



UNIVERSITI PUTRA MALAYSIA

CHEMICAL COMPOSITION AND POTENTIAL OF EUCALYPTUS ESSENTIAL OILS FOR CONTROL OF STORED PRODUCT INSECTS, *Sitophilus oryzae* L. AND *Tribolium castaneum* (HERBST)

MOHAMMAD EBRAHIM FARASHIANI

FP 2014 3



**CHEMICAL COMPOSITION AND POTENTIAL OF *EUCALYPTUS*
ESSENTIAL OILS FOR CONTROL OF STORED PRODUCT INSECTS,
Sitophilus oryzae L. AND *Tribolium castaneum* (HERBST)**

By

MOHAMMAD EBRAHIM FARASHIANI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfillment of the Requirement for the Degree of Doctor of Philosophy**

July 2014

COPYRIGHT

All materials contained within the thesis, including without limitation text, logos, icons, photographs and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

Copyright © Universiti Putra Malaysia



DEDICATION

I would like to dedicate my thesis to my father, my dear mother, my honest brothers and sisters and beloved wife who always inspired and supported me to achieve this goal and my children Zahra and Madiyah.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

POTENTIAL OF *EUCALYPTUS* ESSENTIAL OILS FOR CONTROL OF STORED PRODUCT INSECTS, *Sitophilus oryzae* L. AND *Tribolium castaneum* (HERBST)

By

MOHAMMAD EBRAHIM FARASHIANI

July 2014

Chairperson: Prof. Rita Muhamad Awang, PhD

Faculty: Agriculture

Stored product insects, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst) (Coleoptera.: Tenebrionidae) are among of the most destructive pests of stored products and grains in different parts of the world. Fumigation of pest-infested grains and stored food product with methyl bromide and phosphine has been the most successful method in the control and management of the pests. However, extensive and uncontrolled application of these fumigants has caused serious problems such as resistance in pests and destruction effects on the ozone layer. To overcome such problems, safer options to current fumigants are being investigated. Hence, plant materials with natural pesticide properties are a subject of interest. *Eucalyptus* essential oils are among the safe alternative plant extracts that has the potential to be a natural pesticide. Therefore, this study focuses on identifying the chemical composition of 53 *Eucalyptus* essential oils, studying the effects of carriers and extraction methods, screening fumigant toxicity of the oils, evaluating the influence of environmental factors on the chemical constitution and insecticidal activity of the oils, and testing selected *Eucalyptus* essential oils as biofumigants against *S. oryzae* and *T. castaneum*. Gas chromatography (GC) and Gas chromatography–Mass spectrometry (GC-MS) analysis of 53 *Eucalyptus* essential oils showed that monoterpene compounds (C₁₀ H₁₆) such as 1, 8- cineole, α -pinene and limonene were the major constituents (74%) of the *Eucalyptus* essential oils. Among the 10 chemical solvents examined for the essential oils dilution, Tween 80 was a suitable carrier for diluting the oils. The essential oils (*E. camaldulensis* and *E. globulus*) extracted by hydro-distillation showed the highest fumigant activity (LC₅₀, 24. 89 to 27. 43 μ l/l air) against *S. oryzae* and had the highest percentage composition of 1, 8-cineol (68.75 to 78.23%). Of the 53 *Eucalyptus* species essential oils screened, the oils extracted from *E. camaldulensis* and *E. globulus* had the highest fumigant toxicity (LC₅₀ < 30 μ l/l air) against *S. oryzae*. The main compound of *Eucalyptus* essential oil, 1,8-cineole showed considerable fumigant toxicity against *S. oryzae* (LC₅₀ = 26.59 μ l/l air), and multiple regression analysis revealed a strong correlation (41%) between the fumigant toxicity of essential oils and their 1,8-cineole concentration. Environmental factors such as climate condition and tree age influenced the fumigant toxicity of *E. camaldulensis* and *E. globulus* essential oils against *S. oryzae* significantly. The LC₅₀ values of the oils from the north of Iran (31.72, 27.43 μ l/l air) was significantly higher than the LC₅₀ values of the oils from the south of Iran (41.5, 38.42 μ l/l air). The fumigant toxicity (LC₅₀) of the oils

