

THE FERMENTATION FROM THE LIVING MUSHROOM  
*GANODERMA BONINENSE*, A  
PARASITIC FUNGUS OF PINES.

FARZADIAN CO., LTD.

TEHRAN - IRAN - DAK GENEZOOL  
IRANIAN NATIONAL CENTER FOR DRUG RESEARCH AND ANTI-DRUG

2000

Ch. 4784

1100046019

Perpustakaan  
Universiti Malaysia Terengganu (UMT)

LP 16 FST 3 2006



1100046019

## The effects of extract from free-living amoeba on *Ganoderma boninense*, a pathogenic fungus of plant / Farah Zaidat Mohd Nadzri.



PERPUSTAKAAN

KOLEJ UNIVERSITI SAINS & TEKNOLOGI MALAYSIA  
21030 KUALA TERENGGANU

1100046019

Lihat sebelah

HAK MILIK  
PERPUSTAKAAN KUSTEM

**THE EFFECTS OF EXTRACT FROM FREE-LIVING AMOEBA ON *GANODERMA BONINENSE*, A PATHOGENIC FUNGUS OF PLANT**

By

**Farah Zaidat bt. Mohd Nadzri**

**Research Report submitted in partial fulfillment of  
the requirements for the degree of  
Bachelor of Science (Biological Sciences)**

Department of Biological Sciences  
Faculty of Science and Technology  
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA  
2006

This project should be cited as:

Farah, Z.M.N. 2006. The effect of extract from free-living amoeba on *Ganoderma boninense*, a pathogenic fungus of plant. Undergraduate Thesis, Bachelor of Science (Biological Sciences), Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi Malaysia, Terengganu. 72p.

No part of this project may be produced by any mechanical, photographic, or electronic process, or in the form of phonographic recording, nor may it be stored in a retrieval system, transmitted or otherwise copied for public or private use, without written permission from the author and the supervisor (s) of the project.



JABATAN SAINS BIOLOGI  
FAKULTI SAINS DAN TEKNOLOGI  
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA

PENGAKUAN DAN PENGESAHAN LAPORAN  
PROJEK PENYELIDIKAN I DAN II

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk:

THE EFFECTS OF EXTRACT FROM FREE-LIVING AMOEBAE ON *Ganoderma boninense*,  
A PATHOGENIC FUNGUS OF PLANT

Oleh: Farah Zaidat bt Mohd Nadzri no. matrik: UK 8271

telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperolehi Ijazah Sarjana Muda Sains (Sains Biologi), Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

Disahkan oleh:

Penyelia Utama

Nama: Prof. Madya Dr. Nakisa Mat Amin  
Cop Rasmi:

Tarikh: 27/4/06

PROF. MADYA DR. NAKISA, BT. MAT AMIN  
Penyelia,  
Jabatan Sains Biologi  
Fakulti Sains dan Teknologi  
Kolej Universiti Sains dan Teknologi Malaysia  
21030 Kuala Terengganu.

Ketua Jabatan Sains Biologi

Nama: Prof. Madya Dr. Nakisa Mat Amin  
Cop Rasmi:

Tarikh: 27/4/06

PROF. MADYA DR. NAKISA, BT. MAT AMIN  
Ketua  
Jabatan Sains Biologi  
Fakulti Sains dan Teknologi  
Kolej Universiti Sains dan Teknologi Malaysia  
(KUSTEM)  
21030 Kuala Terengganu.

## **ACKNOWLEDGEMENT**

First of all, with the completion of this project, I would like to express my grateful to God for His Blessings that assist me through the project.

Secondly, I would like to extend my sincere gratitude and my appreciation to my supervisor, Associate Professor Dr. Nakisah bt Mat Amin, whose help, guidance, advice and patience help rendered my progress throughout my project.

Most thanks to Associate Professor Dr. Faridah bt Abdullah, Abg Nazif and Kak Ita from Department of Biology, University Putra Malaysia for helping me providing fungus samples, guidance and give advice for my project. Not forgetting to the all staff and master students of Department of Biological Sciences, KUSTEM and INOS for their assistance in one way or another. Thank you so much to Kak Ina, Kak Tie, Kak Pae, Kak Dah Kak Ilyana and Kak Ida, for the guidance given. Without them my project would never been carried out smoothly.

My thanks also goes to my friends, Wan Azizan, Asmah Jalil, Fatma, Maria, Linda, Diana, JayB, Zairul and Rasul for their moral support and help throughout my campus life in KUSTEM.

Last but not least, I would like to thanks a lot to my parents and relatives for making this a reality and success in my life. They are my pacemaker of my life. Thanks to all.

