

**STUDY ON ANTIBACTERIAL EFFECT OF FUNGUS,  
*TERMITOMYCES* ON DIFFERENT  
TYPES OF BACTERIA**

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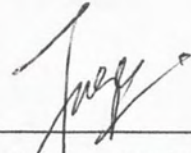
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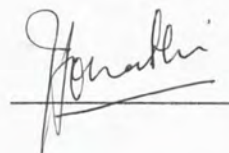
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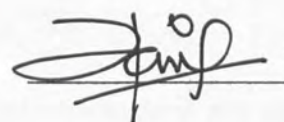
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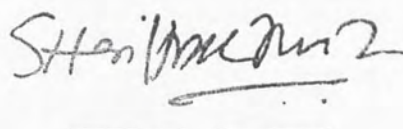
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## ABSTRAK

Kajian ini dijalankan untuk melihat sekiranya fungi, *Termitomyces* (dalam bentuk “mycelia” dan “conidia” mempunyai ciri-ciri antibakteria. Objektif kajian termasuklah menilai potensi fungi *Termitomyces* sebagai agen antibakteria, membandingkan kesan antibakteria fungi *Termitomyces* dalam bentuk kultur “mycelia” dan ekstrak “conidia” dan juga mendapatkan nilai kepekatan terendah ekstrak “conidia” dan kultur “mycelia” yang boleh merencatkan pertumbuhan bakteria yang diuji. Sampel bakteria yang digunakan dalam kajian ini adalah *Bacillus cereus* (gram +ve), *Escherichia coli* (gram -ve), dan *Staphylococcus aureus* (gram +ve). Untuk melihat aktiviti antibakteria, kultur “mycelia” dan ekstrak “conidia” dikaji menggunakan kaedah penyerapan cakera (Disk diffusion method). Kaedah Minimum Inhibitory Concentration (MIC) digunakan untuk mendapatkan kepekatan paling rendah ekstrak “conidia” dan kultur “mycelia” yang boleh merencatkan pertumbuhan bakteria. Untuk ini kepekatan ekstrak “conidia” dan kultur “mycelia” dikurangkan dari  $10^1$  hingga  $10^5$ . Sejumlah 10 sampel ekstrak “mycelia” dan “conidia” digunakan dalam keseluruhan kajian ini. Hasil menunjukkan ekstrak *Termitomyces* dalam kedua-dua bentuk “conidia” dan “mycelia” mempunyai kebolehan merencat pertumbuhan bakteria. Tindakbalas perencatan yang ditunjukkan oleh ekstrak “mycelia” and “conidia” adalah hampir sama. Tindakbalas perencatan yang direkodkan bagi ekstrak “conidia” terhadap *Escherichia coli* adalah yang tertinggi iaitu  $\pm 5.3\text{mm}$ . Perencatan kawasan bakteria sebanyak  $\pm 4.3\text{mm}$  direkodkan bagi ekstrak “conidia” apabila dikaji terhadap *Bacillus cereus*. Semua nilai perencatan bagi ekstrak “conidia” *Termitomyces* adalah lebih tinggi berbanding dengan kultur “mycelia” *Termitomyces* kecuali bagi *Staphylococcus aureus* ( $\pm 1.5\text{mm}$ ). Dalam proses pencairan bersiri, nilai perencatan yang dicatatkan adalah lebih tinggi bagi kultur “mycelia” berbanding dengan ekstrak “conidia”. Tindakbalas perencatan bakteria menggunakan ekstrak “conidia” tidak berlaku pada peringkat pencairan  $10^3$  dan seterusnya. Secara kesimpulannya boleh dikatakan *Termitomyces* dibuktikan mempunyai kesan antibakteria terhadap bakteria yang dikaji.





## ABSTRACT

This study was carried out to identify antibacterial properties of fungus *Termitomyces* in form of mycelia culture and conidial extraction. The objectives of this study were to evaluate the antibacterial potential of fungus, to compare the antibacterial effect of fungus *Termitomyces*, in the form of mycelia culture and conidia extraction and also to find out the lowest concentration of the extraction of conidia and mycelia culture that will inhibit the growth of the tested bacteria. A total of 10 sample each from mycelia and conidial extraction was tested against *Bacillus cereus* (gram +ve), *Escherichia coli* (gram -ve) and *Staphylococcus aureus* (gram +ve). Anti-bacterial activity of the extract was determined using disk-diffusion method (Kirby-Bauer) and minimum inhibitory concentration (MIC) was carried out to identify the lowest concentration needed to inhibit the growth of selected bacteria. Serial dilution method was used with dilution of  $10^1$  till  $10^5$  for the MIC. Mycelia and conidial extraction of the tested *Termitomyces* showed almost same spectrum of antibacterial activity. The highest antibacterial inhibitory activity ( $\pm 5.3\text{mm}$ ) was recorded for the conidial extraction of *Termitomyces* against *Escherichia coli*. The second widest zone of inhibition ( $\pm 4.3\text{mm}$ ) was recorded for the conidial extraction tested against *Bacillus cereus*. All the extraction of mycelia and conidial have an inhibition zone against tested bacteria. The inhibition zone of the conidial extraction was higher compared to the mycelia extractions expect for *Staphylococcus aureus* ( $\pm 1.5\text{mm}$ ). The minimum inhibitory concentration (MIC) of mycelia extraction tested against bacteria showed higher antibacterial inhibitory activity compared to the conidial extraction. For the MIC of the conidial extraction, the inhibition properties stopped at dilution of  $10^3$ . This indicates that increase in dilution of the concentration of the extraction decreases the capability to inhibit the growth of selected bacteria decreases. In conclusion, *Termitomyces* is believed to have antibacterial properties against tested bacteria.