extracted from 20 year old trees (24.45, 26.77 $\mu\text{l/l}$ air) were also notably higher than the oils from five year old trees (30.36, 36.80 $\mu\text{l/l}$ air). Toxicity studies showed that *E. camaldulensis* and *E. globulus* essential oils had strong fumigant toxicity toward adults of *S. oryzae* (LC_{50} , 24.90 - 27.43 $\mu\text{l/l}$ air) and *T. castaneum* (LC_{50} , 37.61 - 38.09 $\mu\text{l/l}$ air), and that they acted very fast (LT_{50} , 2.15 to 3.09 hours) against the insects at 250 $\mu\text{l/l}$ dose. Both oils highly repelled the insects and at 2.5 $\mu\text{l/ml}$ (μl essential oils/ml acetone/40 kernels), their repellency values against *S. oryzae* and *T. castaneum* were more than 90%. Persistency of the oils against the insects was notable and LT_{50} values of the oils at the 100 $\mu\text{l/l}$ dosage were in the range of 5.94 to 7.54 days. Based on the significant insecticidal activities of the selected *Eucalyptus* essential oils, it was evident that *E. camaldulensis* and *E. globulus* essential oils have great potential for future development as safe fumigants.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

**POTENSI MINYAK PATI *EUCALYPTUS* UNTUK MENGAWAL
SERANGGA PEROSAK PRODUK SIMPANAN, *Sitophilus oryzae* L. DAN
Tribolium castaneum (HERBST)**

Oleh

MOHAMMAD EBRAHIM FARASHIANI

Julai 2014

Pengerusi: Prof. Rita Muhamad Awang, PhD
Fakulti: Pertanian

Serangga perosak, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) dan *Tribolium castaneum* (Herbst) (Coleoptera.: Tenebrionidae) merupakan perosak penting bagi produk-produk yang disimpan termasuk juga bijirin di kebanyakan negara di seluruh dunia. Kaedah pengasapan menggunakan metil bromid dan fosfina menjadi kaedah utama yang digunakan untuk mengawal dan menguruskan perosak ini. Akan tetapi, penggunaan kaedah ini yang berlebihan dan tidak terkawal telah mencetuskan beberapa masalah utama seperti keresistenan perosak tersebut dan menyebabkan berlakunya penipisan lapisan ozon. Untuk mengatasi masalah-masalah tersebut, beberapa pilihan yang lebih selamat telah dikaji. Oleh itu, bahan tanaman yang mempunyai sifat pestisida semulajadi telah menjadi subjek utama kajian ini. Minyak pati *Eucalyptus* merupakan alternatif ekstrak tanaman yang selamat dan berpotensi untuk menjadi pestisida semulajadi. Maka, kajian ini bertumpuan untuk mengenal pasti komposisi kimia bagi 53 jenis ekstrak minyak pati *Eucalyptus*, mengkaji kesan pembawa dan kaedah pengekstrakan minyak pati *Eucalyptus*, menguji ketoksikan minyak pati *Eucalyptus*, menilai pengaruh faktor sekitar terhadap jujuk kimia dan aktiviti insektisida ekstrak minyak pati *Eucalyptus* dan juga menguji ekstrak minyak pati *Eucalyptus* yang terpilih sebagai biofumigasi untuk mengawal *S. oryzae* dan *T. castaneum*. Analisis Kromatografi gas (GC) dan Kromatografi gas-spektrometri jisim (GC-MS) bagi 53 jenis minyak pati *Eucalyptus* menunjukkan kompaun monoterpena ($C_{10}H_{16}$) seperti 1, 8- cineole, α -pinene dan limonene merupakan jujuk utama iaitu sebanyak 74% di dalam minyak pati *Eucalyptus*. Antara 10 jenis pelarut kimia yang diuji untuk mencairkan ekstrak minyak pati, Tween 80 tidak sesuai untuk menjadi pembawa bagi melarutkan minyak pati tersebut. Minyak pati (*E. camaldulensis* dan *E. globulus*) yang telah diekstrak menggunakan kaedah penyulingan hidro menunjukkan aktiviti fumigasi yang tertinggi (LC_{50} , 24.89 hingga 27.43 $\mu\text{l/l}$) terhadap *S. oryzae* dan juga mempunyai peratus komposisi 1, 8-cineol (68.75 hingga 78.23%) tertinggi. Berdasarkan ujian yang dijalankan bagi 53 jenis minyak pati dari spesies *Eucalyptus*, minyak pati yang diekstrak dari *E. camaldulensis* dan *E. globulus* mempunyai ketoksikan fumigasi yang tinggi ($LC_{50} < 30 \mu\text{l/l}$) terhadap *S. oryzae*. Kompaun utama, 1,8-cineole, menunjukkan ketoksikan fumigasi yang sewajarnya terhadap *S. oryzae* ($LC_{50} = 26.59 \mu\text{l/l}$), dan analisis regresi pelbagai menunjukkan korelasi yang sangat kuat (41%) antara ketoksikan fumigasi minyak pati dengan kepekatan 1,8-cineole. Faktor sekitar seperti perubahan keadaan iklim

