

## Isolation, Growth and Sporophore Development of *Ganoderma boninense* from Oil Palm in Malaysia

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### ABSTRAK

Banyak miselium bagi *G. boninense* boleh diasingkan daripada tisu-tisu sporofor yang dikulturkan pada potato dextrose agar (PDA) dan juga daripada satu basidiospora yang telah bercambah pada PDA. Suhu optimum untuk tumbesaran miselium ialah 27-29°C dan media yang terbaik ialah lima bean agar. Perkembangan sporofor daripada kultur miselium pada media berlainan tidak berjaya. Kajian tumbesaran bagi sporofor dari batang yang telah dijangkiti menunjukkan tumbesaran perlahan pada minggu 1-9, kemudian tumbesaran bertambah cepat hingga minggu keempat belas, dan kemudian kadar tumbesaran berkurangan semula.

### ABSTRACT

Abundant mycelium of *G. boninense* could be isolated from sporophore tissues cultured on potato dextrose agar (PDA) and from single basidiospore germinating on PDA. The optimum temperature for mycelial growth was between 27-29°C and the best medium was lima bean agar. Sporophore development from mycelial culture was not successful on the various media employed. Studies on the growth of sporophore on infected trunks showed that growth was slow in the first 1-9 weeks, then increased rapidly till the fourteenth week after which growth rate decreased again.

### INTRODUCTION

Various species of *Ganoderma* have been recorded as causal agents of basal stem rot in oil palm in many parts of the world. Much work has been done on disease aetiology (Navaratnam, 1964; Turner 1965a, b) and control (Turner, 1965c, d; Parnata, 1974; Varghese *et al.*, 1976) but basic studies on the biology of *Ganoderma* species are very limited (Bose, 1929, Venkatarayan, 1936, Varghese *et al.*, 1976).

*Ganoderma boninense* Pat. was recently reported as one of the *Ganoderma* species causing basal stem rot of oil palm in some estates in Peninsular Malaysia (Ho and Nawawi, 1985).

The present investigation involved isolation studies, sporophore development and effects of temperature and medium on the mycelial growth of this fungus.

### MATERIALS AND METHODS

#### *Isolation from Young Sporophores and Infected Stem Tissues*

Small pieces (4 mm × 4 mm × 5 mm) of the context layer of button stage sporophore and tissues (10 mm × 10 mm × 15 mm) from the centre of infected oil palm stem were surface sterilised (5 minutes in 10% Chlorox) and plated on to potato dextrose agar (PDA) in petri dishes

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and 250 ml Erlenmeyer flask respectively. The petri dishes and flasks were incubated at  $27 \pm 1^\circ\text{C}$  until there was visible mycelial growth. The mycelium which grew out of the tissues was subcultured immediately on to fresh plates with PDA medium.

#### *Isolation from Single Spore*

Petri dishes with PDA were exposed for about 15–30 seconds under sporulating sporophores. The petri dishes were incubated at  $27 \pm 1^\circ\text{C}$  for about 36 hours after which they were examined under the microscope. Selected germinating spores were isolated singly with a spore cutter and transferred on to a fresh plate with PDA.

#### *Effects of Media and Temperature on Growth*

A disc of mycelium from pure cultures of *G. boninense* growing on PDA was cut out using a No. 1 cork-borer and inoculated on to plates with medium. Growth was compared on nine media *viz* PDA, corn meal agar (CMA), oat meal agar (OMA), lima bean agar (LBA), malt extract agar (MA), prune agar (PA), Czapek agar (CA), carrot dextrose agar (CDA) and rice dextrose agar (RDA). All the media except CDA and RDA were from Difco and were prepared according to the instructions given. CDA was prepared with 200 g carrot, 20 g dextrose, 20 g agar in 1 l distilled water and RDA was prepared with 160 g rice flour, 15 g dextrose, 20 g agar in 1 l distilled water. Five replicates were made for each medium. The plates were incubated at  $27^\circ\text{C}$  and measurements of the colony diameters were recorded daily.

