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Effects of Environment and Nutritional Conditions on mycelial growth of *Ganoderma boninense*

Shih Hao Tony Peng[1]*, Chee Kong Yap[2], Peng Fei Ren[3], Ee Wen Chai[1]

[1]All Cosmos Bio-Tech Holding Corporation, Johor 81700, Malaysia.
[2]University Putra Malaysia, Selangor 43400, Malaysia.
[3]Shandong Academy of Agriculture Sciences, Shandong 250100, China.

ABSTRACT

The basal stem rot disease is caused by the soil-borne pathogen, *Ganoderma boninense*. It is an annihilating and widespread disease in oil palm (*Elaeis guineensis* Jacq.). The nutritional studies were conducted to know the best sources of carbon and nitrogen, ideal pH regimes, the best humidity and optimum temperature required for the mycelial growth of *G. boninense.* Out of six carbon sources tested, fructose and glucose proved to be the best carbon sources for the mycelial growth of *G. boninense.* Out of five nitrogen sources tested, ammonium citrate and ammonium nitrate were noticeably found as the best nitrogen sources for the mycelial growth. Studies on different pH regimes in medium with 83% potatoes and 75% lignocellulosic materials revealed that the ideal pH regimes for the mycelial growth were 4-5. The best humidity for mycelial growth of *G. boninense* was found between 50-60%. It is suitable to grow between 25 and 32 °C, while the optimum temperature is 32 °C. This information can be used as a guideline for *Ganoderma's* disease prevention study and control strategies in the oil palm plantation in the future.

Keywords: *Ganoderma's* disease, mycelial growth, nutritional conditions, oil palm

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is an important crop plantation widely cultivated throughout Southeast Asia countries. Unfortunately, the industry is badly affected by basal stem rot (BSR) disease which is initiated by the soil-borne pathogen namely *Ganoderma boninense* (Ho & Nawawi 1985; Hushiarian *et al.* 2013). This BSR disease is the most devastating and prevalent disease in oil palm (Alexander *et al.* 2017) and has been the root cause for the significant loss of income in Indonesia and Malaysia (Alexander *et al*. 2017; Fowotade *et al*. 2019). The oil palm plantations are suffering significant damage of oil palms at the early stages and this has directly decreased the oil yield (Tan *et al.* 2018). It is therefore, ways of controlling the disease need to be researched and implemented so as to sustain the palm oil industry in this region (Hushiarian *et al.* 2013).

*Corresponding author:
All Cosmos Bio-Tech Holding Corporation, PLO650,
Jalan Keluli, Pasir Gudang Industrial Estate, 81700 Pasir Gudang, Johor, Malaysia.
Email: tonypeng@allcosmos.com

All the fungi have specific nutritional requirement. The mycelial growth of fungi in nature depends on a lot of external and internal factors. These include temperature, humidity, pH, soil structure and the nutrient availability including carbon and nitrogen sources (Lipavska & Konradova 2004; De paiva neto & Otoni 2003; Songulashvili *et al*. 2008; Yassen *et al*. 2013).

It is well reported in the literature that the growth and multiplication of shoots *in vitro* during plant tissue culture techniques are affected by many factors such as carbon and nitrogen sources (Mamoun & Olivier 1991; Panchal & Raol 2007; Li *et al*. 2011; Itoo & Reshi 2014; Lazarević *et al*. 2016) besides temperature and pH (Lazarević *et al*. 2016). Nitrogen and carbon are the indispensable and essential elements, besides others, for the infection, growth and reproduction of the fungi (Lilleskov *et al*. 2002; Yassen *et al*. 2013; Daza *et al*. 2016). The carbon and nitrogen sources greatly affect the growth and establishment of fungi in the field and also *in vitro* under the laboratory condition (Lilleskov *et al.* 2002). For example, Papaspyridi *et al.* (2012) studied the effects of nitrogen and carbon sources on the production of dietary glucans and fibres by *G. australe* and *Pleurotus ostreatus* while Peng *et al.* (2016) investigated the effects of mixed carbon sources by using mannose and galactose in *G. lucidum*. All the above literature pointed to the importance of the optimal nutritional conditions needed by different species of fungus for a variety of beneficial purposes.

From the literature, some preventive treatments to control *Ganoderma* infection in oil palm have been developed such as using the *Ganoderma* selective medium (GSM) (Ariffin & Idris 1992), the GanoCare™ organic (Idris *et al*. 2014) and the GanoCare™ OCSpecial (Idris *et al*. 2015). However, there is no information on the nutritional conditions for mycelial growth of *G. boninense* in which the information can be used in formulating the guideline for *Ganoderma's* disease prevention.

