

THE EFFECT OF EXTRACTS FROM LIVING AGCERBAE
ON PATHOGENIC FUNGI OF PLANTS
RIGIDOPORUS LIGNOSUS

MARIA BINTI EMBONG

FAKULTI SAINS DAN TEKNOLOGI
KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA
2006

THE EFFECT OF EXTRACTS FROM FREE-LIVING AMOEBAE ON
PATHOGENIC FUNGI OF PLANTS, *RIGIDOPORUS LIGNOSUS*

By

Maria Binti Embong

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:

Maria, E. 2006. The Effect of Extracts From Free-Living Amoeba on Pathogenic Fungi of Plants, *Rigidoporus lignosus*. Undergraduate thesis, Bachelor of Science in Biological Sciences, Faculty of Science and Technology, Kolej Universiti Sains dan Teknologi Malaysia, Terengganu.

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

1100046030



**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 EFFECT OF EXTRACTS FROM FREE-LIVING AMOEBIA ON PATHOGENIC FUNGI OF PLANTS, *Rigidoporus lignosus* oleh: Maria Binti Embong no. matrik: UK 8178 telah diperiksa dan semua pembedaan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Biologi sebagai memenuhi sebahagian daripada keperluan memperoleh ijazah Sarjana Muda Sains (Sains Biologi), Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

Disahkan oleh:

Penyelia Utama

Nama: **PROF MADYA DR. NAKISAH BT. MAT AMIN**
Pensyarah,

Cop Rasmi: Jabatan Sains Biologi,
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
21030 Kuala Terengganu.

Tarikh: 4/05/2006

Ketua Jabatan Sains Biologi

Nama: **PROF MADYA DR. NAKISAH BT. MAT AMIN**
Ketua

Cop Rasmi: Jabatan Sains Biologi
Fakulti Sains dan Teknologi
Kolej Universiti Sains dan Teknologi Malaysia
(KUSTEM)
21030 Kuala Terengganu.

Tarikh: 4/05/2006

ACKNOWLEDGEMENTS

Assalamualaikum w.b.t

I would like to especially thank my supervisor, Associate Professor Dr. Nakisah Mat Amin for help, guidance, encouragement, criticism and patient. Without whom I would not be able to go through my final year project successfully. Above all, thanks you so much for being so generous with ideas. I would also like to my utmost appreciation to Prof Madya Dr. Faridah Abdullah, UPM for being very helpful throughout my project period.

To all the staffs of Faculty Science and Technology, KUSTEM, thanks you so much for the guidance given. Not forgetting to all the staffs and master students Laboratory of Mycology, UPM especially to Kak Ita and Abang Nazif, I am truly grateful for the kindness and guidance from which I was able to complete my project work successfully. Special thanks neither also to Kak Iliana and Kak Nor for sharing her knowledge and giving me guidance while doing this project.

I gratefully acknowledge the following people for their time, advice and generous contributions for making this piece of work possible; staff of Biotechnology 3 Laboratory, INOS especially Kak Suhaila; students master who shared working with me at INOS especially Kak Siti Faezah, Kak Dah, Kak Ida, Kak Effa; Cik Ku Naiza, Cik Azlina, Kak Timah from Biochemistry Laboratory; Puan Zarina and Puan Mahidawati from Microbiology Laboratory; and last but not least to the staffs of the Electron Microscopy Preparation Room especially to En. Nasir and Kak Ita. To my fellow group of final year

project; Farah, Diana and Linda, thanks a zillion for sharing the work load of keeping the lab tidy with me.

Special grateful also to my beloved parent, Embong Bin Ibrahim and The Binti Mohd Zain and my siblings who always giving me supports and advices without feeling bored. All the love and support given each time have strengthened up my soul and gave me confident to accomplish this study.

Last but not least, to all my course mates and person who did not mentioned her, thanks for helping and being supportive to me. May Allah bless all of you.