Effects of temperature on growth was conducted on LBA, PDA and RDA in 5 replicates at 6 temperatures *viz*  $21^\circ\text{C}$ ,  $23^\circ\text{C}$ ,  $25^\circ\text{C}$ ,  $27^\circ\text{C}$ ,  $29^\circ\text{C}$  and  $31^\circ\text{C}$ . Measurements of colony diameters were compared 6 days after inoculation.

#### *Cultivation of Sporophore from Pure Cultures of Mycelium*

Pure cultures of *G. boninense* were inoculated

into 1 l Erlenmeyer flasks containing 400 ml of medium. Four different media were used. They were PDA, LBA, modified Elliott's medium (incorporated with 20 g agar per litre) and modified Elliott's medium with 20 g agar and 150 g macerated oil palm trunk tissues in 1 l distilled water. Modified Elliott's medium contained 1.3 g  $\text{KH}_2\text{PO}_4$ , 1.06 g  $\text{Na}_2\text{CO}_3$ , 0.5 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 5 g D-glucose, 1.0 g L-asparagine and 1 ml micronutrients in 1 l distilled water. The micronutrients consisted of 2.86 g  $\text{H}_3\text{BO}_4$ , 1.81 g  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 0.22 g  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.08 g  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.5 g biotin, 0.1 g thiamine and 5 g iron tartrate dissolved in 1 l of distilled water. Five replicates were made for each medium. The flasks were incubated at ambient temperature under diffuse light.

#### *Growth Rate of Sporophore in Infected Palms*

Observations were made on the morphology and growth rate of 20 sporophores from infected field palms and from infected cut stumps incubated in the Botany Garden at University of Malaya. Measurements were made when sporophore was first visible as a very small flat button-like structure. For the first two weeks, measurements were taken on alternate days, but from the third week onwards, records were made weekly until the sporophore stopped growing or was badly damaged by insects and their larvae. The length (parallel to the attachment area) and the width (perpendicular to the attachment area) of sporophore (*Fig. 1*) were measured and where a stipe was present, its length and diameter were also recorded.

## RESULTS AND DISCUSSION

#### *Isolation Studies*

Abundant mycelial growth occurred on PDA after 3–4 days from pieces of sporophore at the button stage. Out of 15 pieces, 11 produced profuse mass of mycelia.

Growth from the infected tissues of oil palm stem cultured in flasks was very poor. Only 2 pieces out of 15 showed some mycelial growth after a week.

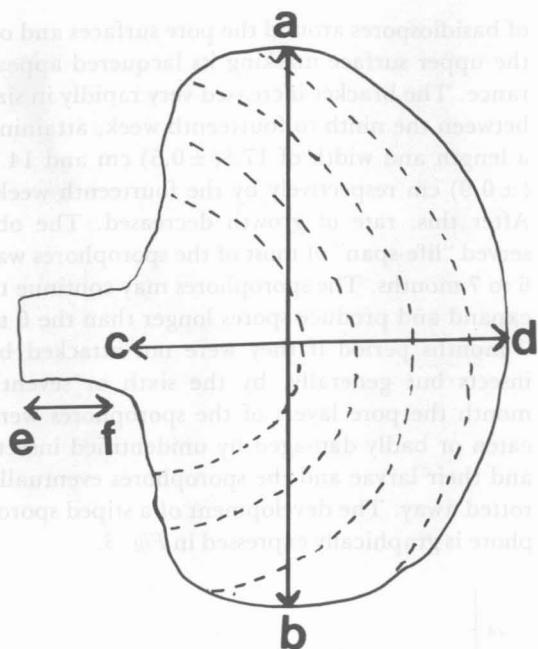


Fig. 1: Graphical representation of sporophore where measurements were made. (a - b), length; (c - d), width; (e - f), stipe of sporophore.