The culture conditions for mycelial growth of *Ganoderma* have been reported for *G. applanatum* (Jo *et al*. 2009; Jeong *et al*. 2009). Goh *et al*. (2016) studied the influence of different medium components (glucose, sucrose and fructose) on the growth of different *Ganoderma* species including *G. boninense*, *G. lingzhi* and *G. australe*. However, specific culture conditions for *G. boninense* has not been reported in the literature. Therefore, the aim of the present study is to evaluate and determine the optimal environment and nutritional conditions for mycelial growth of *G. boninense*.

MATERIALS AND METHODS

The strain used on the present study was *G. boninense* isolated from Malaysia's oil palm plantation.

Determination of the Optimum pH

The ingredients for this study included potato, glucose, hydrochloric acid, sodium hydroxide and agar. An amount of 1000 mL of nutrient solution was prepared from 200 g of potatoes (83%), 20 g of glucose (8.3%) and 21 g of agar (8.7%). An amount of 900 mL medium was prepared and poured into separate conical flasks; each flask contained 150 mL of medium. Medium in the conical flask was adjusted to pH 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14 by using hydrochloric acid and sodium hydroxide. Each pH has 4 replicates. The medium was autoclaved at 121 °C for 40 minutes. After the medium was cooled down to 50 °C, it was poured into a petri dish under sterile conditions. A 0.5cmx0.5cm of *G. boninense* was inoculated and they

were incubated at 25 °C. Mycelial growth of the fungus was observed for every 48 hours.

Since medium with too low pH, the agar could not completely dissolve, lignocellulosic materials were used as a medium for *G. boninense*. This experiment is to investigate whether the mycelia of *Ganoderma boninense* isolated from oil palm plantation able to survive in overly acidic and overly basic condition.

The ingredients for this study included lignocellulosic materials, wheat bran and glucose. The medium formulation consisted of 75% lignocellulosic materials, 24% wheat bran, and 1% glucose. A total of 30 g lignocellulosic materials, 9.6 g wheat bran, 0.4 g glucose were weighed and they were mixed evenly in a ratio of 1:1.4 (1 part of material to 1.4 part of water). The pH of the medium was adjusted using hydrochloric acid and sodium hydroxide and it was put into an 18mmx18mm test tube. The medium occupied half of the test tube space. The steps above were repeated to prepare medium of pH 1, 2, 3, 4, 11, 12, 13 and 14. Each pH value has 7 replicates. Test tubes were filled up with different pH values substrates. The media were autoclaved at 121 °C for 2 hours. *Ganoderma boninense* was inoculated after the tube was cooled down. The test tubes were then incubated at 25 °C for 14 days. Mycelial growth of the fungus was observed for every 48 hours.

Determination of Best Carbon Source

The ingredients for this *in vitro* study included potato, yeast extract, glucose, lactose, fructose, maltose, sucrose, soluble starch and agar. For the use of glucose as a carbon source, 1000 mL of nutrient solution was prepared from 200 g of potatoes, 5 g of yeast extract, 20 g of glucose and 21 g of agar. An amount of 1 500 mL medium was later prepared. For preparation of other carbon sources, glucose in the medium was replaced with an equal amount of lactose, fructose, maltose, sucrose and soluble starch, respectively. An amount of 150 mL was prepared for each carbon source. The medium was autoclaved at 121 °C for 30 minutes. Each carbon source has 4 replicates and they were inoculated with *G. boninense*. The culture plates were incubated at 25 °C and the mycelium growth were observed for every 48 hours.

Determination the Best Nitrogen Source

The nitrogen sources can be divided into: a inorganic sources namely ammonium nitrate and ammonium citrate; and b organic sources namely beef extract, peptone and urea. The ingredients for this *in vitro* study included potato, ammonium nitrate, ammonium citrate, beef extract, peptone, urea and agar. For the use of peptone as a nitrogen source, an amount of 1000 mL of nutrient solution was prepared from 200 g of potatoes, 5 g of peptone, 20 g of glucose and 21 g of agar. For preparation of other nitrogen sources, peptone in the medium was replaced with an equal amount of ammonium nitrate, ammonium citrate, beef extract and urea, respectively. An amount of 150 mL was prepared for each nitrogen source. The medium was autoclaved at 121 °C for 30 minutes. Each nitrogen source has 4 replicates and they were inoculated with *G. boninense*. The culture plates were incubated at 25 °C and the mycelium growth were observed for every 48 hours.

Determination of the Optimum Temperature

The ingredients in this study included potato, yeast extract, glucose, agar and wheat bran. An amount of 1000 mL of nutrient solution was prepared from

200 g of potatoes, 5 g of yeast extract, 20 g of glucose and 21 g of agar and 50 g of wheat bran. According to the formula, 900 mL of medium was prepared and poured into 6 conical flasks; each conical flask contained 150 mL of medium. The medium was autoclaved at 121 °C for 40 minutes. After the medium was cooled down to 50 °C, it was poured into a petri dish under a sterile condition. An area of 0.5cmx0.5cm of *G. boninense* was inoculated and it was incubated at 20 °C, 25 °C, 30 °C, 35 °C incubator, respectively. Each temperature has 4 replicates. Mycelial growth was observed in every 48 hours.

