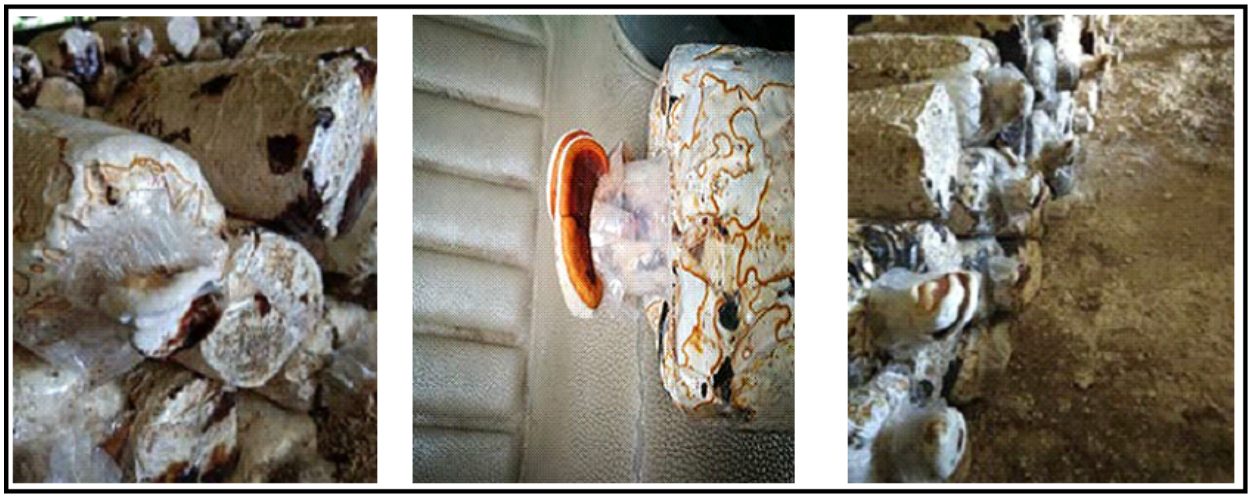

Figure 4. Photos showing the fruiting bodies of *Ganoderma boninense*

RESULTS AND DISCUSSION

From Table 1, the mycelial growth rate of *G. boninense* was found t 0.50 (cm/day), which was considered slow. It took 55 days to full colonization of *G. boninense* in the polypropylene plastic bags. However, the mycelia grew well and there was no contamination during the growing stage. This showed 100% inoculation rates.

Table 1. Mycelial growth status of *Ganoderma boninense* in the polypropylene plastic bags

Mycelial growth rate (cm/day)	Mycelia growth	Complete colonization (day)	Contamination rate
0.5	+++	55	0
Note: Mycelia growth rates for after 7 days of inoculation, 10 polypropylene plastic bags of *Ganoderma boninense* were randomly selected for data collection. The growth rate of mycelia was measured after 5 days			

In this experiment, substrate formulation of corn stalks, LMs, wheat brans and other materials for the cultivation of *G. boninense* has been proven to be successful. Even though the growth rate of mycelia was slow, the mycelia were in good condition. Hence, the fruiting body of *G. boninense* (Figure 3) was found to be visibly produced through artificial cultivation. This is due to the lignin degrading enzymes from *G. boninense*, which are involved in the detoxification and the degradation of lignin in the oil palm [10].

The present use of EFB for the cultivation of fungus has been found in the literature. The EFB has been utilized as substrates for cultivation of mushroom *Pleurotus spp*. [11], black jelly mushroom (*Auricularia polytricha*) [12] and straw mushroom (*Volvariella volvacea*) [13]. Saidu and coauthors [14] reported that the palm oil mesocarp fibre served as a good substrate for the cultivation of oyster mushroom (*Pleurotus spp.*). Thus, the palm oil mesocarp fibre, which is an oil palm waste, can be used as a substrate and source of LMs for the mushroom production. Lau, et al. [12] also reported that the oil palm EFB, serving as a source of LMs, as supplementary substrate can be recommended to black jelly mushroom (*Auricularia polytricha*) growers for improving growth and yield of this mushroom.

