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

JAGEDESWARI A/P MARRIAPPAN

THIS THESIS IS SUBMITTED AS A PARTIAL REQUIREMENT TO OBTAIN BACHELOR OF SCIENCE (Hons.) DEGREE

CONSERVATION BIOLOGY
SCHOOL OF SCIENCE AND TECHNOLOGY
UNIVERSITY MALAYSIA SABAH

2008

PUMS99:1

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS@

JUDUL: STUDY ON ANTIBACTERIAL EFFECT OF FUNGUS, TERMITOMYCES ON DIFFEREN TYPES OF BACTERIA

IJAZAH: BACHELOR OF SCIENCE (Hons) DEGREE

SAYA JAGEDESWARI A/P MARRIAPPAN (HURUF BESAR) SESI PENGAJIAN: 2005/2008

mengaku membenarkan tesis (LPSM/Sarjana/Doktor Falsafah) ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Tesis adalah hakmilik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institutsi pengajian tinggi.
4. Sila tandakan (/)

☐	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau Kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)
☐	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)
☒	TIDAK TERHAD	

Disahkan Oleh

(TANDATANGAN PENULIS)

Alamat Tetap: 299 TMN SAGA
JLN SAGA 6 05400
ALOR MENGKUDU ALOR STAR
KEDAH DARUL AMAN

Tarikh: 10-05-2008

(TANDATANGAN PUSTAKAWAN)

DR HOMA THEVI RAHMAN
Nama Penyelia

Tarikh: 10-05-08

CATATAN:- *Potong yang tidak berkenaan.
**Jika tesis ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa /organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.
@Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan atau disertai bagi pengajian secara kerja kursus dan Laporan Projek Sarjana Muda (LPSM).

STATEMENT

I declare that this thesis is my original work except for quotations and summary that has been cited in reference.

19th March 2008

JAGEDESWARI A/P MARRIAPPAN
HS2005-1349

DECLARATION OF EXAMINERS

DECLARED BY

Signature

1. Supervisor
   (Dr. Homathevi Rahman)

2. Examiner 1
   (Mr. Kueh Boon Hee)

3. Examiner 2
   (Mr. Hairul Hafiz Mahsol)

4. Dean
   (Prof. Madya Dr. Shariff A. Kadir S. Omang)

ACKNOWLEDGEMENT

I would like to take this opportunity to thank all people who have assisted me in the completion of this project. I would like to express my sincere gratitude and appreciation to Dr. Homathevi Rahman, my supervisor, for her constant support and guidance throughout the long course of the entire study. I also would like to thank Prof. Bignell and Prof. Datuk Dr Noramly Muslim for their advice and references.

I also would like to thank to Mr. Chin Thien Pock, Manager of Taritipan and Langkon, Borneo Samudera Plantation, Mr. Boris Soon, Agronomist of Borneo Samudera Plantation, Pn Anifah, Supervisor in Taritipan Oil Palm Plantation and those staff that offer their help during my sampling there. I also offer thanks to En Jeffrey, Pn Dorrin Pn Fatimah and Pak Zainal for their technical help throughout my sampling and laboratory session.

I also would like to thank for the helps and support of my friends especially to Kobi, James and Alfred where without their help in the field, I might face difficulty during the sampling session. I also would like to thank all my best friends and my course mate for their help, advice and motivation throughout this project .Special thanks are given to my family for their love, support, patience and encouragement with out whose help my degree's study would not have been possible.

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 ditunjukan oleh ekstrak "mycelia" and "conidia" adalah hampir sama. Tindakbalas perencatan yang direkodkan bagi ekstrak "conidia" terhadap *Escherichia coli* adalah yang tertinggi iaitu ±5.3mm. Perencatan kawasan bakteria sebanyak ±4.3mm 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* (±1.5mm). 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 (±5.3mm) was recorded for the conidial extraction of *Termitomyces* against *Escherichia coli*. The second widest zone of inhibition (±4.3mm) 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* (±1.5mm). 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.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF SYMBOLS

%	Percent
°C	degree Celsius
g	gram
y^{-1}	per year
ha	hectare
cm	cemtimeter
mm	millimeter
$mgml^{-1}$	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-drived 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 ontogony (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 stilt 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).