Determination of the Best Humadity

The substrate formula used in this study was: 75% lignocellulosic materials, 23% bran, 1% glucose, 1% lime. A total of 30 g of lignocellulosic materials, 9.2 g of wheat bran, 0.4 g of glucose and 0.4 g of lime were mixed evenly with a mix ratio 1:1 (1 part of material to 1 part of water). The substrate was then put in a 20cmx-200cm test tube, which occupied half of the test tube space. The steps above were repeated for the mixture ratio of 1:1, 1:1.2, 1:1.5, 1:1.9 and 1:2.4 in order to obtain the substrate's humidity of 50, 55, 60, 65 and 70%. Test tubes filled with different mixture ratios substrates were autoclaved at 121 °C for 2 hours. Each humidity has 5 replicates of test tube. Later, *G. boninense* was inoculated after the tube was cooled down. The test tubes were then incubated at 25 °C for 14 days, the mycelial growth was observed in every 48 hours.

Statistical Analysis

By using a statistical software (Statsoft STATISTICA version 10 for Windows), inter treatment differences of each parameter in the different nutritional conditions of mycelial growths were analysed using the Post-hoc test (Student-Newman-Keuls) to see if there was any significant difference at $P<0.05$

RESULTS AND DISCUSSION

Effects of pH Regimes

The observational mycelial growths of *G. boninense* in medium with 83% potatoes, under pH regimes of 5, 6 and 7, are shown in Figure 1 while those under pH regimes of 8, 9 and 10 are presented in Figure 2. The effects of different pH regimes (5 to 10) on the mycelial growth of *G. boninense* are presented in Table 1. From Table 1, it shows that mycelia of *G. boninense* are able to grow in medium with 83% potatoes, under the pH regimes of 5, 6, 7, 8, 9 and 10. However, when pH of medium was adjusted to 1, 2, 3, 4, 11, 12, 13 and 14, the agar could not dissolve completely, therefore no mycelial growths of *G. boninense* were observed for the above eight regimes of pH values.

The observational mycelial growths of *G. boninense* in medium with 75% lignocellulosic materials, under acidic conditions, pH regimes of 1, 2, 3 and 4, are shown in Figure 3 while those under alkaline conditions, pH regimes of 11, 12, 13 and 14, are presented in Figure 4. The effect of different pH values on the mycelia growth of *G. boninense* in medium with 75% lignocellulosic materials are presented in Table 2. From Table 2, pH 1 and 2 resulted in no sign of mycelium growth while pH 4 showed best mycelium growth rate with mycelia did not turn to yellow color. Growth rates in pH 3, 11, 12, 13 and 14 did show mycelium growth with mycelia colors ranging from obviously in yellow color to slight turn to yellow color. This shows that mycelia of *G. boninense* are able to grow in medium with 83% potatoes, under the pH regimes of 3, 11, 12, 13 and 14, with the best growth rate found in pH 4.

Figure 1 The observational mycelial growths of *Ganoderma boninense* in medium with 83% potatoes, under pH regimes of a 5, b 6 and c 7.

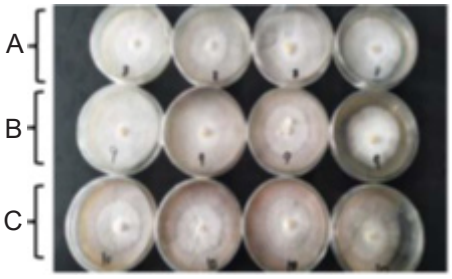

Figure 2 The observational mycelial growths of *Ganoderma boninense* in medium with 83% potatoes, under pH regimes of a 8, b 9 and c 10.

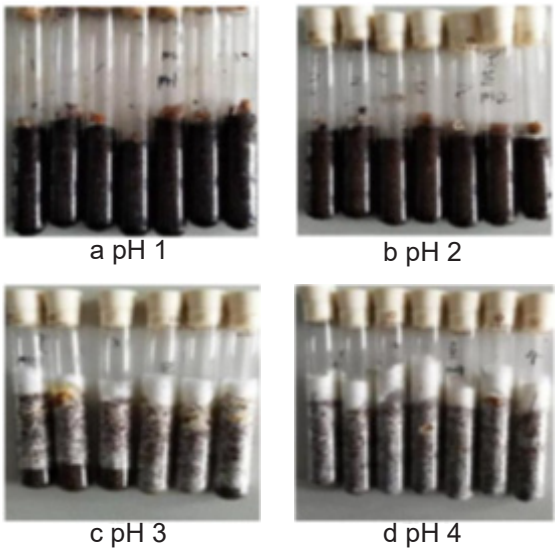

Figure 3 The observational mycelial growths of *Ganoderma boninense* in medium with 75% lignocellulosic materials under acidic conditions, pH regimes of a 1, b 2, c 3, c one replicate is not available and d 4.

