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Effect of Temperature and pH on Growth Pattern of *Ganoderma boninense* from Oil Palm in Peninsular Malaysia

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ABSTRAK

Tumbesaran miselum G. boninense *dari cakera inokulum terapung yang dieram dalam kelalang, diputarkan setiap hari, jauh lebih banyak daripada tumbesaran miselum G. boninense dari cakera inokulum yang tenggelam dan tidak digerakkan. Suhu optima untuk tumbesaran miselum adalah antara 27°C-30°C dalam medium pepejal dan cecair. Pada peringkat permulaan (1-3 hari) terdapat kurang tumbesaran dalam pola tumbesaran miselum itu. Kemudian terdapat satu peringkat di mana tumbesarannya cepat (6-18 hari) disusuli dengan satu peringkat autolisis dan kejatuhan berat keringnya. pH medium akan berubah mengikut perubahan dalam pola tumbesaran itu. Apabila kadar tumbesaran miselum meningkat, pH medium menurun. Apabila kadar tumbesaran miselum menurun semasa pengeraman dilanjutkan, pH medium meningkat. pH optima bagi tumbesaran miselum adalah antara 3.7-5.0.*

ABSTRACT

Mycelial growth of G. boninense *from floating inoculum disks incubated in flasks swirled daily was significantly more abundant than that from submerged inoculum disks in static flasks. The optimum temperatures for mycelial growth in both solid and liquid media were between 27-30°C. The mycelial growth pattern in liquid medium showed an initial lag phase of little growth (1-3 days), then a phase of rapid growth (6-18 days) and finally a phase of autolysis and decline in dry weight. The pH of the medium changed correspondingly with the changes in the growth pattern. As mycelial growth increased, the pH of the medium decreased and when mycelial growth decreased during prolonged incubation, pH of the medium increased. Optimum pH for mycelial growth was between 3.7-5.0.*

INTRODUCTION

Basal stem rot of oil palm (*Elaeis guineensis* Jacq.), caused by species of *Ganoderma* Karsten, has been reported from this country as early as 1931 by Thompson. The disease, mainly confined to palms above twenty-five years of age, was not considered economically important until the mid-1960s when a large number of palms of 10-15 years were infected (Turner, 1981). The area under oil palm cultivation in Malaysia has expanded rapidly in recent years. With this rapid expansion in oil palm cultivation under monocultural conditions, the incidence of basal stem rot has continued to be on the increase, particularly along coastal areas, and a higher percentage of young palms (as young as one year old) have been found recently to be attacked by the fungus.

The most prevalent and widespread *Ganoderma* species associated with the disease in Peninsular Malaysia is *G. boninense* Pat. (Ho & Nawawi, 1985). Although some basic work has been carried out on the fungus with respect to sporophore development (Ho & Nawawi, 1986a), diurnal periodicity of basidiospore discharge (Ho & Nawawi, 1986b) and germination and viability

of basidiospore (Ho & Nawawi, 1986c), information on conditions of growth in culture is lacking.

The present study was initiated to provide some information on the growth pattern of *G. boninense* in culture and the effects of temperatures and pH on its growth.

MATERIALS AND METHODS

The isolate of G. *boninense* used in this study originated from sporophore growing out of an oil palm trunk naturally infected by the fungus at Highland Estate, Kelang. The method of isolation was similar to that given by Ho & Nawawi (1986a). Stock cultures were maintained on lima bean agar in test-tubes or MacCartney's bottles, incubated at 25°C and stored at 4°C.

A glucose-asparagine (GA) medium was used as the standard basal culture medium unless stated otherwise. It consists of the following: 12.5g D-glucose; 2.68 g L-asparagine; 2.0g KH_2PO_4; 0.5g $MgSO_4.7H_2O$; 2 mg thiamine-HCl; 10 ml trace element solution and distilled water made up to one litre. The trace element solution consisted of 0.25 g $FeSO_4.7H_2O$; 0.22 g $ZnSO_4.7H_2O$; 0.04 g $CuSO_4.5H_2O$; 0.04 g $MnSO_4.4H_2O$; and 0.05 g $NaMoO_4.2H_2O$ dissolved in one litre distilled water. Sufficient sulphuric acid was added to keep the iron in solution.

Aliquots of GA medium (usually 25 ml) were dispensed into each of 150 ml Erlenmeyer flasks, stoppered with non-absorbent cotton wool and autoclaved for 15 min at 15 p.s.i. The pH of the medium ranged between 4.5-4.8. Where a solid medium was required, 15 g/l Difco Bacto agar was added.