REFERENCE

Annan Peter C.T.H., 2002. *Guidelines and Recommendations for Antimicrobial Minimum Inhibitory Concentration (MIC) testing against Veterinary Mycoplasma species*.www.edpscience.org

Aanen, D. K., Ros, V.I.D., Licht, H.H., Mitchell, J. Slipper, B.Lefevre, C.R., Boomsma, J.J. 2007. *Pattern of Interactions specificity of Fungus- growing termites and Termitomyces symbionts in South Africa*. BMC Evolutionary Biology.

Aanen, D.K., Eggleton, P, P. Lefebvre, C.R., Frøslev, T.G., Rosendahl, S., and Boomsma.J.J. 2002. *The evolution of fungus-growing termites and their mutuality fungal symbionts*. United States of America.

Abe, T., Bignell, D.E. & Higashi, M. 2000. *Termites: Evolution, Sociality, Symbiosis, Ecology*. Kluwer Academic Publishers, Dordrecht.

Abe, T., Kirtibutr, N., & Holt, J. A. 1997. *Global diversitification of termites-Its pattern and causal mechanisms from April 1995 to March 1997*.International Scientific Research (Joint Research) of the Japanese Ministry of Education, Science, Sports and Culture.

Batra, L .R. & Batra S. W. T. 1979. Termite-Fungus Mutualism: In *Insect-Fungus Symbiosis*. John Wiley and sons, New York

Bently, R, Meganathan, R., 1982. *Biosynthesis of Vitamin K (menaquinone) in Bacteria*, Bacteriological Reviews. Vol 46(3):241-280.

Bignell, D.E. 2000. Soil-feeding termites: biology, microbial associations and digestive mechanisms. *In Termites: Evolution, Sociality, Symbioses, Ecology* (eds, T. Abe, D.E. Bignell and M. Higashi), Kluwer Academic Publishers, Dordrecht, Netherlands.

Bignell, D.E. 2006. *Intestinal Microorganisms Soil Invertabrates* (eds König.H & Varma A.) Springer- Verlag Berlin Heidelberg.

Botha, W.J & Eicker.A. 1992. *Nutritional value of termitomyces mycelial protein and growth of mycelium on natural substrates*. Cambridge Unit Press, Britain

Bourtzis & Miller T.A.2003. *Insect symbiosis*, CRC Press, United States of America.

Carlie J. M ., Watkinson S.C ., & Gooday, W.G. 2001. *The Fungi.* 2nd ed. Academic Press. United Kingdom.

Cooke, R.G. & Rayner, A. 1984. *Ecology of Saprotrophic fungi.* Longman Inc, USA

Darlington, J.P.E.C.1994. Nutrition and Evolution in Fungus–growing Termites .In: Hunt, J.H., & Nalepa, C.A. *Nourishment & Evolution in Insect Societies*, West view Press, United States of America.

Duponnois, R., Thioulouse J.,Paugy M.,Masse D., Lepage M .2004. *Functional diversity of soil microbial community, rock phosphate dissolution and growth of Acacia seyal as influenced by grass-litter and soil feeding termite nest structure amendments*. Elsevier B.V; www.sciencedirect.com

Duur, A. K. 2006. *Stability of termite-fungus mutualism*. Biology Letters, The Royal Society, Britain.

Ehling-Schulz M, Fricker M, Scherer S. 2004.*Bacillus cereus, the causative agent of an emetic type of food-borne illness.* Mol Nutr Res Vol 48(7): 479-87
PMID 15538709

Farb, P.1980. *The Insects*.2nd ed. Life Nature Library (Time-Life book/ Hong Kong) United States of America

Feng P, Weagant S, Grant, M. 2002. *Enumeration of Escherichia coli and the Coliform Bacteria.* Bacteriological Analytical Manual (8th ed.). FDA/Center for Food Safety & Applied Nutrition.

Frøslev, T.G Aanen.D.K, Laessoe, T. and Rosendahl, S. 2003. *Phylogenetic relationships of Termitomyces and related taxa*, Mycological Research, The International Journal of Fungal Biology, Volume *107,Part III*, Cambridge University Press, United Kingdom

Freeman, P. 1979. *Insects; an illustrated survey of the most successful animals on earth*, Grosset & Dunlap, New York.