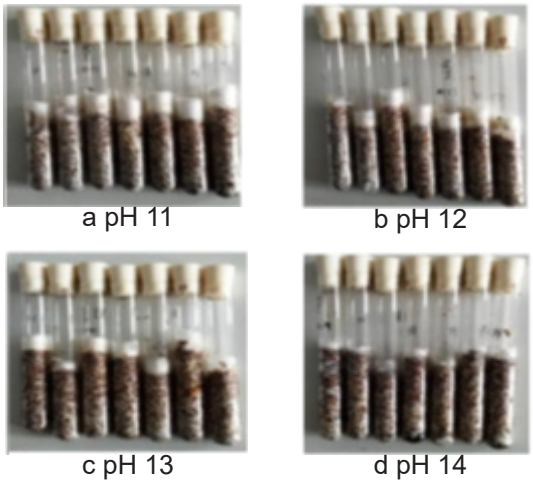

Figure 4 The observational mycelial growths of *Ganoderma boninense* in medium with 75% lignocellulosic materials under alkaline conditions, pH regimes of a 11, b 12, c 13 and d 14.

Table 1 The effects of different pH regimes on the mycelial growth (mean ± SE, cm) of *Ganoderma boninense* in medium with 83% potatoes

pH value	Day 2	Day 4	Day 6	Mycelial density
5	1.67 ± 0.07^{e}	4.30 ± 0.01^{b}	7.65 ± 0.05^{b}	+++
6	1.55 ± 0.05^{d}	4.45 ± 0.05^{d}	7.55 ± 0.05^{b}	++
7	1.28 ± 0.08^{ab}	3.78 ± 0.01^{a}	6.53 ± 0.02^{a}	++
8	1.43 ± 0.03^{c}	4.40 ± 0.01^{cd}	7.80 ± 0.01^{c}	++
9	1.20 ± 0.01^{a}	4.55 ± 0.03^{e}	7.93 ± 0.03^{d}	++
10	1.30 ± 0.01^{b}	4.35 ± 0.04^{bc}	7.83 ± 0.03^{c}	++

*Note: The more "+", the higher mycelial density
Values sharing a common letter in the post hoc column are not significantly different (P>0.05).

Table 2 Effect of different pH values on the mycelia growth of *Ganoderma boninense* in medium with 75% lignocellulosic materials

pH values	Mycelia color	Growth rate
1	-	-
2	-	-
3	++	+
4	+++	+++
11	++	++
12	+	++
13	+	++
14	+	+

Note:
Mycelial color: "-" mycelia did not grow; "+" mycelia obviously in yellow color; "++" mycelia slightly turn to yellow color; "+++" mycelia did not turn to yellow color
Growth rate: The more "+", the better mycelial growth.

Studies on different pH regimes in medium with 83% potatoes and 75% lignocellulosic materials, revealed that the ideal pH for the mycelial growth were 4-5. Deshmukh *et al*. (2012) also reported that the optimum pH for the fungus *C. gloeosporioides* to produce the maximum dry mycelial weight and sporulation were at pH 5.5 and pH 6.5 in liquid media, respectively. Favorable mycelial growth of *G. boninense* was obtained at the pH range of 5 to 9 (Table 1). Therefore, the results indicated that *G. boninense* can grow at broad range of pH, from pH 5 to 10. This result also indicated that different *G. boninense* prefer different pH values tending toward neutrality.

The present result was supported by mycelial growth of *G. lucidum* can be observed at the pH range of 5 to 9. Jonathan *et al*. (2004) and Fasola *et al*. (2007) obtained very good mycelial growth of *Volvariella esculenta* and *V. speciosa,* respectively, over a wide range of pH regimes but optimum growth was obtained at pH 6. Likewise Adejoye *et al*. (2007) also reported *Schizophyllum commune* exhibited a favorable growth at pH 5.5 while Akinyele and Adetuyi (2005) reported that pH range for the mycelial growth of *V. volvacea* was between 5.5 and 8.5. All of their results suggested that mushrooms may have a broad pH range for their favorable mycelial growth.

Effect of Carbon Sources

The observational mycelial growths of *G. boninense* under lactose, soluble starch and fructose as carbon sources are given Figure 5 while those under maltose, glucose and sucrose as carbon sources are presented in Figure 6. The effects of different carbon sources on the mycelial growth of *G. boninense* are shown in Table 3. From Table 3, among all the carbon sources, the order of mycelial growths were observed as glucose and fructose>maltose and soluble starch>sucrose and lactose. This indicated noticeably that

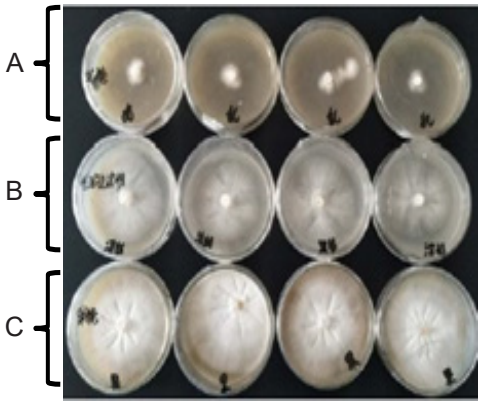

Figure 5 The observational mycelial growths of a *Ganoderma boninense* under lactose, b soluble starch and c fructose as carbon sources.