Each flask of liquid GA medium was inoculated with a 6 mm mycelial disk cut out from the margins of actively growing colonies of the fungus on solid GA medium. To ensure that the disks remained afloat, they were first transferred to fresh solid GA medium and allowed to grow overnight at 25°C before transferring them to the flasks. Air trapped among the growing mycelium helped to keep the disks afloat. Disks can be submerged by pushing them to the bottom of the flasks. Inoculated flasks were incubated at 29 + 1°C in the dark and gently swirled daily. At the end of the incubation period, mycelial mats were separated from the liquid medium by filtration through fine meshed nylon cloth, washed with distilled water, oven dried at 70° C overnight and then weighed.

Different Inoculum Treatments

In this preliminary experiment, inoculum disks were made to float or submerged and the flasks gently swirled daily or remained static. Four replicates were used for each treatment. Dry weights of mycelial mats were determined after 15 days incubation.

Temperature and Mycelial Growth

Mycelial growth was determined on solid and liquid GA at 16°C, 20°C, 27°C, 30°C, 34°C and 39°C. Agar plates, each containing 20 ml solid medium, were inoculated centrally and the colony diameters were measured daily. Four plates were used for each temperature.

Each flask with liquid medium was inoculated with a floating disk and swirled daily. Four flasks were used for each temperature and dry weights of mycelial mats and final pH of the medium determined after 15 days incubation.

Mycelial Growth and pH Changes

Flasks containing 25 ml liquid GA medium at pH 4.5 were inoculated as mentioned above, incubated and swirled daily. Mycelial mats were harvested every 3 days for 27 days and their dry weights and final pH of the meidum determined. Four replicates were made for each harvesting.

pH and Mycelial Growth

In this experiment, the carbon and nitrogen sources were substituted by sucrose (11.89 g/l) and peptone (3.40 g/l) respectively. Citrate - phosphate buffer using appropriate volumes of 0.5M sodium phosphate (Na_2HPO_4) and 0.5M citric acid was employed to obtain a pH range of 3.1-7.5. Flasks containing 25 ml of the medium were inoculated as above and incubated for 15 days. Four replicates were made for each pH value.

RESULTS AND DISCUSSIONS

Effect of Different Inoculum Treatments

The effects of different inoculum treatments on the mycelial growth of *G. boninense* are shown in Table 1. Mycelial growth from floating inoculum

disks was significantly more abundant than that from submerged inoculum disks. This indicates that *G. boninense*, like most other fungi, requires at least some free oxygen in the medium for growth. Thus, colonies grown floating on the surface of the medium and in close contact with available air in the flasks grew better than those submerged in the medium.

Mycelial growth was further increased when the flasks were swirled daily. In a swirled culture, not only was there increased aeration, but metabolites excreted during the growth of the fungus were also prevented from accumulating around the mycelium.

TABLE 1
Effects of inoculum treatments on mycelial growth

		Dry weight* (mg)	Initial pH of medium	Final pH of medium
Floating inoculum disk	Flask swirled	73 ± 5.7	4.8	3.7
	Flask static	55 ± 3.2	4.8	4.0
Submerged inoculum disk	Flask swirled	42 ± 3.7	4.8	4.2
	Flask static	25 ± 1.6	4.8	4.5

* After 15 days of incubation at 29 ± 1°C.

Effect of Temperatures on Growth

Growth of *G. boninense*, in both liquid and solid media, increased with increasing temperatures until 27-30°C where maximum growth was obtained *(Fig. 1)*. When temperatures increased above this optimum range, growth rate declined again. Similar optimum temperatures (25-29°C) for growth of *G. boninense* and *Ganoderma* spp. from oil palm had been reported earlier by Ho & Nawawi (1986a) and Varghese *et al* (1976). The slow growth outside the range of 16°C and 39°C is quite characteristic of many fungi, as is the rapid, almost linear growth reponse at temperatures 16-30°C.

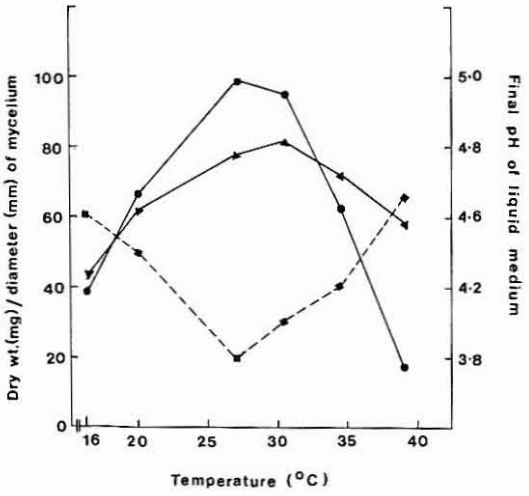

Fig. 1: Effect of temperature on mycelial growth ●——● *liquid medium,* ▲——▲ *solid medium,* ■- - -■ *change in pH.*