Gbolagade, J. Kigigha, L. & Ohimain E. 2007. *Antagonistic effect of Extracts of Some Nigerian Higher Fungi against Selected Pathogenic Microorganisms.* American-Eurasian Journal: Agriculture &Environment Science. IDOSI Publication

Gillott, C. 2005. *Entomolgy*. Third edition, Published by Spinger, Netherlands

Ghosh A.K & Sengupta T. 1987. *The mutualistic association between Macrotermitinae and Termitomyces*. In: Wilding, N, Collins, N.M., Hammond, USA.

Grizimek B.1975. *Grizimek's Animal Life Encyclopedia 2*: Insects.1st ed in English. Van Nostrand Reinhad Ltd.

Gullan, P.J. & Craston, P.S. 2005. *The Insects, an Outline of Entomology.* 3rd Edition Blackwell Publishing.

Hawksworth, D.L., 2005. *Mushroom polysaccharides in Human Health Care.* In: Deshmuki, S.K., & Rai, M.K. (pnyt.) *Biodiversity of Fungi; Their Role in Human Life.* United States of America.

Heim, R. 1977. In McCulloch M. M., 1992. *The Evolution of Insect-Fungus Associations: From Contact to Stable Symbiosis.* American Society of Zoologists. Boston.

Higashi, M. & Abe, T. 1996. *Phylogeny, biogeography and species diversity; Global diversification of termites driven by the evolution of symbiosis and sociality.* International Scientific Research (Joint Research) of the Japanese Ministry of Education, Science, Sports and Culture.(Project No. 07044193)

Holt J A & Abe T. 1997. In Batra, L .R. & Batra S. W. T. (1979). Termite-Fungus Mutualism: In *Insect-Fungus Symbiosis*. John Wiley and sons, New York

Hudault, S. J., Guignot and A.L. Servin. July 2001. *Escherichia coli strains colonizing the gastrointestinal tract protect germfree mice against Salmonella typhimurium infection.* Gut 49: 47-55.PMID 11413110

Hunt J.H., and Nalepa C.H. 1994. *Nourishment & Evolution in Insect Societies*. West view Press, United States of America.

Hyodo, F., Tayasu, I. Azuma, I. Kudo,T. & Abe.2003. *Differential role of symbiotic fungi in lignin degradation and food provision for fungus-growing termites* (Macrotermitinae: Isoptera). Journal on Functional ecology,Vol 17, 186-193.

Ingold, C.T. & Hudson, H.J. 1993. *Biology of Fungi*.6th Ed. Chapman &Hall, United Kingdom

Inoue, T., Vijarnsorn, P & Abe, T. 1997. *Mound structure of the fungus-growing termite Macrotermes gilvus in Thailand.* Journal of Tropical Ecology, Cambridge University Press.

Isaac, S., Frankland, J.C., Walting, R., & Whalley, A. 1992. Aspect of Tropical Mycology. Cambridge University Press

Jeffries P. & Pierce, G.C. July 1987. *The Mycologist Bulletin of British Mycological Society*. Volume 21, Cambridge University Press.

Jenkins N, Heviefo G, Langewald J, Cherry A & Lomer C. 1998. *Development of mass production technology for aerial conidial for use as mycopesticides.* Biocontrol News and Information. Vol 19,No 1:21N-31N.Cab international.

Johnson R.A., Thomas R.J., Wood T.G., & Swift M.J., 1981. *The inoculation of the fungus comb in newly founded colonies of some species of the Macrotermitinae (Isoptera) from Nigeria.* Journal of Natural History. Taylor and Francis Ltd.

Kotiranta A, Lounatma K, Haapasalo M.2000. *Epidemiology and pathogenesis of Bacillus cereus infections.* Microbes Infect Vol 2(2): 189-98 PMID 10742691

Krishna, K., & F.M. Weesner, 1969. *Biology of Termites*, Vol 1. Academic Press INC New York.