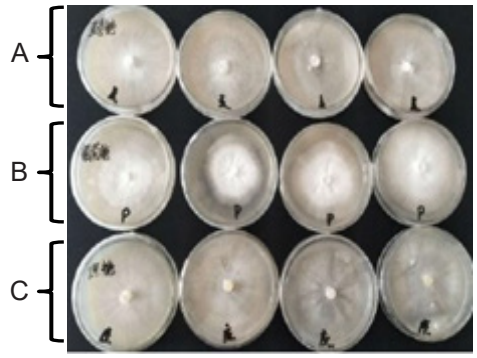

Figure 6 The observational mycelial growths of a *Ganoderma boninense* under maltose, a glucose and c sucrose as carbon sources.

Table 3 The effects of different carbon sources on the mycelial growth (mean ± SE, cm) of *Ganoderma boninense*

Carbon sources	Day 2	Day 4	Day 6	Mycelial growth
Glucose	2.10 ± 0.02^{b}	4.30 ± 0.03^{b}	7.00 ± 0.02^{bc}	+++
Maltose	2.80 ± 0.02^{d}	6.00 ± 0.02^{d}	8.00 ± 0.03^{d}	++
Fructose	2.50 ± 0.01^{c}	5.40 ± 0.02^{c}	7.50 ± 0.04^{c}	+++
Sucrose	2.30 ± 0.03^{bc}	5.00 ± 0.03^{c}	7.60 ± 0.02^{c}	+
Lactose	1.80 ± 0.01^{a}	1.80 ± 0.02^{a}	1.80 ± 0.01^{a}	+
Soluble starch	1.90 ± 0.01^{a}	4.10 ± 0.02^{b}	6.50 ± 0.03^{b}	++

* Note: The more "+", the better mycelial growth
Values sharing a common letter in the post hoc column are not significantly different (P>0.05).

glucose and fructose are better carbon sources for mycelial growth of *G. boninense.*

Out of six carbon sources tested, fructose and glucose had been found as the best for the mycelial growth. This indicated noticeably that fructose and glucose are better carbon sources for mycelial growth of *G. boninense*. Sangeetha & Rawal (2008) reported that mannitol was found to be the best source of carbon for the growth and sporulation of the *Colletotrichum gloeosporioides* (mango disease), followed by fructose and sucrose. According to Bhojwani and Rajdan (1996), glucose and fructose can hold up for the growth of some tissues during tissue culture. The carbon sources of the medium can supply energy and uphold the osmotic potential (De paiva neto & Otoni 2003). Based on different carbon sources on *in vitro* micropropagation of *Oxalis corniculata*, Swetha Prasuna and Srinivas (2016) reported that fructose was the best carbon source with maximum increase of shoot multiplication. Panchal and Raol (2007) reported that mannitol was found as the best carbon source for the *in vitro* vegetative growth of *Fusarium moniliforme* isolated from wilted sugar cane fields. In comparison to that reported by Deshmukh *et al*. (2012), they reported that starch and xyllose were the best carbon sources for the growth and sporulation of *C. gloeosporioides*, followed by glucose and sucrose. All the reviewed information above indicated different species of fungus has the different optimal carbon sources for the mycelial growth of the fungus.

Dextrin was the best carbon source for mycelial growth of *G. lucidum*. However, found sorbitol lactose and glucose showed slow level of mycelial growth in *G. lucidum.* However, in case of IUM 938 strain of *G. lucidum*, optimum mycelial growth was found on fructose, agreeing with the present result for *G. boninense*. Chandra and Purkayastha (1977) had previously reported that most of the tropical edible macrofungi were in favor of utilizing glucose than other carbon sources. The preference of glucose over other carbon compounds may be due to the fast metabolization of glucose by the fungi to produce cellular energy easily (Garraway & Evans 1984). Griffin (1994) suggested that mannose and fructose are the most commonly utilized sugars after glucose. Those results are partially similar to our findings, but they showed that mycelial density in all carbon sources is thin where our result is opposite.

Effect of Nitrogen Sources

The observational mycelial growths of *G. boninense* under ammonium citrate, beef extract and urea as nitrogen sources are given in Figure 7 while those under ammonium nitrate and peptone as nitrogen sources are presented in Figure 8. The effects of different nitrogen sources on the mycelial growth of *G. boninense* are presented in Table 4. According to the experimental results, *G. boninense* showed optimum and best mycelial growth on ammonium citrate and ammonium nitrate. Except for urea, peptone and beef extract also facilitated considerable mycelial growth of *G. boninense* (Table 4).

