UNIVERSITI PUTRA MALAYSIA

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

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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

DEDICATION

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

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)

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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, thisstudy 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 (C_{10} H_{16}) 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 (LC_{50}, 24. 89 to 27. 43 µl/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 (LC_{50} < 30 µl/l air) against*S. oryzae*. The main compound of *Eucalyptus* essential oil, 1,8-cineole showed considerable fumigant toxicity against *S. oryzae* (LC_{50} = 26.59 µl/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 LC_{50} values of the oils from the north of Iran (31.72, 27.43 µl/l air) was significantly higher than the LC_{50} values of the oils from the south of Iran (41.5, 38.42 µl/l air). The fumigant toxicity (LC_{50}) of the oils

extracted from 20 year old trees (24.45, 26.77 µl/l air) were also notably higher than the oils from five year old trees (30.36, 36.80 µl/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 µl/l air) and *T. castaneum*(LC_{50} , 37.61 - 38.09µl/l air), and that they acted very fast (LT_{50} , 2.15 to 3.09 hours) against the insects at 250 µl/l dose. Both oils highly repelled the insects and at 2.5 µl/ml (µl essential oils/ml acetone/40kernels), 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 µl/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 bromidadan fosfina menjadi kaedah utama yang digunakan untuk mengawal dan menguruskan perosak ini. Akan tetapi, penggunaan kaedah ini yang berlebihan dan tidak terkawal telah mancetuskan 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 juzuk 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–spektometri jisim (GC-MS)bagi 53 jenis minyak pati*Eucalyptus*menunjukkan kompaun monoterpene (C_{10} H_{16}) seperti 1, 8- cineole, α-pinene dan limonene merupakan juzuk 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 µl/l) terhadap *S. oryzae* dan juga mempunyai peratus komposisi 1, 8-cineol (68.75 hingga78.23%) tertinggi. Berdasarkan ujian yang dijalankan bagi 53 jenis minyak pati dari spesis *Eucalyptus*, minyak pati yang diekstrak dari *E. camaldulensis*dan*E. globulus* mempunyai ketoksikan fumigasi yang tinggi (LC_{50} < 30 µl/l) terhadap*S. oryzae*. Kompaun utama, 1,8-cineole,menunjukkan ketoksikan fumigasi yang sewajarnya terhadap *S. oryzae* (LC_{50} = 26.59 µl/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 bagiminyak 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 µl/l) lebih tinggi berbanding dari selatan Iran (41.5, 38.42 µl/l). Ketoksikan fumigasi (LC_{50}) bagi ekstrak dari pokok yang berusia 20 tahun (24.45, 26.77 µl/l) juga lebih tinggi berbanding ekstrak dari pokok berusia lima tahun (30.36, 36.80 µl/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 µl/l) dan *T. castaneum*(LC_{50} , 37.61 -38.09µl/l),malah juga, ia bertindak lebih pantas (LT_{50} , 2.15 to 3.09 hours) terhadap serangga perosak tersebut pada dos 250 µl/l. Kedua-dua minyak pati bertindak untuk menghalau serangga perosakdan pada2.5 µl/ml (µl minyak pati/ml acetone/40kernel), nilai penolakan (repellency value) minyak pati terhadap*S. oryzae* dan*T. castaneum* adalah melebihi 90%. Ketegaran (persistency) minyak pati terhadap serangga perosak adalah ketara dan nilai LT_{50} bagi minyak pati pada dos 100 µl/l berjulat dari 5.94 ke 7.54 hari. Berdasarkan aktiviti insektisida yang ketara bagi ekstrak minyak pati dari spesis *Eucalyptus,* ia telah membuktikan bahawa minyak pati dari ekstrak *E. camaldulensis* dan *E. globulus*mempunyai potensi yang tinggi untuk dijadikan sistem fumigasi yang selamat.

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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.

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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:

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DECLARATION

Declaration by graduate student

I hereby confirm that:

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This is to confirm that:

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