UNIVERSITI PUTRA MALAYSIA

CHEMICAL PROFILING OF SELECTED MEDICINAL PLANTS AND THEIR NANOEMULSION FORMULATION AGAINST PAPAYA MEALYBUG (*Paracoccus marginatus* Williams & Granara de Willink)

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By

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

October 2018

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DEDICATION

To my family with love

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

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Chairman : Professor Dzolkhifli Omar, PhD
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The papaya mealybug, *Paracoccus marginatus* (Hemiptera: Pseudococcidae), is considered a treacherous pest for many tropical and subtropical vegetables, fruits and ornamental plants. Synthetic insecticides are one of the methods available for controlling the papaya mealybugs. Nevertheless, mealybugs have developed a genetic resistance to synthetic insecticides which adversely affects the environment by polluting the air, soil, and water. The alternative approach to control the pest with minimum harmfulness is to use bioactive compounds present in plants. Many studies have revealed the possibility of utilizing essential plant oils to control insects as an eco-friendly pesticides. Therefore, the study endeavours to prepare and determine the effectiveness of nanoemulsion formulations of *Artemisia herba-alba*, *Myrtus communis*, *Mentha longifolia* and *Salvia spinosa* essential oils against papaya mealybugs, *P. marginatus.* The essential oils of selected medicinal plants were extracted by hydrodistillation and solvent extraction methods, and their chemical profiles were identified using GC-MS system. The insecticidal activities of the essential oils were firstly evaluated against female adults of the papaya mealybug. Then, the most effective essential oil of each plant was selected to prepare water in oil (W/O) nanoemulsion formulations. Three mineral oils as carriers and 18 types of surfactants were screened to prepare and characterize nanoemulsion formulations, and their toxicity was determined against the papaya mealybugs. The GC-MS analysis exhibited that the essential oils of the plants contain various active components such as; eucalyptol, piperitone, cineron, dehydroabietic acid, α.terpineol α-pinene, linderol and alcanfor. The quality and quantity of these components depend on the type of the plant and the method of extraction. The biological screening of the essential oil toxicity of *A. herba-alba, M. longifolia,* and *M. communis* extracted by hydrodistillation, and *S. spinosa* essential oil obtained by solvent extraction showed a high toxicity against papaya mealybug. After the miscibility screening, three

surfactants (EW70, Termul 3540 and DB10) and two mineral oils (paraffin and methyl ester) were chosen to prepare nanoemulsion formulation of the selected plants essential oil. Three ternary phase diagrams were constructed from these materials, and three points (FN5, FN8, and FN11) were selected from each ternary phase diagrams. After miscibility and stability test, it was prepare nine formulations with 20% of *A. herba-alba, M. longifolia. M. communes* essential oil and three formulations with 15% of *S. spinosa* essential oil. The results of characterization tests confirmed that all formulations with essential oils were nanoemulsion formulations within nano-particle size at range 26.94 to 108.00mm. The formulations exhibited high stability under centrifugation and storage conditions with good physical characterizations. The toxicity of the essential oil nanoformulations against *P. marginatus* showed the nano formulation code FN5 (paraffin oil and DB10 surfactant) achieved the highest toxicity against papaya mealybugs and largest droplet spreading area on papaya leaves followed by nano formulation codes FN8 (methyl ester and Termul 3540 surfactant) and FN11 (methyl ester and EW70 surfactant) with all selected plants essential oils. The nanoemulsion formulation code FN5 with *M. longifolia* essential oil revealed the best results compared with other nanoemulsion formulations with selected plant essential oils. The present study found that nanoemulsion formulations of essential oils of *A. herba-alba, M. communis, M. longifolia* and *S. spinosa* are safe and eco-friendly insecticides. These botanical pesticides can be utilized as an alternative approach to control the papaya mealybug.