Out of five different nitrogen sources from the present study, the best nitrogen sources for mycelial growth of *G. boninense* are ammonium citrate and ammonium nitrate. Sangeetha and Rawal (2008) reported that ammonium nitrate supported good growth and sporulation the *C. gloeosporioides*, agreeing with the present finding. Deshmukh *et al*. (2012) found that potassium nitrate as the best nitrogen source for the growth and sporulation of the pathogen *C. gloeosporioides*.

Similarly, eight strains of *G. lucidum* showed optimum mycelial growth on ammonium acetate while two strains of *G. lucidum*

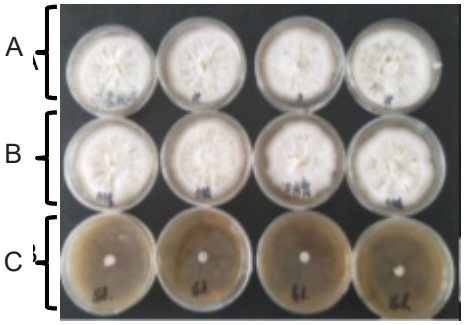

Figure 7 The observational mycelial growths of a *Ganoderma boninense* under ammonium citrate, b beef extract and c urea as nitrogen sources.

Figure 8 The observational mycelial growths of a *Ganoderma boninense* under ammonium nitrate and b peptone (one replicate is not available) as nitrogen sources.

Table 4 Effects of different nitrogen sources on the mycelial growth (mean ± SE, cm) of *Ganoderma boninense*

Nitrogen sources	Day 2	Day 4	Day 6	Mycelial growth
Peptone (organic)	1.50 ± 0.02[a]	3.63 ± 0.01[b]	5.67 ± 0.07[bc]	++
Beef extract (organic)	1.50 ± 0.02[a]	3.50 ± 0.01[b]	6.18 ± 0.03[c]	++
Ammonium nitrate (inorganic)	1.50 ± 0.02[a]	3.50 ± 0.02[b]	6.75 ± 0.06[d]	+++
Ammonium citrate (inorganic)	1.50 ± 0.02[a]	3.50 ± 0.03[b]	6.58 ± 0.05[d]	+++
Urea (organic)	1.50 ± 0.01[a]	1.50 ± 0.01[a]	1.50 ± 0.01[a]	-

* Note: The more "+", the better mycelial growth
Values sharing a common letter in the post hoc column are not significantly different (P>0.05).

(IUM 757 and 1027) showed no mycelial growth on urea. Therefore, present result indicated that inorganic nitrogen sources (ammonium citrate and ammonium nitrate) enhanced the mycelial growth of *G. boninense*, supported by the finding by *G. lucidum*. The present findings are very similar to the observations *V. esculenta* (Jonathan *et al*. 2004). Hence, mycelial growth of *G. boninense* was more favorable on the culture media containing inorganic nitrogen sources than organic nitrogen sources.

Effect of Temperature

After 5 days of inoculation, the observational mycelial growths of *G. boninense* at 15 °C, 20 °C, 25 °C, 30 °C and 35 °C are presented in Figure 9 while those at 28 °C, 30 °C and 32 °C are given in Figure 10, those at 25 °C, 28 °C, 30 °C, 32 °C and 34 °C are shown in Figure 11, and those at 34 °C, 37 °C, 40 °C and 43 °C are given in Figure 12. Table 5 shows the effects of different temperature on the mycelial growth of *G. boninense* after five days of inoculation.

As shown in Figures 9, 10, 11 and 12, and Table 5, temperature between 25-35 °C, after 5 days of inoculation, the mycelia had the fastest growth rate at 30 °C, followed by 25 °C. *Ganoderma boninense* is not suitable to grow at 15 °C, 20 °C, and 35 °C while there was no sign of mycelium growth at 35 °C.

Figure 11 shows that when temperature at 25 °C, 28 °C, 30 °C, 32 °C and 34 °C, mycelia had better growth rate at 32 °C, however mycelia grew slowly at 34 °C. As shown in Figure 12 when temperature was set at 34 °C, 37 °C, 40 °C, 43 °C, mycelia only grew at 34 °C after 10 days of inoculation. However, the hyphal growth was inhibited at this temperature, growth was slow, and mycelia were sparse. It is observed that temperature at 37 °C and above resulted in no sign of mycelium growth. As a conclusion, mycelia of *G. boninense* are suitable to grow between 25 and 32 °C, while the optimum temperature is 32 °C. Temperature at 34 °C and above will inhibit the growth

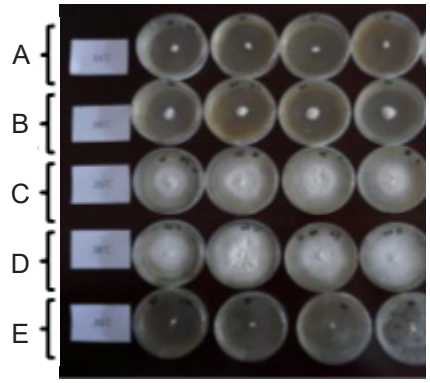

Figure 9 The observational mycelial growths of *Ganoderma boninense* at a 15 °C, b 20 °C, c 25 °C, d 30 °C and e 35 °C, after 5 days of inoculation.