Basidiospores collected from exposing petri dishes with PDA under sporulating sporophores germinated after 30 hours. Single germinating spore transferred to fresh PDA plates grew to a colony of 16 mm in diameter in 6 days.

#### Effect of Medium

The results on the rate of mycelial growth are given in Fig. 2. Growth rates varied among the different media. LBA was the most suitable for growth followed by RDA and PDA. Growth was significantly poorer ( $P = 0.05$ ) on CA. Mycelium on PA was sparse, appressed and thinly spread out on the medium but those on all the other media were abundant and floccose. Recent studies by Tseng *et al.*, (1984) on *G. lucidum* (Leys) Karst. showed that generally, mycelial growth was better in complete media than in synthetic media such as Czapek medium.

#### Effect of Temperature

Mycelial growth rates at different temperatures on the 3 media are given in Table 1.

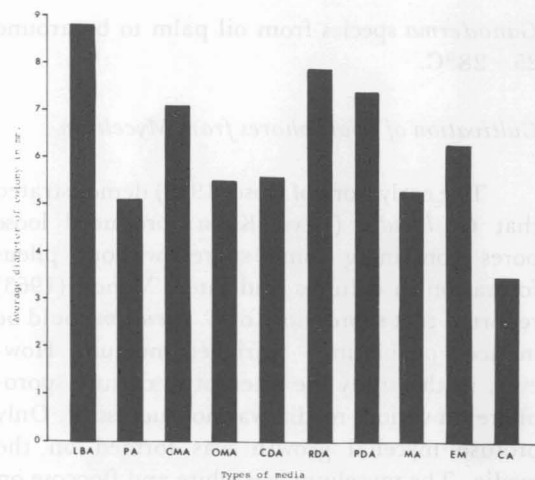


Fig. 2: Growth of *G. boninense* 6 days after incubation on various media. Lima bean agar (LBA), prune agar (PA), corn meal agar (CMA), oat meal agar (OMA), carrot dextrose agar (CDA), rice dextrose agar (RDA), potato dextrose agar (PDA), malt agar (MA), modified Elliott's agar (EM), Czapek agar (CA).

TABLE 1  
Effect of temperature on mycelial growth of *G. boninense* on three selected media

Temperature °C	Mean colony diameter (mm after 6 days)		
	LBA	RDA	PDA
21	40 <sup>a</sup>	33 <sup>a</sup>	35 <sup>a</sup>
23	51 <sup>b</sup>	47 <sup>b</sup>	46 <sup>b</sup>
25	70 <sup>c</sup>	63 <sup>c</sup>	59 <sup>c</sup>
27	89 <sup>d</sup>	79 <sup>d</sup>	70 <sup>d</sup>
29	83 <sup>d</sup>	75 <sup>d</sup>	71 <sup>d</sup>
31	73 <sup>c</sup>	66 <sup>c</sup>	60 <sup>c</sup>

\*For each medium (vertical column) mean values with no letters in common are significantly different ( $P = 0.05$ ) as determined by Duncan New Multiple Range Test.

Optimum growth was obtained between 27–29°C and at 21°C growth was significantly ( $P = 0.05$ ) poorer. Varghese *et al.*, (1976) also found the optimum temperature for mycelial growth of

*Ganoderma* species from oil palm to be around 25 – 28°C.

#### Cultivation of Sporophores from Mycelium

The early work of Bose (1929) demonstrated that *G. lucidus* (Leys) Karst. produced loose pores containing some spores without pileus formation in cultures and later, Menon (1963) reported that sporophore of *G. lucidum* could be induced on biotin – enriched medium. However, in this study the attempt to culture sporophore on various media was not successful. Only profuse mycelial growth was formed on the media. The mycelium was white and floccose on the surface of the medium but dark brown and thick lower down. Small peglike projections were formed by the mycelium in about 2 months but these projections did not develop further into sporophores even after 4 months.