TABLES OF CONTENTS

| | Page |
|--|-------------|
| ACKNOWLEDGEMENTS | ii |
| LIST OF TABLES | vii |
| LIST OF FIGURES | viii |
| LIST OF ABBREVIATIONS | x |
| LIST OF APPENDICES | xi |
| ABSTRACT | xiii |
| ABSTRAK | xiv |
| CHAPTER 1 INTRODUCTION | 1 |
| 1.1 Introduction | 1 |
| 1.1.1 The controlling of <i>Rigidoporus lignosus</i> | 2 |
| 1.2 Importance of Study | 4 |
| 1.3 Objectives of study | 4 |
| CHAPTER 2 LITERATURE REVIEW | 5 |
| 2.1 Introduction of amoebae | 5 |
| 2.2 Classification of <i>Acanthamoeba</i> | 6 |
| 2.2.1 The Role of <i>Acanthamoeba</i> | 7 |
| 2.3 Plant pathogenic fungi | 9 |
| 2.4 <i>Rigidoporus lignosus</i> and white root disease of rubber trees | 10 |
| 2.5 Control of <i>Rigidoporus lignosus</i> by fungicide | 11 |

| | |
|--|--------|
| CHAPTER 3 METHODOLOGY | 15 |
| 3.1 Amoebae | 15 |
| 3.1.1 Samples of amoebae | 15 |
| 3.1.2 Cultivation Amoebae | 15 |
| 3.1.3 Extract Amoebae | 16 |
| 3.1.3a Determination of Protein Concentration | 17 |
| 3.1.3b Determination of Absorbance Values | 17 |
| 3.1.3c Determination the Volume of Amoebae Extract With Various Concentration | 18 |
| 3.2 Fungi | 21 |
| 3.2.1 Samples of fungi | 21 |
| 3.2.2 Cultivation fungi | 21 |
| 3.3 Treatment of <i>Rigidoporus lignosus</i> on PDA with the amoebae extracts | 21 |
| 3.4 Observation on the growth of <i>Rigidoporus lignosus</i> | 22 |
| 3.5 Data Analysis | 26 |
| CHAPTER 4 RESULTS | 27 |
| 4.1 Observation on Radial Growth of <i>Rigidoporus lignosus</i> after treatment with <i>Acanthamoeba</i> extracts; AK and P1 extracts | 27 |
| 4.2 Determination of Percentage Inhibition of Radial Growth (PIRG) of <i>Rigidoporus lignosus</i> after treated by 2 <i>Acanthamoeba</i> extracts; AK and P1 | 35 |
| CHAPTER 5 DISCUSSION | 37 |
| CHAPTER 6 CONCLUSION | 41 |
| REFERENCES | 42 |

APPENDICES

46

CURRICULUM VITAE

64

LIST OF TABLES

| Table | Page |
|---|------|
| 4.1 Mean Radial Growth of <i>Rigidoporus lignosus</i> after being exposed to two <i>Acanthamoeba</i> extracts; AK and P1 | 28 |
| 4.2 Percentage Inhibition of Radial Growth (PIRG) of <i>Rigidoporus lignosus</i> after treatment with 2 <i>Acanthamoeba</i> extracts; AK and P1 | 36 |

LIST OF FIGURES

| Figure | Page |
|--|------|
| 3.1 Cultures of amoebae in a culture flask | 19 |
| 3.1(a) AK isolate | 20 |
| (b) P1 isolate | 20 |
| 3.2 Cultures of <i>Rigidoporus lignosus</i> in a universal bottle; as a source pathogenic fungus | 23 |
| 3.3a Plug of <i>Rigidoporus lignosus</i> in PDA agar | 23 |
| 3.3b Plugs of <i>Rigidoporus lignosus</i> incubated with different concentration of P1 extract | 24 |
| 3.3c Plugs of <i>Rigidoporus lignosus</i> with different protein concentration <i>Acanthamoeba</i> sp (AK) extract | 25 |
| 4.1 Radial growths of <i>Rigidoporus lignosus</i> grown with AK and P1 extract | 29 |
| 4.1a The Growth of <i>Rigidoporus lignosus</i> in 6 days without the extracts (control) | 30 |
| 4.1b The Growth of <i>Rigidoporus lignosus</i> in 6 days with the 2000 µg/ml <i>Acanthamoeba</i> sp (AK) extracts | 30 |
| 4.1c The Growth of <i>Rigidoporus lignosus</i> in 6 days with the 1000 µg/ml <i>Acanthamoeba</i> sp (AK) extracts | 31 |
| 4.1d The Growth of <i>Rigidoporus lignosus</i> in 6 days with the 500 µg/ml <i>Acanthamoeba</i> sp (AK) extracts | 31 |
| 4.1e The Growth of <i>Rigidoporus lignosus</i> in 6 days with the 250 µg/ml <i>Acanthamoeba</i> sp (AK) extracts | 32 |
| 4.1f The Growth of <i>Rigidoporus lignosus</i> in 6 days with the 2000 µg/ml <i>Acanthamoeba</i> sp (P1) extracts | 32 |
| 4.1g The Growth of <i>Rigidoporus lignosus</i> in 6 days with the 1000 µg/ml <i>Acanthamoeba</i> sp (P1) extracts | 33 |

