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Novel Media for Cultivation of Fruiting Body of Ganoderma boninense for Medicinal Benefits

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Ganoderma boninense is a well-recognized causal pathogen of basal stem rot (BSR) of oil palm with intensive economic losses by reducing yield of fresh fruit bunches (FFB) and killing the palms. However, recent reports shown *G. boninense* also possess great potential in pharmaceutical. Literatures show that it exhibits strong antimicrobial, antifungal and antimalaria activities. Moreover, *in-vivo* toxicity studies show that the extracts are relatively safe for oral administration. Hence, this plant pathogen has potential for drug development. Therefore, cultivation of this species is crucial to obtain standardized quality of raw material for the medicinal research of *G. boninense* in future. In this study, the commercial mushroom polypropylene bag technique was adapted with modification by addition of blended medium mixtures for the cultivation purpose. Wood sawdust was designed with different protocols such as by adding mineral-rich palm kernel cake (PKC), polyphenol-rich selected local herbs and sugar element-rich cracked corn to investigate the growth of the fruiting bodies. Finding shows that additional of PKC affects the number of successful germination bag and number of pin head formation while the morphology and colour of the fruiting bodies remain unchanged. This study serves as preliminary finding for the future development of commercial cultivation of *G. boninense*.

Keywords: *Ganoderma boninense*; fruiting body cultivation; mushroom commercial production; agro-industrial residues; potential drug development

I. INTRODUCTION

Ganoderma boninense, the basal stem rot (BSR) disease remains the most devastation white fungus disease in oil palm plantation. This pathogen spreads in the soil through root to root contact, infects the healthy trees, decays the bottom of the stem and roots, and further inhibits the water and nutrients uptake of the palm. The uncontrollable widespread and difficulty in early detection of *Ganoderma spp* disease causes it become the major concern for oil palm industry (Chong, 2017).

To date, most of the researchers are focusing on the pathogenic study of *G. boninense* disease such as changes of cell wall lipids metabolites during infection (Alexander *et al*., 2017); leaf proteomic study of infected oil palm (Al-Obaidi *et al*., 2016); enzyme activities of *G. boninense* in oil palm and their interactions (Naher *et al*., 2012) and many more. On the other hand, researchers are searching for a solution to inhibit and suppress the growth of this fungus by introducing multiple biological agents in suppressing basal stem rot (BSR) disease (Alexander *et al*, 2017; Chong *et al*, 2017); formulation of phenolic compounds as treatment (Surendran *et al*, 2017; Chong *et al*, 2012) and many others. Despite causing severe profitability losses in oil palm industry, very few researchers are looking at the pharmaceutical potential of this "destructive" species of *Ganoderma*.

G. boninense, a basidiomycete white rot fungus which is a species of Ganodermataceae family, having phylogenic

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relationship with *Ganoderma lucidum* (Jargalmaa *et al*, 2017), which is a well-known medicinal mushroom. It had been used as traditional remedy in Asia for century and had also become an economic valuable (~2.5 billion) in the health industry for Western (Bishop *et al*, 2015) because of the potential in treatment of modern diseases. Recent reports revealed that solvent extract of *G. boninense* possess antimicrobial activity against standard and clinical isolates nosocomial infectious bacteria such as *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Streptococcus pyogene, Staphylococcus aureus* as well as Methicillin-resistant *Staphylococcus aureus* (MRSA) (Abdullah *et al*, 2018; Ismail *et al*, 2014). Ganoboninketal, fractions of ethyl acetate extract of *G. boninense* also exhibits antiplasmodial activity and agonistic activity against liver X receptor β (LXRβ) (Ma *et al*, 2015). *In-vivo* toxicity tests on the extracts and isolated compounds proved *G. boninense* is safe for oral administration and several studies convince the potential of *G. boninense* for future drug development (Abdullah, 2018; Sasidharan *et al*, 2010). Thus, cultivation methodology of *G. boninense* is important in order to obtain the standardized and sustainable raw material and also to eliminate the identification problem because of high similarity of the morphology characteristics among *Ganoderma* species.

To date, there is no report investigating the potential of cultivating the fruiting body of *G. boninense*. As this species also posses pharmaceutical potential, the method of cultivation should be developed like other medicinal mushroom such as *Ganoderma lucidum*, *Lignosus rhinoceros*, and *Hericium erinaceus*. Thus, this paper presents the first pilot cultivation technique of *G boninense* for the production of its fruiting body.