Lamberty, M., Zachary, D., Lanot, R., Bordereau, C., Robert, A., Hoffmann, J.A., & Bulet, P. 2000. Constitutive expression of a Cysteine-rich antibacterial peptide in a termite insects. The American Society for Biochemistry and Molecular Biology, JBC Papers in Press.www.jbc.org

Lee, K.E & Wood, T.G.1971.*Termites and soils*, Academic Press, New York.

Leun M, Chang C, Huang S & Chen C. 2003. *Antioxidant properties of methanolic extracts from Grifola frondosa, Morchella esculenta and Termitomyces albuminosus mycelia.* Elsevier Ltd.

Leuthold R.H., Badertscher.S, & Imboden.H. 1989. *The Inoculation of Newly Formed Fungus Comb with Termitomyces in Macrotermes Colonies (Isoptera, Macrotermitinae).* Insectes Sociaux, Paris Volume 36, 328-338

Lefévre, C. & Bignell, D.E. 2006. *Cultivation of symbiotic fungi by termites of the subfamily Macrotermitinae.* Kluwer Academic Publishers, Dordrecht.

Liu GY ,Essex A, Buchanan JT, Datta V, Hoffman HM, Bastian JF, Fierer J, Nizet V 2005. *Staphylococcus aureus golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. J Exp Med* 202 (2)

Matheny P.B., Curtis J.M., Hoftetter V., Aime, M.C., Moncalvo, J-M., Ge, W., Yang, Z-L.,Slot, S., Aanen, D.K.,& Hibbett D.S.2006.Major clades of Agaricales: a

multilocus phylogenetic overview. Mycologia 98(6)in www.pnas.org/cgi/doi/10.1073/pnas.222313099.

Marx & Irene. *Method for enhancing cellobiase activity of Termitomyces clypeatus using a glycosylation inhibitor.* Harness Dickey & Pierce, PLC. United States Patent 6946277. http:www.freepatentsonline.com

McKillip J.L., 2000. *Prevalence and expression of enteroxins in Bacillus cereus and other Bacillus spp., a literature review.Antonie Van Leewenhoek* : 77(4). PMID 10959569.

Moriya.S., Inoue.T., Ohkuma,M., Yaovapa,T., Johjima,T., Suwanarit, P., Sangwanit,U.,Vongkaluang,C., Noparatnaraporn,N.,& Kudo,T.2005. Fungal Community Analysis of Fungus Gardens in Termite Nests. Vol 20 http://wwwsoc.nii.ac.jp/jsme2/

Mukherjee S & Khowala S. 2002. *Regulation of Cellobiase Secretion in Termitomyces clypeatus by Co-Aggregation with Sucrase.* Current Microbiology Vol.45. pp 70-73.Springer-Verlag New York Inc.2002.

Munyanziza E. 1996. *Domestication of Mushrooms from the Miombo woodlands; current status and crucial issues for agro forestry.* FAO Corporate Document Repository.

Mueller, U.G. & Gerardo, N 2002. *Fungus-farming insects: Multiple origins and diverse evolutionary histories.* PNAS online article, United States of America. (http://www.pnas.org/)

Ohkuma M., Maeda Y., Johjima T., & Kudo T., 2001. *Lignin degradation and roles of white rot fungi: Study on an efficient symbiosis system in fungus-growing termites and its application to bioremediation.* RIKEN review N0.42.

Okech. M.A & Kotengo M.O. 1988. *Culture, isolation and microscopic studies on Termitomyces species from the fungus- comb of Macrotermes minhaelseni (Isoptera, Macrotermitinae)*, Mushroom Journal for the tropics, formerly The Mushroom Newsletter for the Tropics. Vol 8 (2), Published by International Mushroom Society for the Tropics.

Pierce G.D. 1987. *The Genus Termitomyces in Zambia*, The Mycologist Bulletin of Mycologist Society. Vol 21(3), Cambridge University Press.

Purkayastha R.P & Chandra,A. 1985. *Manual of Indian Edible Mushroom.* Today & tomorrow's Printers & Publishers, New Delhi.

Rajan, S. 2001. *Introduction to Fungi*. Ammal Publication PVT.LTD, New Delhi

Reid, G., J.Howard and B. S. Gan. 2001. *Can bacterial interference prevent infection.* Trend in Microbiology Vol 9: 424-428. PMID 11553454.