| | |
|--|----|
| 4.1h The Growth of <i>Rigidoporus lignosus</i> in 6 days with the 500 µg/ml <i>Acanthamoeba</i> sp (P1) extracts | 33 |
| 4.1i The Growth of <i>Rigidoporus lignosus</i> in 6 days with the 250 µg/ml <i>Acanthamoeba</i> sp (P1) extracts | 34 |
| 4.2 The Percentage Inhibition of Radial Growth (PIRG) of <i>Rigidoporus lignosus</i> by 2 <i>Acanthamoeba</i> extracts; AK and P1. | 36 |

LIST OF ABBREVIATIONS

| | | |
|--------------------|---|--------------------------|
| abs | - | absorbance |
| nm | - | nanometer |
| % | - | percentage |
| $^{\circ}\text{C}$ | - | Degree Celcius |
| ANOVA | - | Analysis of Variance |
| g | - | gram |
| ml | - | milliliter |
| mg | - | milligram |
| mg/ml | - | milligram per milliliter |
| $\mu\text{g/ml}$ | - | microgram per milliliter |
| μm | - | micron meter |
| L | - | liter |
| μL | - | micron liter |
| cm | - | centimeter |

LIST OF APPENDICES

| Appendix | Page | |
|----------|--|----|
| A | a) Determining of protein concentration extracts <i>Acanthamoeba</i> sp (P1) and <i>Acanthamoeba</i> sp (AK) | 46 |
| | b) Determining the volume of protein amoebae extract using the formula. | 47 |
| B | Table 1 Radial Growth of <i>Rigidoporus lignosus</i> against 250, 500, 1000 and 2000 ($\mu\text{g/ml}$) concentrations of <i>Acanthamoeba</i> sp (AK). | 48 |
| | Table 2 Radial Growth of <i>Rigidoporus lignosus</i> against 250, 500, 1000 and 2000 ($\mu\text{g/ml}$) concentrations of <i>Acanthamoeba</i> sp (P1). | 48 |
| | Table 3 Percentage Inhibition of Radial Growth (PIRG) of <i>Rigidoporus lignosus</i> against 250, 500, 1000 and 2000 ($\mu\text{g/ml}$) concentrations of <i>Acanthamoeba</i> sp (AK) extracts. | 49 |
| | Table 4 Percentage Inhibition of Radial Growth (PIRG) of <i>Rigidoporus lignosus</i> against 250, 500, 1000 and 2000 ($\mu\text{g/ml}$) concentrations of <i>Acanthamoeba</i> sp (P1) extracts. | 50 |
| | Figure 1 The effect of different concentration of AK extracts on radial growth of <i>Rigidoporus lignosus</i> . | 51 |
| | Figure 2 The effect of different concentration of P1 extracts on radial growth of <i>Rigidoporus lignosus</i> . | 51 |
| C | a) Determination of radial growth on <i>Rigidoporus lignosus</i> by different concentrations of <i>Acanthamoeba</i> sp (AK). | 52 |
| | b) Determination of radial growth on <i>Rigidoporus lignosus</i> by different concentrations of <i>Acanthamoeba</i> sp (P1). | 55 |
| D | Figure 1: Autoclave | 58 |
| | Figure 2: Biomedical freezer (Minus 20) | 58 |