All the above fungal cultivations are successful because of the presence of LM in EFB which is essential for growth of the fungus. In particular, the present finding was well supported by Sudirman and coauthors, who used EFB as substrates for cultivation of *G. boninense* [15]. Jumali and Ismail reported that *G. boninense* is able to degrade lignin from oil palm EFB [16]. The process of lignin degradation is affected by the number of spores and period of incubation of the fungus, *G. boninense* [16].

MANAGEMENT OF OIL PALM EMPTY FRUIT BUNCHES

The present study indicates the LM from EFB can promote can promote the growth of the oil palm disease *Ganoderma boninense* in the oil plantation. Therefore, a strategy to manage the EFB is recommended.

The EFB should be well managed by not a simple disposal of EFB on the oil palm plantation areas as biomass wastes. EFB is abundant in palm-oil-producing countries and can cause environmental problems [17]. Since the EFB consists of a huge amount of LM [18], it is the biomass wastes which are categorized as organic wastes that are environmentally degradable. Much of this residual waste is not used but contributes to severe environmental problems when left in processing factories. However, owing to the large quantities generated, these wastes have the potential to pollute the environment.

The oil palm EFB has traditionally been burnt in incinerator of palm oil mill and their ash recycled into the plantation as fertilizer, either left in the plantations or burned illegally. However, due to the environmental problem, the incineration of oil palm EFB has been discouraged [19]. According to Sumanthi and coauthors [20], oil palm cultivation generates a significant amount of lignocellulosic biomass derived mainly from EFB. Small amounts are being utilised for fibre production and energy generation [18]. Hence, in order to protect the environment and to ensure the sustainability of the oil palm industry, the lignocellulosic biomass waste from EFB must be fully exploited for commercial uses.

Through intensive research and development attempts, the world's oil palm biomass has been commercialized in a variety of biomass-based products. For example, the conversion of oil palm EFB into lignocellulosic chemicals with special attention on various extraction processes [21]. Therefore, proper management of EFB disposal should be wisely considered.

In view of the environmental problem, full exploitation of LM from the EFB is highly recommended. LM is the most favourable feedstock because of its low cost and high availability [22]. Because the palm fruit consists mainly of the pulp and the seeds (kernal oil), the residual EFB lipids may have different compositions [17]. Furthermore, building block of oil palm EFB fibre comprises of mainly cellulose, hemicellulose and lignin. It composed approximately around 44.2% cellulose, 33.5% hemicellulose and 20.4% of the lignin [23]. These percentages are comparable to those reported by Virginia and co-authors [24], who reported cellulose (46%), hemicellulose (34%) and lignin (20%). These complex components must be degraded into smaller molecules before further downstream bioconversion. This is being due to the fact of the high availability of EFB and its biodegradable nature is among the factors that act as catalysts to promote the use of LM as a value-added product. Another study by Nurkaya, et al. [25] who investigated the utilization of EFB waste for enzyme cellulase production.

Therefore, EFB is a major source of LM for industrial use due to the fact of being economically viable. Besides, the collection of EFB for the production of LM can be environmental solution to the disposal problem [26]. According to Tanaka, et al. [27], EFB will become the most abundant and available woody biomass in the oil palm plantation area, so that it is desirable to have an efficient usage of EFB for the sustainability of palm oil industries [28]. The LM from EFB possesses significant potential applications in food, chemicals and pharmaceuticals industries [21]. Hence, it is believed that the above two management strategies are useful in tackling the BSR disease in the oil palm plantation.

CONCLUSION

From this in vitro experiment, it can be concluded that *G. boninense* can be successfully cultivated in a medium of different LMs sampled from EFB of oil palm. Substrate formulation of corn stalks, LMs (sampled from EFB), wheat brans and other materials for the cultivation of *G. boninense* has been proven to be successful. The EFB are sources of LMs that can promote the growth of *G. boninense* in the oil palm plantation area. This study pointed to the need of management of oil palm EFB in the plantation in order to control BSR disease in the oil palm plantation. The management strategy is recommended as proper disposal of EFB in the oil palm plantation and full exploitation of LM from the EFB into value-added products. This is important to avoid disposing the oil palm EFB at the oil palm plantation because the natural decomposition of EFB can promote the growth of *G. boninense*.

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