Growth and Changes in pH of Medium

The mycelial growth pattern of *G. boninense* in liquid GA medium incubated for 27 days is given in *Fig. 2*. The fungus exhibited an initial lag phase of little growth (1-3 days), then a phase of rapid and approximately linear growth (6-18 days) and finally a phase of declining growth and autolysis. Growth was slow during the initial period because hyphae in the inoculum disk were usually damaged and some time was required before growth began again. When growth began, it was relatively slow at first and then increased rapidly, resulting in a phase of approximately

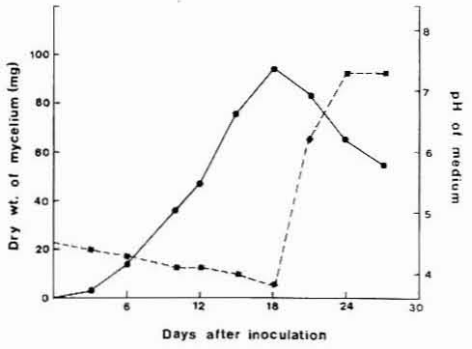

Fig. 2. Growth pattern and pH changes in medium. ●——● mycelial growth, ■ --■ final pH of medium.

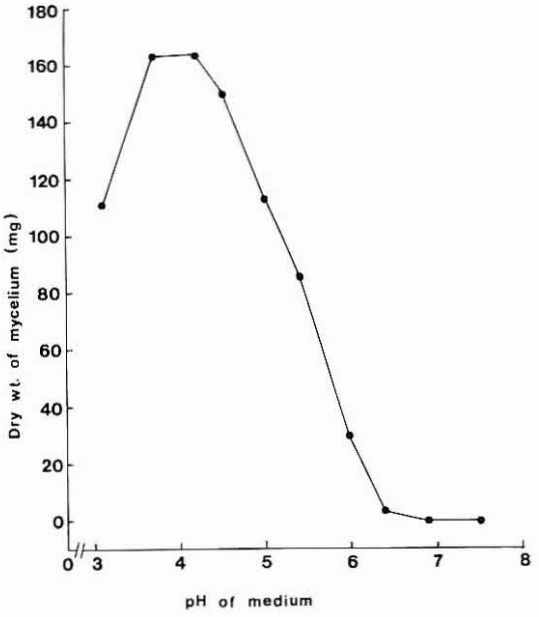

Fig. 3. Effect of pH on mycelial growth.

linear growth. Chemical events during this phase of rapid growth were not studied in this investigation, but had been reported for other fungi where there was rapid utilization of carbohydrate, nitrogen and phosphate and respiratory activity was at maximum (Cochrane 1958, Griffin 1981). In culture, where no fresh nutrients were supplied, the phase of rapid growth could not be maintained and a phase of deceleration or decline began. The onset of this phase could be due to some nutrients reaching a limiting concentration, to unfavourable changes in the pH or oxygen levels or to accumulated metabolic products excreted by the fungus reaching a toxic level. In this phase, growth not only ceased but antolysis of mycelium resulted in an actual loss of weight so that the growth curve based on dry weight measurements fell.

Mycelial growth in unbuffered medium would invariably alter the pH of the medium. *Fig. 2* shows the mycelial growth of *G. boninense* and the shift in pH of the medium. As mycelial growth increased, the pH of the medium correspondingly decreased reaching a minimum value of 3.8 when mycelial growth was maximum (18 days). After this, the pH increased very rapidly to 7.3 as mycelial growth decreased again during prolonged incubation (27 days).

Metabolic activities during growth were responsible for the shift in pH of the medium. Generally, decrease in pH was more in medium which supported abundant mycelial growth of *G. boninense* (Table 1, *Figs. 1 & 2*). The decrease in pH was probably due to the accumulation of organic acids, amino acids and carbonic acid (from carbon dioxide which dissolved into the medium) formed from the metabolism of sugars during growth. Decrease in pH was smaller in medium with less mycelial growth. Increase in pH was found in medium where there was autolysis of mycelium as during the last phase of prolonged growth of *G. boninense* in culture. The release of autolytic products into the medium probably caused the pH of the medium to rise.

Effect of pH on Growth

The effect of pH on the mycelial growth of *G. boninense* is shown in *Fig. 3*. Abundant mycelial growth was found to be in the pH range of 3.7-5.0. Growth was very poor at pH 6.4 and negligible at pH 6.9-7.5. Venkatarayan (1936) had also reported negligible growth of *G. lucidum* at pH 7.6.

The pH-growth curve of *G. boninense* showed a single optimum pH range for growth. However, for *G. lucidum*, a double pH optimum for growth (at pH 6.6 and pH 5.1) has been observed (Venkatarayan 1936).

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