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

PEMPROFILAN KIMIA TUMBUHAN UBATAN TERPILIH DAN FORMULASI NANOEMULSI TERHADAP KOYA-KOYA BETIK (*Paracoccus marginatus* Williams & Granara de Willink)

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Koya-koya betik iaitu, *Paracoccus marginatus* (Hemiptera: Pseudococcidae), telah dianggap perosak berbahaya bagi kebanyakan sayuran tropikal dan subtropikal, buah-buahan dan tumbuhan hiasan. Racun sintetik merupakan salah satu cara yang sedia ada bagi mengawal koya-koya betik. Walau bagaimanapun, koya-koya telah membentuk suatu ketahanan genetik terhadap racun sintetik yang memberikan kesan pada alam sekitar melalui pencemaran udara, tanah, dan air. Pendekatan alternatif bagi mengawal perosak dengan kemudaratan yang minimum adalah dengan menggunakan sebatian bioaktif yang wujud dalam tumbuhan. Banyak kajian telah memperlihatkan kemungkinan menggunakan minyak pati tumbuhan bagi mengawal serangga sebagai racun perosak mesra alam. Oleh sebab itu, kajian ini bertujuan untuk menyedia dan menentukan keberkesanan formulasi nanoemulsi *Artemisia herba-alba*, *Myrtus communis*, *Mentha longifolia* dan minyak pati *Salvia spinosa* terhadap koya-koya betik, *P. marginatus*. Minyak pati bagi tumbuhan ubatan terpilih telah diekstrak melalui kaedah hidropenyulingan dan pengekstrakan solven, dan profil kimia mereka telah dikenal pasti menggunakan sistem GC-MS. Aktiviti racun serangga minyak pati, pertamanya telah dinilai pada betina dewasa koya-koya betik. Kemudian, minyak pati yang paling efektif bagi setiap tumbuhan telah dipilih bagi menyediakan formulasi nano air dalam minyak (W/O). Tiga minyak sebagai pembawa dan 18 jenis surfaktan telah disaring bagi menyedia dan mencirikan formulasi nanoemulsi dan ketoksikan mereka telah ditentukan pada koya-koya betik. Analisis GC-MS menunjukkan bahawa minyak pati tumbuhan mengandungi pelbagai komponen aktif seperti; eukliptol, piperiton, asid dehidrobietik cineron, terpineol α-pinena, linderol, dan alkanfor. Kualiti dan kuantiti komponen tersebut bergantung s tumbuhan dan kaedah pengekstrakanpada jeni. Penyaringan biologikal bagi ketoksikan minyak pati *A. herba-alba, M. longifolia*, dan *M. communis* yang diekstrak melalui hidropenyulingan, dan minyak pati *S. spinosa* yang diperoleh melalui

pengekstrakan solven menunjukkan ketoksikan yang tinggi terhadap koya-koya betik. Selepas penyaringan kelarutcampuran, tiga surfaktan (EW70, Termul 3540 dan DB10) dan dua minyak mineral (parafin dan ester metil) telah dipilih bagi menyediakan formulasi nanoemulsi bagi minyak pati tumbuhan terpilih. Tiga gambar rajah fasa ternar telah dibentuk daripada bahan tersebut, dan tiga titik (FN5, FN8, dan FN11) telah dipilih setiap dari gambar rajah fasa ternar. Selepas kelarutcampuran dan ujian stabiliti, ia telah menyediakan sembilan formulasi dengan 20% *A. herba-alba*, *M. longifolia.*, minyak pati *M. communes* dan tiga formulasi dengan 15% minyak pati *S. spinosa*. Dapatan ujian pencirian mengesahkan bahawa semua formulasi dengan minyak pati merupakan formulasi nanoemulsi dalam lingkungan saiz partikel berjulat antara 26.94 hingga 108.00mm. Formulasi tersebut memperlihatkan stabiliti yang tinggi di bawah keadaan emparan dan penyimpanan dengan pencirian fizikal yang baik. Ketoksikan minyak pati nanoformulasi terhadap *P. marginatus* menunjukkan nanoformulasi kod FN5 (minyak parafin dan surfaktan 10) memperoleh ketoksikan yang paling tinggi terhadap koya-koya betik , kawasan penyebaran titisan paling besar ke atas daun betik, diikuti oleh nanoformulasi kod FN8 (ester metil dan surfaktan Termul 3540) dan FN11 (ester metil dan surfaktan EW70) dengan semua minyak pati tumbuhan terpilih. Formulasi nanoemulsi kod FN5 dengan minyak pati *M. longifolia* memperlihatkan hasil yang paling baik berbanding dengan formulasi nanoemulsi minyak pati terpilih lain. Kajian ini mendapati bahawa formulasi nanoemulsi minyak pati *A. herba-alba, M. communis, M. longifolia* dan *S. spinosa* adalah selamat dan racun mesra alam. Racun serangga botanikal tersebut dapat digunakan sebagai pendekatan alternatif bagi mengawal koya-koya betik.