dan usia pokok mempengaruhi ketoksikan fumigasi bagi minyak pati *E. camaldulensis* dan *E. globulus* terhadap *S. oryzae* dengan ketara. Nilai LC_{50} bagi ekstrak minyak pati dari utara Iran (31.72, 27.43 $\mu\text{l/l}$) lebih tinggi berbanding dari selatan Iran (41.5, 38.42 $\mu\text{l/l}$). Ketoksikan fumigasi (LC_{50}) bagi ekstrak dari pokok yang berusia 20 tahun (24.45, 26.77 $\mu\text{l/l}$) juga lebih tinggi berbanding ekstrak dari pokok berusia lima tahun (30.36, 36.80 $\mu\text{l/l}$). Ujian toksisiti menunjukkan bahawa minyak pati dari *E. camaldulensis* dan *E. globulus* mempunyai ketoksikan fumigasi yang lebih kuat terhadap serangga dewasa *S. oryzae* (LC_{50} , 24.90 - 27.43 $\mu\text{l/l}$) dan *T. castaneum* (LC_{50} , 37.61 - 38.09 $\mu\text{l/l}$), malah juga, ia bertindak lebih pantas (LT_{50} , 2.15 to 3.09 hours) terhadap serangga perosak tersebut pada dos 250 $\mu\text{l/l}$. Kedua-dua minyak pati bertindak untuk menghalau serangga perosak dan pada 2.5 $\mu\text{l/ml}$ (μl minyak pati/ml acetone/40 kernel), nilai penolakan (repellency value) minyak pati terhadap *S. oryzae* dan *T. castaneum* adalah melebihi 90%. Ketegaran (persistence) minyak pati terhadap serangga perosak adalah ketara dan nilai LT_{50} bagi minyak pati pada dos 100 $\mu\text{l/l}$ berjulat dari 5.94 ke 7.54 hari. Berdasarkan aktiviti insektisida yang ketara bagi ekstrak minyak pati dari spesies *Eucalyptus*, ia telah membuktikan bahawa minyak pati dari ekstrak *E. camaldulensis* dan *E. globulus* mempunyai potensi yang tinggi untuk dijadikan sistem fumigasi yang selamat.

ACKNOWLEDGEMENTS

In the name of Allah, The Most Merciful and the Most Compassionate

This dissertation would not have been finished if it were not for the help and support of following individuals, faculty and institutions.

I am particularly thankful to my chief supervisor Prof. Dr. Rita Muhamad Awang for her thoughtful guidance, sincere encouragement and continuous support in the Ph.D. program. I wish to express my warm thanks to worthy members of my supervisory committee: Prof. Dr. Dzolkhifli Bin Omar, whose helpful guidance and insightful comments in supervisor committee meetings and support during the program, Prof. Dr. Mohammad Hasan Asareh his warm encouragement and discerning comments as well as his support during laboratory and field works of my thesis. I would like to express my thanks to Prof. Dr. Mawardi Rahmani for his kindness and helps. I owe my deepest gratitude to Prof. Dr. Fatemeh Sefidkon who has been unsparing in her efforts to make my Ph.D. program such a success.

I would like to thank all the staff from Department of plant protection for their kindness and helps.

I thank to Eng. Vahid Reza Moniri, Dr. Mohammad Jafar Farsi, Eng. Sattar Zeinali, and my other colleagues for their friendship assistance during laboratory works in Entomology Laboratory of Iranian Research Institute of forests and rangelands.