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## LIST OF SYMBOLS

%	Percent
°C	degree Celsius
g	gram
y <sup>-1</sup>	per year
ha	hectare
cm	centimeter
mm	millimeter
mgml <sup>-1</sup>	miligram per mililiter
C	Carbon
H	Hidrogen
N	Nitrogen
Mg	Magnesium
Ca	Calcium
C-N	Carbon-Nitrogen
NA	Nutrien Agar
PDA	Potato Dextrose Agar
Π	pi
D	diameter
Vol	volume



## CHAPTER 1

### INTRODUCTION

#### 1.1 *Termitomyces*

*Termitomyces* is the fungus that grows in association with termite subfamily Macrotermitinae, Termitidae, Isoptera. *Termitomyces*, Class Basidiomycotina, Family Tricholomataceae (Okech & Kotengo, 1988), has mutuality symbiosis between the fungus growing termites and they are dependable on each other. *Termitomyces* depends on termites for growth and protection while the termites use the fungus as their main food (Duur, 2006).

*Termitomyces* also helps the termite to degrade the plant-driven material. As *Termitomyces* have high content of nitrogen, other nutrients and possess digestive enzymes, they are capable in degradation process of the litter (Bourtzis & Miller, 2003). Occasionally the fruiting bodies of *Termitomyces* develop from the nodules of *Termitomyces* and out of the termite mound during rainy season (Weeber, 1989).





Fungus comb, the habitat for *Termitomyces* is a special structure in the termite mounds that formed with termite faecal pellet. Fungus comb consists of many thin vertical laminae linked together, forms a reddish- brown compact sponge-shaped comb (Gillott, 2005; Hunt & Nalepa, 1994). Small spheres or nodules on the fungus comb also known as conidial nodules and synnemata consisting of spherocytocysts bearing conidia of blastosporic ontogeny (Wood & Thomas, 1989).

## 1.2 Research justification

The aim of this research is to gather information on the antibacterial values of *Termitomyces*. This study scope is to identify the capability of *Termitomyces* in form of conidia and mycelia extraction to inhibit the growth of selected bacteria; *Bacillus cereus*, *Escherichia coli*, and *Staphylococcus aureus*.

Research conducted by Gbolagade *et al.*, (2007) to investigate antagonistic effects of higher fungi extract against selected microorganisms which includes *Termitomyces microcarpus* and *Termitomyces robustus* indicates that *Termitomyces* species do have antibacterial values. This research is to identify whether the conidial and the mycelia of *Termitomyces* also have the same properties.



### 1.3 Objectives

The objectives of this study are:

- a. To evaluate the antibacterial potential of fungus, *Termitomyces* in view of the limited scientific information on their medical value.
- b. To compare the antibacterial effect of fungus *Termitomyces*, in form of mycelia culture and conidia extraction.
- c. To determine the lowest concentration of the extraction of conidia and mycelia culture that will inhibit the growth of the tested bacteria.

### 1.4 Hypothesis

The hypothesis of the study is that, there is an antibacterial value in *Termitomyces* either in form of conidia and mycelia culture. Minimum inhibitory concentration will be providing information on the minimum quantity of extraction needed to inhibit bacteria growth.



## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Background on Macrotermitinae

Termites (Isoptera) consist of 2200 species (Higashi & Abe, 1996) and seven families divided into two groups of lower termites and higher termites. Six out of the seven families of termites are in the lower termites groups: Mastotermitidae, Kalotermitinae, Termopsidae, Hodotermitidae, Rhinotermitinae, and Seritermitidae (Higashi & Abe, 1996). Subfamilies of higher termites include Termitinae, Apicotermitinae, Nasutitermitinae and Macrotermitinae (Higashi & Abe, 1996).