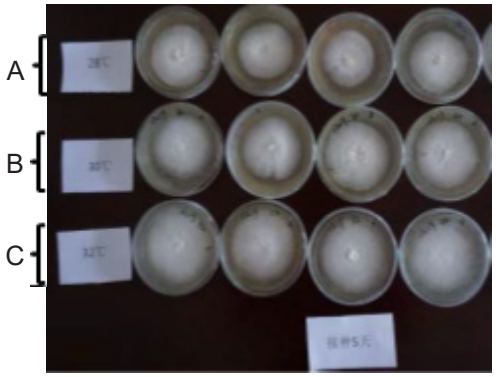

Figure 10 The observational mycelial growths of *Ganoderma boninense* at a 28 °C, b 30 °C and c 32 °C, after 5 days of inoculation.

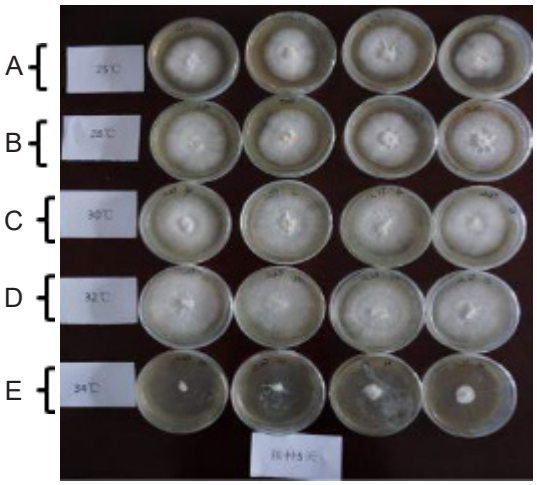

Figure 11 The observational mycelial growths of *Ganoderma boninense* at a 25 °C, b 28 °C, c 30 °C, d 32 °C and e 34 °C, after 5 days of inoculation.

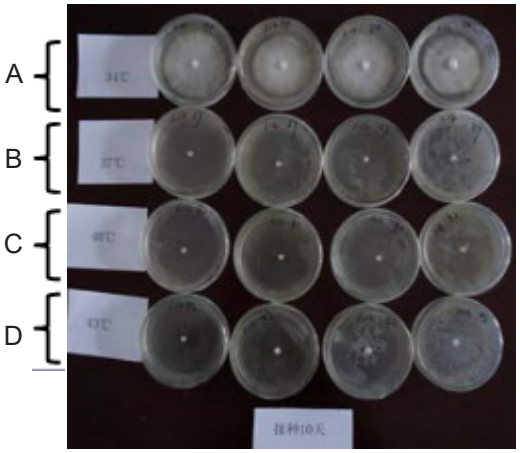

Figure 12 The observational mycelial growths of *Ganoderma boninense* at a 34 °C, b 37 °C, c 40 °C and d 43 °C, after 5 days of inoculation.

Table 5 The effects of different temperatures on the mycelial growth RATE (mean ± SE, cm/day) of *Ganoderma boninense* after five days of inoculation

Number	Temperature	Growth rate (cm g^{-1})	Growth potential
1	15 °C	0.22 ± 0.01[a]	+
2	20 °C	0.52 ± 0.02[b]	+
3	25 °C	0.79 ± 0.03[c]	+++
4	28 °C	0.83 ± 0.04[c]	+++
5	30 °C	0.88 ± 0.09[c]	++++
6	32 °C	0.89 ± 0.07[c]	++++
7	34 °C	0.30 ± 0.01[a]	++
8	35 °C	No sign of mycelium growth	No sign of mycelium growth

* Note: The more "+", the better mycelial growth
Values sharing a common letter in the post hoc column are not significantly different (P>0.05).

of mycelia. Previously, Ho and Nawawi (1985) reported the optimum temperature for mycelial growth of *G. boninense* from Peninsular Malaysia were between 27-29 °C and significantly poorer at 21 °C.