My special thanks to Prof. Dr. Hosein Sardabi, *Eucalyptus* taxonomy specialist of Iranian Research Institute of forests and rangelands, for his helpful advice about *Eucalyptus* species to conduct this study. I would like to express my thanks to my colleagues in North and South of Iran for their helping in collecting the plants material from field.

Finally, I wish to express my thankfulness to my family also brothers and sisters for love and encouragement and for my darling mother, for her prayers and moral support, for my father who raised me with a love of science and supported me in all my pursuits. In addition, the most of all for my loving, supportive, encouraging, and patient wife Mojgan Sadat and my children Zahra and Mahdiyeh whose faithful support during the stages of this PhD is so appreciated.

I certify that a Thesis Examination Committee has met on 10 July 2014 to conduct the final examination of Mohammad Ebrahim Farashiani on his thesis entitled "Chemical Composition and Potential of *Eucalyptus* Essential Oils for Control of Stored Product Insects *Sitophilus oryzae* L. and *Tribolium castaneum* Herbst" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Zainal Abidin bin Mior Ahmad, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Kamaruzaman bin Sijam, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Hafidzi bin Mohd Noor, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Ghulam Hussain Abro, PhD

Professor
Sindh Agriculture University
Pakistan
(External Examiner)



NORITAH OMAR, PhD

Associate Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 18 August 2014

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Rita Muhamad Awang, PhD

Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairperson)

Dzolkhifli Omar, PhD

Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Mawardi Rahmani, PhD

Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Mohammad Hasan Asareh, PhD

Professor
Iranian Research Institute of forests and rangelands
Tehran, Iran
(Member)

BUJANG BIN KIM HUAT, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

Declaration by graduate student

I hereby confirm that:

- This thesis is my original work;
- Quotations, illustrations and citations have been dully referenced;
- This thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- Intellectual properly from the thesis and copyright of thesis are fully owned by Universiti Putra Malaysia, as according to the University Putra Malaysia (Research) Rules 2012;
- Written permission must be obtain from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- There is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: Mohammad Ebrahim Farashiani, GS24785

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision,
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____

Name of
Chairman of
Supervisory

Committee: **Rita Muhamad
Awang, PhD**

Signature: _____

Name of
Member of
Supervisory

Committee: **Dzolkhifli Omar, PhD**

Signature: _____

Name of
Member of
Supervisory

Committee: **Mawardi Rahmani,
PhD**

Signature: _____

Name of
Member of
Supervisory

Committee: **Mohammad Hasan Asareh,
PhD**

TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	iii
ACKNOWLEDGEMENTS	v
APPROVAL	vi
DECLARATION	viii
LIST OF TABLES	xiii
LIST OF FIGURES	xv
LIST OF ABBREVIATIONS	xvi
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	3
2.1 Stored product insects	3
2.1.1 The Rice Weevil, <i>Sitophilus oryzae</i> (L.)	3
2.1.2 The red flour beetle, <i>Tribolium castaneum</i> (Herbst)	4
2.2 Control of stored product insects	4
2.3 Overview of Essential Oils	6
2.3.1 Constituents of Essential Oils and their uses	6
2.3.2 Constituents of <i>Eucalyptus</i> essential oils and their uses	6
2.3.3 <i>Eucalyptus</i> Essential oils in Iran	7
2.3.4 <i>Eucalyptus</i> Essential Oil Extraction	8
2.3.5 Isolation, purification and quantification of bioactive compounds	10
2.4 Insecticidal activity of essential oils	10
2.4.1 Mode of action	11
2.4.2 Insecticidal properties of <i>Eucalyptus</i> and other plant essential oils on stored product insects	11
2.5 Influence of different factors on the quantity and quality of essential oils	16
2.5.1 Genetic of plant species	16
2.5.2 Climate conditions	16
2.5.3 Extraction methods of essential oils	17
2.5.4 Harvesting season	17
2.5.5 Plant age	18
3 EFFECT OF EXTRACTION METHOD AND CARRIER ON COMPOSITION AND TOXICITY OF <i>EUCALYPTUS</i> ESSENTIAL OILS	19
3.1 Introduction	19
3.2 Materials and Methods	19
3.2.1 Effect of distillation method on essential oil content and composition	19
3.2.2 Effect of distillation method on essential oils fumigant toxicity	22
3.2.3 Effect of carrier on fumigant toxicity of the essential oils	23
3.3 Results and discussion	23