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LIST OF ABBREVIATIONS

%	Percentage
a.i	Active Ingredient
ANOVA	Analysis of Variance
C. R. D.	Completely Randomized Design
C.L.	Confidence Limit
cm	Centimeter
df	Degree of Freedom
EC	Emulsifiable *Concentrate*
EO	Essential Oil
g	Gram
GC-MS	Gas Chromatography–Mass Spectrometry
h	Hour
HLB	Hydrophilic-Lipophilic Balance
IPM	Integrated Pest Management
L:D	Light: Dark
LC_{50}	Lethal Concentration 50
LT_{50}	Lethal Time 50
m	Meter
mg/l	Milligrams per Liter
min.	Minute
ml	Milliliter
mm	Millimeters
mm^2	Square Millimeter
mN/m	Millinewton Per Meter
mPa s	Millipascal Per Second
mv	Millivolt

NIST	National Institute of Standards and Technology
nm	Nanometer
O/W	Oil in Water
ºC	Celsius
RH	Relative Humidity
rpm	Rotation Per Minute
RT	Retention Time
SAS	Statistical Analysis Software
S.E.	Standard Error
UV	Ultraviolet Visible
v/v	Volume to Volume
Vis	Visible
W/O	Water in Oil
w/w	Weight to Weight
x^2	Chi-*Square*
µl	Microliter
Mm	Micrometer
Ache	Acetylcholinesterase
Ach	Acetylcholin

CHAPTER 1

INTRODUCTION

Papaya fruits (*Carica papaya* L.) are economically important and popular fruits of subtropical and tropical and regions. They are consumed world-wide as fresh fruits or as processed products (Silva *et al.,* 2007). After, Mexico and Belize, Malaysia is the third major papaya fruit exporting country in 2010. Annually, it exports more than 57.45 % with a total value of 9.17 million US$ of global production (FAOSTAT, 2013). In fact, the fruit production has many constraints. One of them is papaya mealybug, *Paracoccus marginatus* (Williams and Granara de Willink). It is considered as a major economic pest where it is introduced to new locations without their natural enemies (Miller *et al*., 2002; Muniappan, 2009).

Papaya mealybugs are invasive and polyphagous insects. They attack over 86 host plants belonging to more than 35 plant families. It is a soft-bodied and sap-sucking insect pest; both adults and nymphs are the most deleterious and damaging stages. They secrete honeydew which further promotes the sooty mould on a plant that decreases photosynthesis process which results in leaf yellowing, defoliation, retardation in plant growth and eventually causes the death of the plant in severe infestation (Tanwar *et al.,* 2010; Schneider & Lapolla, 2011; Santos *et al.,* 2013). Several management tools have been previously applied, but still, it is one of the most challenging and difficult pests to control. Their thick, waxy body cover does not only create delinquency in penetration of pesticide, but also protects them from natural enemies. Moreover, they are always present in the bottom surface of the leaves, cracks and under the bark of the plant as protection from control agents. The chemical control remains the first choice for papaya mealybugs management. Nevertheless, due to the risk of chemical pest control methods to the public health; there has been an on-going attempt to reduce the use of harmful pesticides. This has led to the development of more targeted compounds that exhibit less side effect (Galanihe *et al.,* 2011; Saengyot & Burikam, 2011).