Jonathan and Fasidi (2003) found that Psathyrella atroumbonatai grew fairly well within the temperature range of between

25 and 35 °C. the best mycelial growth *G. lucidum* was found at 30 °C. Shim *et al*. (2003) reported that the mycelial growth of Paecilomyces fumosoroseus was favourable at the range of between 20 and 25 °C and had been expedited in proportion to the rise of temperature. However, the mycelial growth appeared to be inhibited at the temperature higher than 30 °C. Jonathan *et al*. (2004) also reported that the growth of Schizophyllum commune was inhibited at 45 and 50 °C. This could be attributable to the denaturation and inactivation of important enzymes which catalyse metabolic processes of the experimented fungus. Therefore, similar explanation can be provided for temperature at 34 °C and above that inhibited the mycelial growth of *G. boninense.*

Effect of Humidity

The observational mycelial growths of *G. boninense* under humidity of 50%, 55% and 60% are presented in Figure 13 while those under humidity of 65% and 70% are given in Figure 14. The effects of different humidity on the mycelial growth of *G. boninense* are presented in Table 4. Therefore, from Figures 13 and 14, and Table 6, it shows that the best humidity for mycelial growth of *G. boninense* is between 50-60%. There is limited reported study on the effect of humidity on the mycelial growth of *G. boninense.*

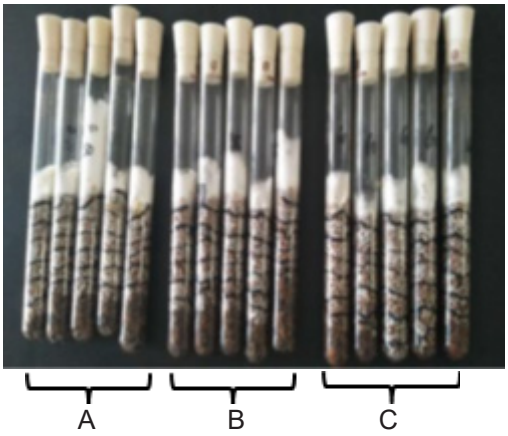

Figure 13 The observational mycelial growths of *Ganoderma boninense* under humidity of a 50%, b 55% and c 60%.

Figure 14 The observational mycelial growths of *Ganoderma boninense* under humidity of a 65%; b one replicate is not available) and 70%.

Table 6 The effects of different humidity on the mycelial growth (mean ± SE, cm) of *Ganoderma boninense*

%	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Mycelia growth
50	0.66 ± 0.02^{a}	1.80 ± 0.05^{a}	2.72 ± 0.02^{a}	3.78 ± 0.04^{a}	4.82 ± 0.05^{a}	6.02 ± 0.06^{a}	+++
55	0.78 ± 0.03^{b}	1.96 ± 0.03^{a}	2.96 ± 0.05^{ac}	3.94 ± 0.05^{a}	5.04 ± 0.04^{a}	6.12 ± 0.05^{a}	+++
60	0.90 ± 0.02^{c}	2.22 ± 0.01^{b}	3.38 ± 0.05^{cd}	4.64 ± 0.04^{b}	6.06 ± 0.05^{b}	7.02 ± 0.04^{b}	+++
65	0.74 ± 0.03^{b}	2.26 ± 0.02^{b}	3.60 ± 0.06^{d}	4.98 ± 0.02^{b}	6.40 ± 0.04^{cd}	7.64 ± 0.06^{c}	++
70	0.90 ± 0.02^{c}	2.30 ± 0.04^{b}	3.90 ± 0.07^{e}	6.10 ± 0.05^{c}	6.96 ± 0.07^{d}	8.30 ± 0.05^{d}	++

* Note: The more "+", the better mycelial growth

Values sharing a common letter in the post hoc column are not significantly different (P>0.05).

CONCLUSIONS

In the present *in vitro* study, fruiting bodies of *G. boninense* were collected from oil palm plantation in Malaysia. Isolation and subculture were done to get the pure culture of *G. boninense.* The effects of different carbon sources, nitrogen sources, humidity, temperature and medium with different pH regimes on the mycelial growth of *G. boninense* were investigated. In medium with 83% potatoes, mycelia of *G. boninense* could be able to grow in pH regimes of 5, 6, 7, 8, 9 and 10. In pH 5, mycelia had the best growth rate. Therefore, the optimum pH for mycelia growth of *G. boninense* in medium with 83% potatoes is pH 5. However, mycelia grew well in medium with 75% lignocellulosic materials of pH 4. This indicated better mycelia color and growth rate in pH 4. Therefore, the ideal pH regimes for the mycelial growth were 4-5.

The results showed that the best carbon sources for the mycelial growth of *G. boninense* were glucose and fructose, while the best nitrogen sources were ammonium citrate and ammonium nitrate. The optimum humidity for the mycelia growth was between 50-60%. It is suitable to grow between 25 and 32 °C, while the optimum temperature is 32 °C. Temperature at 34 °C and above will inhibit the growth of mycelia.

This optimal nutritional conditions and environment can be fully utilized as a guideline for *Ganoderma's* prevention study and for spawn production in the future. Hence, the use of nutritional conditions with fructose and glucose as carbon sources, with ammonium citrate and ammonium nitrate bases, and slightly acidic soils can promote the growth of *G. boninense* in Malaysia.

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