II. MATERIALS AND METHODS

A. Preparation of G. boninense Mycelial Culture

G. boninense culture was collected in Genetic lab, Faculty of Science and Natural Resources, Universiti Malaysia Sabah. The culture was periodically subculture in PDA agar. Inoculum was prepared by cutting and transferring of 9- mm diameter mycelia from the periphery of a seven days old colony growing on PDA agar in petri dish.

B. Spawn Preparation

Seven days old *G. boninense* mycelial culture was grown on PDA agar in petri dish. Cracked corn was used as the sole media for spawn preparation. Briefly, media was soaked overnight with distilled water and water was strained before fill into 18x150mm test tube to a height of 80-mm. The test tubes were covered with cotton plug and then autoclaved at 121 °C for 15 mins. The test tubes were left to cool before inoculated with one 9-mm diameter mycelia plug. The test tubes were incubated in dark, room temperature (27±2°C) for two weeks for full spawn development.

C. Preparation of Substrate Formulation for Mycelial Growth

Media being investigated consists of lignocellulosic-rich wood sawdust, rice mill byproduct rice bran, mineral-rich palm kernel cake (PKC), polyphenol-rich selected local herbs (*Blumea balsamifera* & *Clinacanthus nutans*), sugar element-rich cracked corn. Single and mixed formulations were investigated according to the percentage as shown in Table 1. The moisture content was adjusted to 75%. The media mixture was filled in polypropylene bag (120x130x500mm) with total dry weight of 500g. The bag was covered with cotton plugged-plastic cap with holes.

Table 1. Formulation and percentage (%) of prepared substrate for cultivation of fruiting body of *G. boninense*.

Sawdust	Palm kernel cake (PKC)	*Blumea balsamifera*	*Clinacanthus nutans*	Rice bran
80	-	-	-	20
60	20	-	-	20
60	-	20	-	20
60	-	-	20	20

D. Mycelium Incubation and Development of Fruiting Body

Fifty replicate bags for each formulation using different composition as Table 1 were prepared and sterilized by autoclaving at 121 °C, 15 psi for 2 hrs. Upon cooling they were inoculated with 14 days old *G. boninense* spawn, sealed with cotton-plugged plastic cap with holes. The plastic cap consists of five holes with 5 mm diameter each. The inoculated bags were incubated at room temperature (27±2°C) in the dark. After 30 days of incubation in dark, the plastic cap was removed and the mycelia-colonized bags were cultivated for development of fruiting body. The cultivation room was ventilated, maintained at (26±2°C) with 6 hours of fluorescent Day-Light/day and watered daily.

III. RESULTS AND DISCUSSION

Selection and combination of substrate as media for cultivation of mushroom is one of the most crucial factors that affects the mycelia growth and fructification of mushroom (Oyetayo *et al*, 2013). *Ganoderma spp*., a white rot fungus secretes extracellular ligninolytic enzymes and laccases to breakdown the cellulose, hemicellulose and lignin contents in substrate (Zhou *et al*, 2013). Hence, lignocellulosic biomass is always the major substrate in mushroom cultivation. In this study, the wood sawdust was added as the highest substrate percentage (80%) while supplemented with 20% of rice bran for nitrogen source and some with additional 20% of variable supplements (60% of sawdust in this case).

Substrate mixture with additional of palm kernel cake (PKC) gave the highest number of fruiting bags and prompted to develop more fruiting body by forming more than one pinhead (Figure 1). The detail comparison of fruiting bags, pin head formation and colour changes of cultivation of *G. boninense* in different media is shown in Table 2. To date, there is no artificial cultivation system of *G. boninense* has been reported unlike other *Ganoderma spp*. and edible mushroom. Inorganic salts such as $CaSO_4$ and $MgSO_4$, are good additives for cultivation of *G. lucidum* at most up to 1% of total substrate weight which can improved the mycelial growth rate (Zhou *et al*, 2012). Moreover, minerals such as phosphorus, zinc, magnesium, calcium, copper, manganese, sulfur enhance the production of mushroom *Pleurotus spp*. (Alananbeh *et al*, 2014). Essential oils like coixenolide and citrus oil have been reported as an additive to improve the mycelial growth rate and metabolites biosynthesis in cultivation of *G. lucidum* and *Antrodia cinnamomea* respectively (Zhou *et al*, 2014; Ma *et al*, 2014).