## TABLE OF CONTENTS

	Page
<b>ACKNOWLEDGEMENTS</b>	ii
<b>LIST OF TABLES</b>	vii
<b>LIST OF FIGURES</b>	viii
<b>LIST OF ABBREVIATIONS</b>	ix
<b>LIST OF APPENDICES</b>	x
<b>ABSTRACT</b>	xi
<b>ABSTRAK</b>	xii
<b>CHAPTER 1 INTRODUCTION</b>	1
1.1 Introduction	1
1.2 Importance of Study	2
1.3 Objectives	2
<b>CHAPTER 2 LITERATURE REVIEW</b>	4
2.1 Identification of <i>Acanthamoeba</i> spp	4
2.1.1 Taxonomy and nomenclature of <i>Acanthamoeba</i> spp	5
2.1.2 Morphological features of <i>Acanthamoeba</i>	6
2.1.3 Life cycle	7
2.2 The Roles of <i>Acanthamoeba</i>	8
2.3 Previous studies of Bioactive Compound on Protozoa	8

2.4	Identification of <i>Ganoderma</i> spp	10
2.4.1	Taxonomy and nomenclature of <i>Ganoderma</i> spp	10
2.4.2	Morphological features of <i>Ganoderma boninense</i>	12
2.4.3	Cultural characteristics	13
2.5	<i>Ganoderma boninense</i> and Basal Stem Rot (BSR) disease	14
2.6	Previous study on <i>Ganoderma boninense</i>	15
<b>CHAPTER 3 METHODOLOGY</b>		17
3.1	Source of <i>Ganoderma boninense</i> culture	17
3.1.1	Receiving and maintaining the fungus cultures samples	17
3.1.2	Culture media preparation: Potato-Dextrose-Agar (PDA)	20
3.2	Source of <i>Acanthamoeba</i> (AK) and <i>Acanthamoeba</i> (PI)	20
3.2.1	Culture media preparation	20
3.3	Preparation of Amoeba Extract	21
3.3.1	Determination of protein concentration	22
3.3.2	Determination the volume of amoeba extract with various concentration	23
3.4	Treatment of <i>Ganoderma boninense</i> with amoebae extracts	25
3.4.1	Preparation of PDA media containing amoeba extract	25
3.4.2	Assay of fungal sensitivity to the amoeba extract	25
3.5	Data Analysis	28

<b>CHAPTER 4 RESULTS</b>	29
4.1 Sensitivity of <i>Ganoderma boninense</i> to the two amoeba extract	29
4.1.1 Determination of Radial Growth of <i>Ganoderma boninense</i> by <i>Acanthamoeba</i> extracts; AK and P1	29
4.1.2 Determination of Percentage of Inhibition of Radial Growth (PIRG) of <i>Ganoderma boninense</i> after treatment with <i>Acanthamoeba</i> extracts; AK and P1	35
<b>CHAPTER 5 DISCUSSION</b>	
5.1 Sensitivity of <i>Ganoderma boninense</i> to the two amoeba extracts	38
5.2 Radial Growth and Percentage Inhibition of Radial Growth (PIRG) of <i>Ganoderma boninense</i> by two <i>Acanthamoeba</i> extracts	39
<b>CHAPTER 6 CONCLUSION</b>	44
<b>REFERENCES</b>	45
<b>APPENDIXES</b>	51
<b>CURICULUM VITAE</b>	59

## LIST OF TABLES

<b>Table</b>		<b>Page</b>
4.1	Mean Radial Growth of <i>Ganoderma boninense</i> by 2 <i>Acanthamoeba</i> extracts; P1 and AK	31
4.2	Percentage Inhibition of Radial Growth (PIRG) of <i>Ganoderma boninense</i> by 2 <i>Acanthamoeba</i> extracts; P1 and AK	36

## LIST OF FIGURES

<b>Figure</b>		<b>Page</b>
3.1	Cultures of <i>Ganoderma boninense</i> in a petri dishes	19
3.2	Cultures of <i>Ganoderma boninense</i> in a universal bottle; as source	19
3.3	Trophozoite of <i>Acanthamoeba</i> – Type AK under inverted microscop (Magnification x100)	24
3.4	Trophozoite of <i>Acanthamoeba</i> – Type P1 under inverted microscop (magnification x100)	24
3.5	0.7 cm agar plug from <i>Ganoderma boninense</i> at the center of solidified agar PDA with amoeba extract	27
3.6	<i>Ganoderma boninense</i> at first day after treatment	27
4.1a	The effect of different concentration of AK extracts on radial growth of <i>Ganoderma boninense</i>	32
4.1b	The effect of different concentration of P1 extracts on radial growth of <i>Ganoderma boninense</i> .	32
4.2	The difference in radial growth of <i>Ganoderma boninense</i> treated with AK and P1 extracts	33
4.3a	<i>Ganoderma boninense</i> growth 8 days after treatment. Solidified agar with <i>Acanthamoeba</i> (AK) extract	34
4.3b	<i>Ganoderma boninense</i> growth 8 days after treatment. Solidified agar with <i>Acanthamoeba</i> (PI) extract	34
4.4	Percentage Inhibition of Radial Growth (PIRG) of <i>Ganoderma boninense</i> by 2 <i>Acanthamoeba</i> extracts; AK and P1	37