Macrotermitinae includes of 12 genera with seven from the Ethiopian region only, two (*Hypotermes* and *Euscaiotermes*) from the Oriental and other three (*Macrotermes*, *Odontotermes*, *Microtermes*) from both region of Ethiopian and Oriental





(Batra & Batra, 1979). Phylogenetic studies using Bayesian analyses of DNA sequences supports earlier assumption that fungus growing termites are African originate (Aanen *et al.*, 2002).

In Malaysia there are only four genera of fungus growing termites recorded; *Macrotermes*, *Odontotermes*, *Hypotermes* and *Microtermes* (Yoshiaki Hashimoto *et al.*, 2006). *Macrotermes gilvus* is chosen as the fungus supplier in this study as they are disturbance tolerant. They can be found commonly in disturbed areas as rubber and oil palm plantation and also in the exploited lands (Abe *et al.*, 1997)

Macrotermitinae has undergone evolution lacking of endosymbionts; (Ingold & Hudson, 1993) the wood-digesting protozoa, but retain a bacterial flora in the gut. Endosymbionts are microorganisms that found in the guts of lower termites capable of breaking down the cellulose and degrade the lignin (Hyodo *et al.*, 2003). They are generally detritivores, feeding mainly on dead wood, dead grass, and dung and often plays dominant role in litter removal (Duponnois *et al.*, 2005). Macrotermitinae with their special ability to cultivate fungus cultivates sponge mycelia in the fungus comb from the conidia nodules (Ingold & Hudson, 1993).



## 2.2 Biology of Macrotermitinae

### 2.2.1 Colony structure of Macrotermitinae

Termites are eusocial, polymorphism Isoptera where the adult stage represented up to five morphological distinct types and caste. The termite colony also known as the termitaria consist of king and queen as their main reproductives, followed by eggs, larvae, workers, soldiers and nymphs. Termites have two fundamental characteristics that differentiate them from Hymenoptera, where they are hemimetabolous and basically bisexual (Roisin, 2000).

Queens and kings are the primary reproductivators where they are safely protected by the soldiers. The queen never leaves the chamber but continually produces eggs. The soldiers have strong sclerotized head with enlarged mandibles, and frontal gland producing defensive secretion. Workers are helper that look for food and build the termite mound to ensure continuous survival of the colonies. *Termitomyces* spherules or “mycotetes” is fed to the queen and eaten by the workers and young nymphs.

Termite colony passes through various life stages which are the juvenile phase, then the adult phase and thirdly the senile phase. In the juvenile phase high percentage of larvae will be formed and the size of the mould grows according to the size of the colony, formation of alates and nuptial flights take place in the adult phase. Reductions in number



of individuals take place in the senile phase. After the senile phase most of the mould will be abandoned by the termites.

Colony foundation is a crucial aspect of the termite-fungus relationship. Each year sexually matured, winged males and females are produced at specific seasons, often during the raining season. Successful paired alates are potential to build new colonies once they have burrowed into the soil. Without the comb being inoculated with *Termitomyces* the colonies will die (Wood & Thomas, 1989).

### **2.2.2 Termitaria of Macrotermitinae**

Macrotermitinae constructs large clay-rich earthen mounds made from degraded organic matter, feces and organic-rich saliva together with the nursery and fungus combs (Freeman, 1979). Macrotermitinae mounds have no galleries and they focus mainly in providing protection and insulation for the termite population. The colony of Macrotermitinae receives the nutrients from the saliva and faecal materials used during the mound construction (Cooke & Rayner, 1984).

This contrast with other mound building termites where their mounds are compose of highly organic fecal materials and uningested soil that act as main nutrient sources within the landscape (Holt & Abe, 1997). Selective feeding and sorting of mineral fraction in the gut may contribute to the difference in the nutrient content of





various termite mound. For example the mound of soil feeders are richer in silt and clay fraction compare to the surrounding soils (Bignell, 2006).

### 2.2.3 Termitaria of *Macrotermes gilvus*

The mounds of *Macrotermes gilvus* is made up of bare soil, where the outer wall is around 13- 48 cm thick. The hive is the heart of the termitaria that enclose of the royal chamber, nursery area, food store and fungus comb. There are sets of scattered chambers excavated inside the *M. gilvus* mound. Assumption was made from present studies that these empty chambers may support the elevation of the hive and fungus comb chambers with the enlargement of the mound (Inoue *et al.*, 1997).

Mounds of *M. gilvus* have two interesting features. The hive will be located higher as the mounds grow larger and wider. This supports the utilization of the upper part of the mound as they grow larger. Secondly the mound lacks of a clear air passage system. Since there is no ridge for diffusion of the respiration gases, chambers were located near to the surface. Cracks usually occur on the mound surface probably to accelerate the respiration circulation (Inoue *et al.*, 1997).





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