3.3.1 Effect of distillation method on the content and composition of extracted <i>Eucalyptus</i> essential oils	23
3.3.2 Effect of distillation method on essential oils fumigant toxicity	25
3.3.3 Effect of essentials oil dilution with carier on fumigant toxicity of them against <i>Sitophilus oryzae</i>	26
3.4 Conclusion	27
4 EXTRACTION AND ANALYSIS OF CHEMICAL CONSTITUENTS IN 53 EUCALYPTUS SPP.	29
4.1 Introduction	29
4.2 Materials and Methods	29
4.2.1 Plant materials	29
4.2.2 Extraction procedure	29
4.2.3 Identification of chemical components	30
4.2.4 Statistical Analysis	31
4.3 Results and Discussion	31
4.3.1 Quantity of essential oils extracted from the <i>Eucalyptus</i> spp.	31
4.3.2 Chemical composition of essential oils extracted from 53 <i>Eucalyptus</i> spp.	34
4.4 Conclusion	44
5 FUMIGANT TOXICITY OF EUCALYPTUS ESSENTIAL OILS AGAINST RICE WEEVIL, <i>Sitophilus oryzae</i> L.	45
5.1 Introduction	45
5.2 Materials and Methods	45
5.2.1 Plant materials	45
5.2.2 Chemical materials	45
5.2.3 Insects	45
5.2.4 Toxicity bioassay	46
5.2.5 Statistical Analysis	46
5.3 Results and discussion	46
5.3.1 Fumigant toxicity of 53 <i>Eucalyptus</i> species essential oils against <i>S. oryzae</i>	46
5.3.2 Relationship between the major components of <i>Eucalyptus</i> essential oils and their insecticidal activity against <i>S. oryzae</i>	48
5.3.3 Fumigant toxicity of the the three pure main components of <i>Eucalyptus</i> essential oils (1,8-cineol, limonene and α -pinene) on <i>S. oryzae</i>	52
5.4 Conclusion	54
6 EFFECT OF CLIMATE, AGE OF TREES AND HARVESTING SEASON ON CHEMICAL COMPOSITION AND TOXICITY OF <i>Eucalyptus</i> ESSENTIAL OILS	55
6.1 Introduction	55
6.2 Materials and Methods	55

6.2.1 Plant materials	55
6.2.2 Insects	58
6.2.3 Toxicity bioassay	58
6.2.4 Statistical Analysis	58
6.3 Results and Discussion	58
6.3.1 Effect of climate conditions on chemical composition and fumigant toxicity of <i>Eucalyptus</i> essential oils	58
6.3.2 Effect of trees age on chemical composition and fumigant toxicity of <i>Eucalyptus</i> essential oils	61
6.3.3 Effect of harvesting season on chemical composition and insecticidal activity of Eucalyptus essential oils	64
6.4 Conclusion	67
7 TOXICITY OF SELECTED EUCALYPTUS ESSENTIAL OILS AGAINST TWO IMPORTANT STORED PRODUCT INSECTS, <i>Sitophilus oryzae</i> AND <i>Tribolium castaneum</i>	68
7.1 Introduction	68
7.2 Materials and Methods	68
7.2.1 Plant materials	68
7.2.2 Insects	69
7.2.3 Fumigant toxicity bioassay	69
7.2.4 Time response (LT ₅₀) bioassay	70
7.2.5 Repellency bioassay	70
7.2.6 Persistency	72
7.3 Results and Discussion	72
7.3.1 Fumigant toxicity of the selected essential oils on <i>S. oryzae</i> and <i>T. castaneum</i>	72
7.3.2 Time response	76
7.3.3 Repellent activity	80
7.3.4 Persistency	84
7.4 Conclusion	87
8 SUMMARY, GENERAL CONCLUSIONS AND RECOMMENDATIONS	88
8.1 Summary	88
8.2 General conclusions	91
8.3 Recommendations for Future Research	92
REFERENCES	94
APPENDICES	105
BIODATA OF STUDENT	111
LIST OF PUBLICATIONS	112