## **LIST OF ABBREVIATIONS**

%	percentage
°C	Degree Celcius
ANOVA	Analysis of Variance
g	gram
ml	mililiter
mg	milligram
mg/ml	miligram per mililiter
µg/ml	microgram per milliliter
µm	micron meter
L	liter
µL	micron liter
cm	centimeter
MICs	Minimum Inhibition Concentrations
PDA	Potato-Dextrose-Agar

## LIST OF APPENDICES

<b>Appendix</b>		<b>Page</b>
A	Preparation of Amoeba Extracts with Various Concentrations	52
B	Table 1: Radial Growth of <i>Ganoderma boninense</i> against 250, 500, 1000 and 2000 µg/ml concentrations of AK extracts.	55
	Table 2: Radial Growth of <i>Ganoderma boninense</i> against 250, 500, 1000 and 2000 µg/ml concentrations of P1 extracts.	55
C	Table 3: Percentage Inhibition of Radial Growth (PIRG) of <i>Ganoderma boninense</i> against 250, 500, 1000 and 2000 µg/ml concentrations of AK extracts.	56
	Table 4: Percentage Inhibition of Radial Growth (PIRG) of <i>Ganoderma boninense</i> against 250, 500, 1000 and 2000 µg/ml concentrations of P1 extracts.	57
D	a) ANOVA single factors: Determination of radial growth on <i>Ganoderma boninense</i> by different concentrations of AK extracts	58
	b) ANOVA single factors: Determination of radial growth on <i>Ganoderma boninense</i> by different concentrations of P1 extracts	58

## **ABSTRACT**

A study has conducted to see the effect of two amoeba extracts on a plant pathogenic fungus; *Ganoderma boninense*. Two amoeba species isolated from marine environment designed as *Acanthamoeba* (P1) and *Acanthamoeba* (AK) were used in this study. The extracts of amoebae were labeled as P1 and AK extracts, accordingly and were obtained by sonication of the amoeba cell's pellets. Antifungal activities of the amoeba extracts were tested against *Ganoderma boninense* by spreading the extracts on PDA which later was used to culture the fungus. The amount of protein in the extracts was measured to inoculate the concentration of extracts exerted in this study. The concentration of extracts used in this studied were 250 µg/ml, 500 µg/ml, 1000 µg/ml and 2000 µg/ml. The growth of *Ganoderma boninense* was observed after eight days. Radial of the mycelial growth (in cm) were taken and recorded. Results of this study show that all the extracts at various concentration were gave a little effects on the growth of *Ganoderma boninense*, suggesting that both amoeba extracts used in this study do not have potential to been used as antifungal agents for *Ganoderma boninense*.

## **KESAN EKSTRAK DARIPADA AMOEBA BEBAS-HIDUP KE ATAS *Ganoderma boninense*, KULAT PATOGENIK TUMBUHAN**

### **ABSTRAK**

Kajian telah dijalankan untuk melihat kesan dua ekstrak ameba ke atas kulat patogenik tumbuhan; *Ganoderma boninense*. Dua spesis ameba yang digunakan dalam kajian ini adalah diasingkan dari sekitaran marin dikenali sebagai *Acanthamoeba* (P1) dan *Acanthamoeba* (AK). Ekstrak ameba dilabelkan sebagai P1 dan AK dan diperolehi daripada pemecahan pelet sel ameba. Aktiviti antikulat ekstrak ameba diuji ke atas *Ganoderma boninense* dengan meletakkan ekstrak ameba ke atas PDA yang mana kemudian akan digunakan untuk mengkultur kulat tersebut. Jumlah protein dalam ekstrak diukur untuk mendapatkan ekstrak dengan kepekatan yang dikehendaki dalam kajian ini. Kepekatan yang digunakan ialah 250 µg/ml, 500 µg/ml , 1000 µg/ml dan 2000 µg/ml. Perkembangan *Ganoderma boninense* diperhatikan selepas lapan hari. Perkembangan jejari miselia (dalam cm) diambil dan direkodkan. Hasil kajian ini menunjukkan semua ekstrak dengan pelbagai kepekatan memberi sedikit kesan pada perkembangan *Ganoderma boninense*, mencadangkan kedua-dua ekstrak ameba tidak berpotensi untuk digunakan sebagai agen antikulat ke atas *Ganoderma boninense*.