#### Growth Rate of Sporophore in Infected Palms

The first sign of sporophore formation in the infected trunk was the appearance of a flat or slightly raised white button-like structure which became prominently dome-shaped within 2 days. This structure elongated very rapidly and by 7 days developed into a slender white column of 1.1(±0.2) cm long and 1.0(±0.2) cm wide. Within 14 days the column – like structure hardened and turned brown except at the growing apical end which remained white. This structure became the stipe of the sporophore. Once the stipe reached a length of about 1.6 – 2.0 cm it ceased to increase in length. Instead, the apical end expanded giving rise to a bracket. The bracket was white when first formed but as its length and width increased rapidly, the colour of the upper surface became light-brown and then deepened to a shiny dark-brown with concentric rings. The undersurface remained white and the growing margin was either white or orange. The bracket measured 3.9(±0.3) cm in length and 2.3(±0.6) cm in width with pores formed on the undersurface by the fourth week. Basidiospores were observed to be liberated on the fifth week. In the sixth and seventh week, formation and profuse liberation of basidiospores were visibly indicated by brown deposits

of basidiospores around the pore surfaces and on the upper surface masking its lacquered appearance. The bracket increased very rapidly in size between the ninth to fourteenth week, attaining a length and width of 17.8(±0.5) cm and 14.6(±0.9) cm respectively by the fourteenth week. After this, rate of growth decreased. The observed "life-span" of most of the sporophores was 6 to 7 months. The sporophores may continue to expand and produce spores longer than the 6 to 7 months period if they were not attacked by insects but generally, by the sixth or seventh month the pore layers of the sporophores were eaten or badly damaged by unidentified insects and their larvae and the sporophores eventually rotted away. The development of a stiped sporophore is graphically expressed in Fig. 3.

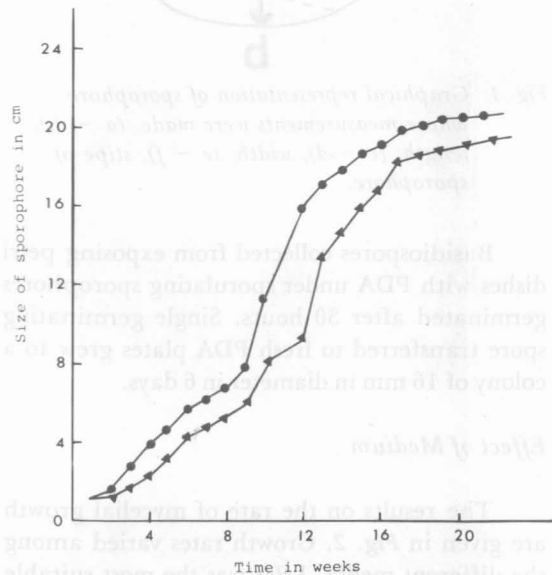


Fig. 3: Rate of sporophore development in *G. boninense*. (●—●), length; (▼—▼), width of sporophore.

The development of sessile sporophore also began as a flat, white button-like structure but instead of elongating to a stipe, the button-like structure expanded to form a small bracket by the second week. The morphology and development of these sessile brackets were similar to those of the stiped sporophores already described above.

Sporophores which form on the horizontal

surface (such as cut stumps) of infected oil palm trunks normally develop long stipes. The stipes in these sporophores raise the pileus or bracket away from the substrate surface. Sporophores which develop on the vertical sides of the infected trunks usually do not possess stipes.

Measurements of stipes and the length and width of sporophores show that there is a correlation between the size of the sporophore and the length of the stipe. Larger sporophores are observed to have stouter and shorter stipes than those that are smaller. Hawker (1950) suggested that decrease in length of stipe with increase in size of sporophore reduced the danger of the stipe breaking as a result of the weight of large sporophore. The relatively long, thin stipes of smaller sporophores are sufficiently strong to carry the light bracket.

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