Table 2. Comparison on number of fruiting bags, pinhead formation and colour changes of cultivation of *G. boninense* in different media.

Media	No. of fruiting bags	No. of pinhead formation	Colour changes from young to mature
Sawdust	11	1	White to dark brown
Added palm kernel cake	26	1-2	White to dark brown
Added *Bluemea basamifera*	13	1-2	White to dark brown
Added *Clinacanthus nutans*	12	1	White to dark brown
Added cracked corn	8	1	White to dark brown

Initiation of fruiting body generally happens upon the onset of nutrients starvation or induces by harsh or drastic alteration in circumstances that unfavorable the mycelia growth. Besides the intrinsic factors, numerous extrinsic factors were reported to involve in the primordial initiation such as humidity, rate of oxygen and carbon dioxide, light intensity and others (Zhou *et al*, 2012). In brief, the development of fruiting body is divided into several stages: primordial initiation, elongation, cap formation and growth, and maturation (Sanodiya *et al*, 2009). In this study, primordial initiation was observed approximately after ten days of transferred for germination. Fructification usually is induced after the development of vegetative mycelium contributed by the environmental changes (Kües *et al*, 2000). In this study, removal of plastic cap to reduce the CO_2 concentration and watering to increase the humidity

acts as the drastic change extrinsic cultivation factors that affect the primordial formation. Following the primordial initiation, the fruiting body is gradually grown and elongated upward and reached it maturation after 4-5 months (Figure 2) as the fruiting body turned to dark brown in colour. During the initiation state, the pinheads of *G. boninense* were soft and white, followed by the continuously grow of dense white layer. During the course of development, the dense white mycelium layer changed their colour to light brownish and eventually turned to dark brown. At this point, the fruiting body had begun to harden and mature.

Figure 1. Formation of fruiting body of *G. boninense* in media blended with 20 % of palm kernel cake (PKC) with more than one primordial.

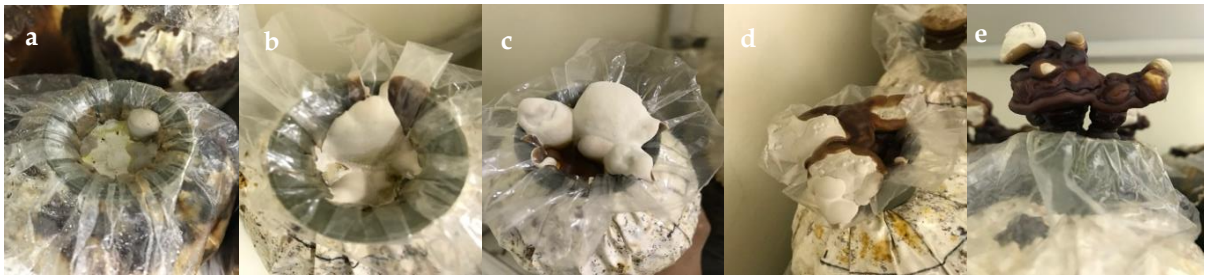

Figure 2. Major developmental stages of fruiting body of *G. boninense*. (a) Primordial initiation; (b) white mycelium dense; (c, d & e) elongation and maturing of fruiting body.

Figure 3. Fruiting body of *G. boninense* cultivated with different blended media. Formulated with 20% of *Blumea basamifera* (a, b, c); 20% of *Clinacanthus nutans* (d, e, f); 20% of cracked corn (g, h, i); control (j, k, l).

IV. CONCLUSION

This paper serves as the first report of successful artificial cultivation of *G. boninense* fruiting body using designed media mixture with recycle agricultural and industrial wastes. Wood sawdust and palm kernel cake (PKC) supplemented with rice bran were formulated into artificial media which were able to support the growth of major developmental stages of *G. boninense* until maturation of fruiting body. Preliminary study to develop cultivation method of *G. boninense* which is effective, economic and easily adopted by local mushroom growers is crucial for the future potential of drug development using *G. boninense*.

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