Rogers J, Ju Y, & Lehmann J.2005. *Some Xylaria species on termite nests.* The Mycological Society of America,Vol 97(4) Lawrence,KS.

Roisin Y., 2000. Diversity and Evolution of caste patterns In: In Termites: Evolution, Sociality, Symbioses, Ecology (eds, T. Abe, D.E. Bignell and M. Higashi), Kluwer Academic Publishers, Dordrecht, Nertehrlands

Rouland-Lefévre, C. & Bignell, D.E. 2001. *Cultivation of symbiotic fungi by termites of the subfamily Macrotermitinae in Symbiosis: Mechanisms and Model systems* (ed J.Seckbach). Kluwer Academic Publishers, Dordrecht.

Sands, W.A., 1969. The association of termites and fungi In Biology of Termites; Krishna K. & Weesner F.M., Vol 1, Academic Press, New York and London.

See, L.S., Noraswati Mohd Rashid, Staines, H., Azanto, M., Siller, I. 2005. *Utilization of fungi by Rural Communities for food and medicine in Peninsular Malaysia.* FRIM Research Report, Kementerian Sumber Asli & Alam Sekitar.

See, L.S., Shyun, C.Y., & Noraswati Mohd Rashid. 2006. *Common edible mushrooms of Orang Asli communities in Peninsular Malaysia.* Perpustakaan Negara Malaysia.

Seiber R.1983. *Establishment of Fungus Comb in Laboratory Colonies of Macrotermes michaelseni and Odontotermes montanus (Isoptera, Macrotermitinae).* Vol 30. Insectes Sociaux, Paris. Printed in Masson, Paris.

Srinivasan, M.C. 2004. *Practical Mycology for Industrial Biotechnologists.* Tata McGraw-Hill Publishing Company Limited, New Delhi, India.

Sumbali, G. 2005. *The Fungi.* Alpha Science International Ltd. Harrow, United Kingdom

Taprab, Y., Ohkuma, M., Johjima, T., Maeda, Y., Moriya, S., Inoue, T., Suwanarit, P., Noparatnaraporn, N., & Kudo,T. 2002. *Molecular Phylogeny of Symbiotic Basidiomycetes of Fungus-growing Termites in Thailand and Their Relationship with the Host.* Biosci. Biotechnol. Biochem Journal.

Thapa, R.S. 1981.*Termites of Sabah: Sabah Forest Record Number 12.*Sabah Forest Department.

Thomas, R.J., 1987. *Distribution of Termitomyces and other fungi in the nests and major workers of several Nigerian Macrotermitinae*. Vol. 19, Soil Biology and Biochemistry Journals. Mycologist Society.

Tortora, G.J., Funke, B.R., & Case, C.L.2005. *Microbiology An Introduction.* Pearson Education, Inc., Benjamin Cummings. United State of America

Vogt, R.L., L.Dippold. (2005) *Escherichia coli 0157:H7 outbreak associated with consumption of ground beef.* Public Health Reports Vol 2:174-178. PMID 15842119

Wasser, P.S., Didukh, M.Y. 2005. Mushroom polysaccharides in Human Health Care. In: Deshmuki, S.K., & Rai, M.K. (pnyt.) *Biodiversity of Fungi; Their Role in Human Life.* Inc (NH), United States of America.

Wasser, P. S.,2007. Herbagram; How Mushroom can help save the world. The Journal of American Botanical Council. Issue 76, American Botanical Council Press. United States of America.

Wood, T. G. & Thomas, R. J.1989. The mutualistic association between *Macrotermitina* and *Termitomyces*. In: Wilding, N, Collins, N.M., Hammond,

Webber, J.F. *Insect-fungus Interactions*.14th Symposium of the Royal Entomology Society of London in Collaboration with the British Mycotological Society Academic Press London.

Yongabi, K., Agho, M, & Carrera, D.M. 2004. *Enthomological studies on wild mushrooms in Cameroon*, Central Africa. Micologia Aplicada Internacional Colegio de Postgraduados, Mexico

Yoshiaki, H., Homathevi, R., & Maryati, M. 2006. *Inventory & Collection; Total protocol for understanding of biodiversity*, 2nd Ed. Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah, and Japan International Cooperation Agency (JICA).