| | |
|---|----|
| Figure 3: Laminar flow to pour the media | 59 |
| Figure 4: Laminar flow to culture the fungus, <i>Rigidoporus lignosus</i> | 59 |
| Figure 5: Incubator 50 ⁰ C | 60 |
| Figure 6: Laminar Flow to culture the amoebae | 60 |
| Figure 7: Refrigerator | 61 |
| Figure 8: Incubator 30 ⁰ C to keep the amoebae culture | 61 |
| Figure 9: Centrifuge 5804 R | 62 |
| Figure 10: Centrifuge (mikro 20) | 62 |
| Figure 11: Sonicator to lyse the cell of amoebae extracts | 63 |
| Figure 12: Spectrophotometer to determine the absorbance of protein amoebae extracts | 63 |

ABSTRACT

In this study extracts of two free-living amoebae; *Acanthamoeba keratitis* (AK) and *Acanthamoeba* sp (P1) were tested on *Rigidoporus lignosus*, a pathogenic fungus. *Acanthamoeba keratitis* (AK) was a clinical isolate and *Acanthamoeba* sp (P1) was isolated from marine environment. *Rigidoporus lignosus* was taken from a rubber tree infected by white root disease. To observe the anti fungal preparation of the extract, the culture of *Rigidoporus lignosus* was grown on PDA with spread the amoebae extracts at different concentrations and the observation the growth of the fungus was done after 6 days. The protein concentration of the amoebae extracts measured were starting from 2000 µg/ml, 1000 µg/ml, 500 µg/ml and 250 µg/ml. Results in this study show that all the extracts used gave a little effected on concentration the growth of *Rigidoporus lignosus*. Both of the amoebae extracts; AK and P1 were observed to inhibit radial mycelia growth of *Rigidoporus lignosus* at concentration of 2000 µg/ml which the Percentage Inhibition of Radial Growth (PIRG) by AK extract was 4.62% and P1 extract was 4.44%. At lower concentration of amoebae extracts 250 µg/ml, there was no inhibit on radial mycelia growth observed and the PIRG, both of the amoebae extracts were 0.26%. The results obtained from this current study clearly demonstrated there is no different in anti fungal activities in both amoebae extracts used in this study.

KESAN EKSTRAK DARIPADA AMEBA HIDUP KE ATAS KULAT PATOGEN, *RIGIDOPORUS LIGNOSUS*

ABSTRAK

Dalam kajian ini, dua ekstrak ameba hidup; AK dan P1 telah diuji ke atas kulat patogen, *Rigidoporus lignosus*. AK adalah pengasingan klinikal dan P1 adalah diasingkan daripada persekitaran marin. *Rigidoporus lignosus* telah diambil daripada pokok getah yang dijangkiti oleh penyakit pada akar putihnya. Untuk melihat penyediaan ekstrak anti-kulat, kultur *Rigidoporus lignosus* telah ditumbuhkan di atas PDA yang diratakan dengan ekstrak ameba pada kepekatan berbeza dan pertumbuhan kulat diperhatikan selepas 6 hari. Kepekatan protein ekstrak ameba telah diukur bermula daripada 2000 µg/ml, 1000 µg/ml, 500 µg/ml dan 250 µg/ml. Keputusan dalam kajian ini menunjukkan semua ekstrak yang digunakan pada kepekatan berlainan membawa kesan yang sedikit ke atas pertumbuhan *Rigidoporus lignosus*. Kedua-dua ekstrak ameba; AK and P1 telah diperhatikan perencatan pertumbuhan jejari miselia *Rigidoporus lignosus* pada kepekatan 2000 µg/ml di mana peratus perencatan AK adalah 4.62% dan P1 adalah 4.44%. Pada kepekatan rendah iaitu 250 µg/ml, tiada perencatan ke atas miselia yang diperhatikan dan peratus perencatannya bagi kedua-dua jenis ameba adalah 0.26%. Keputusan yang didapati daripada kajian ini jelas ditunjukkan tiada perbezaan aktiviti anti-kulat dalam kedua-dua ameba ekstrak yang digunakan dalam kajian ini.