Essential oils are one of the most crucial materials found in the aromatic plants and commonly obtained by hydrodistillation, solvent extraction and other methods (Bakkali *et al.,* 2008; Ntalli & Menkissoglu-Spiroudi, 2011). Owing to their insecticidal activities, i.e. growth regulator, repellent, adulticidal, ovicidal and fumigant against numerous insect pests, these oils are of high importance as botanical insecticidal agents. There are a large number of components in these essential oils that can help in reducing the resistance or adaptation of insects (Regnault-Roger *et al.,* 2012; Peschiutta *et al.,* 2017).

Various studies on the insecticidal possibility of essential oils have been conducted regarding *Artemisia herba-alba, Mentha longifolia, Myrtus communis and Salvia spinosa* against various insect pests. Therefore, the essential oils of these plants have

been considered as an imperative biological source to control the different number of insect pests. The insecticidal activities of these essential oils are due to the presence of various active chemical compounds like terpenes and terpenoid (Isman, 2006; Bassolé & Juliani, 2012). Both these compounds (terpene and terpenoid) are volatile and lipophilic that can readily penetrate into insect cuticle and intervene with their physiological process (Tayoub *et al.*, 2012; Sağlam & Özder, 2013). Yet, the quantity and quality of these oils are highly affected by plant age, parts, harvesting time, extraction method and the geographical location of the plant (El-Zaeddi *et al.*, 2016). Despite the promising characteristics and advantages of these natural oils, few problems that limit or decrease the shelf-life of these oils and prevent them from becoming potential agents for pesticides still exist, such as: volatilization, rapid breakdown, high sensitivity to the light and UV, the ability for oxidation and weak water solubility (Isman & Grieneisen, 2014; Peschiutta *et al.*, 2017).

Hence, the formulated essential oil is considered as an efficient, safe and feasible approach to modulate oil release. It increases the chemical and physical stability of essential oil and decreases the interactions between the environmental factors and the active components. The formulation also makes storage, packaging and application processes easier and low cost (Markus & Linder, 2006; Chen *et al.*, 2013; Ghosh *et al.*, 2013). The formulation process of plant materials as nanoemulsion is a highly credible and effective technique to develop numerous botanical pesticides (Margulis-Goshen & Magdassi, 2013; Bilia *et al.*, 2014). The physical and chemical properties of nanoemulsion system such as high stability, decreased droplet size (20 – 200 nm) and reduced turbidity have led to expanding the utilization in different fields like agriculture, cosmetics, food and medicine (Asmawati, *et al.*, 2014; Ramar *et al.*, 2017).

Due to their thick, waxy body, found in mass-groups under leaves, cracks and the bark of plants, the papaya mealy-bug is considered as a highly difficult pest to control by traditional insecticide. Many investigations suggested that the nanotechnology has improved pesticide release, water solubility, the physicochemical stability of the active ingredients by protecting the active substances from the environmental influences and their interactions. Therefore, it increases the activity and penetration of the treated insects (Margulis-Goshen & Magdassi, 2013; São Pedro *et al*., 2013; Ghosh *et al.*, 2013). In fact, there are not any studies conducted regarding the observation of insecticidal activity of a plant's essential oil and its nanoemulsion application against P. marginatus. Hence, the present study aimed to prepare and determine the insecticidal activity of nanoemulsion formulation of *A. herba-alba, M. longifolia, M. communis* and *S. spinosa* essential oils extracted by hydrodistillation and solvent extraction through the following objectives:

1. To recognize the chemical profile of the selected plant essential oils obtained by hydrodistillation and solvent extraction methods using GC-MS technique.
2. To screen of the insecticidal activity of the selected plant essential oils obtained by hydrodistillation and solvent extraction methods against *P. marginatus*
3. To prepare the nanoemulsion formulations of essential oils and characterize the formulations
4. To determine the toxicity effects of prepared formulation against *P. marginatus*.

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LIST OF PUBLICATIONS

Aldosary, N. H., Omar, D., Awang, R. M., & Adam, A. A. (2018). Chemical Profiling and Insecticidal Activity of Artemisia herba-alba Essential Oil Against Papaya Mealybug Paracoccus marginatus (Hemiptera: Pseudococcidae). *Research Journal of Applied Sciences, Engineering and Technology*, 15(7): 261-269.

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