CONTENTS

Acknowledgements	c
Abstract in Thai	e
Abstract in English	h
List of Tables	0
List of Figures	r
List of Abbreviations	W
List of Symbols	у
Statements of Originality in Thai	Z
Statements of Originality in English	aa
Chapter 1 Introduction	1
1. General introduction	1
1.1 Statement and significance of problem	1
2. Literature review	4
2.1. Mosquitoes	4
2.2. Mosquitocidal property of plant products	7
2.3. Mosquitocidal actions of plant products	11
2.4. Petroselinum crispum, the most effective plant sample	14
3. Purposes of the study	16
4. Usefulness of the study	16
Chapter 2 Materials and Methods	17
2.1 Materials	17
2.1.1 Plant materials	17

	2.1.2 Experimental animals	19
	2.1.2.1 Mosquitoes	19
	2.1.2.2 Albino rats (Ratus ratus)	19
	2.1.3 Chemicals	20
2.2	Methods	21
	2.2.1 Mosquito rearing	21
	2.2.2 Plant preparations	22
	2.2.2.1 Preparation of crude plant extracts	22
	2.2.2.1.1 Steam distillation	22
	2.2.2.1.2 Solvent extraction	23
	2.2.3 Investigation of the insecticidal activity of plant products	23
	2.2.3.1 Preliminary screening for larvicidal activity	23
	2.2.3.2 Dose-response larvicidal bioassay	24
	2.2.3.3 Antimosquito bioassays of plant products and	
	conventional synthetic insecticides	24
	2.2.3.3.1 Larvicidal bioassay	24
	2.2.3.3.2 Adulticidal bioassay	24
	2.2.4 Determination of mosquitocidal actions of the most	
	Co effective plant by Chiang Mai University	25
	2.2.4.1 Behavioral response observation	25
	2.2.4.2 Physical change observation	26
	2.2.4.2.1 Light microscopic (LM) study	26
	2.2.4.2.2 Scanning electron microscopic (SEM) study	26
	2.2.4.3 Biochemical change observation	27
	2.2.4.3.1 Determination of lethal threshold time for	27
	mortality of larvae and adults	27
	2.2.4.3.2 Preparation of whole body homogenates	27

2.2.4.3.3 Determination of protein concentrations	28
2.2.4.3.4 Determination of enzyme activity	28
2.2.4.3.4.1 Glutathione S-transferases (GSTs)	28
2.2.4.3.4.2 Esterases (α - and β -esterases)	29
2.2.4.3.4.3 Mixed-function oxidases (MFO)	29
2.2.4.3.4.4 Acid and alkaline phosphatases	30
(ACP and ALK)	
2.2.4.3.4.5 Acetylcholinesterase (AChE)	31
2.2.5 Gas chromatography-mass spectrometry (GC-MS) analysis	31
2.2.6 Statistical analysis	33
Chapter 3 Results	34
3.1 Preparation of plant products	34
3.2 Investigation of insecticidal activity of plant products	36
3.2.1 Preliminary screening for larvicidal activity against	
Ae. aegypti	36
3.2.2 Dose-response larvicidal bioassay	36
3.2.3 Antimosquito bioassays of P. crispum oil and synthetic	
insecticidal compounds	39
3.2.3.1 Larvicidal bioassay	39
3.2.3.2 Adulticidal bioassay	39
3.3 Determination of mosquitocidal actions of P. crispum oil	48
3.3.1 Behavioral response observation	48
3.3.1.1 Larvae	48
3.3.1.2 Adults	48
3.3.2 Physical change observation	49

3.3.2.1 Light microscopic (LM) study	49
3.3.2.1.1 Larvae	49
3.3.2.1.2 Adults	50
3.3.2.2 Scanning electron microscopic (SEM) study	52
3.3.2.2.1 Larvae	52
3.3.2.2.2 Adults	52
3.3.3 Biochemical change observation	57
3.3.3.1 Biochemical assay on mosquito larvae	57
3.3.3.1.1 Enzyme activity in untreated larvae	
(0-h time point)	57
3.3.3.1.2 Threshold time for lethal effect of <i>P. crispum</i>	
oil on larvae	58
3.3.3.1.3 Effects of P. crispum oil on biochemical	
changes in larvae	59
3.3.3.1.3.1 GSTs activity	59
3.3.3.1.3.2 Esterases activity	61
3.3.3.1.3.3 MFO activity	64
3.3.3.1.3.4 ACP and ALK activity	65
3.3.3.1.3.5 AChE activity	68
3.3.3.2 Biochemical assay on mosquito adults	69
3.3.3.2.1 Enzyme activity in untreated adults	
(0-h time point)	69
3.3.3.2.2 Threshold time for lethal effect of <i>P. crispum</i>	
oil on adults	71

Page

3.3.3.2.3 Effects of P. crispum oil on biochemical	
changes in adults	72
3.3.3.2.3.1 GSTs activity	72
3.3.3.2.3.2 Esterases activity	73
3.3.3.2.3.3 MFO activity	76
3.3.3.2.3.4 ACP and ALK activity	78
3.3.3.2.3.5 AChE activity	80
3.4 Chemical composition of <i>P. crispum</i> oil	82
Chapter 4 Discussion	84
Chapter 5 Conclusion	99
References	100
List of Publication	120
Appendix	121
Curriculum Vitae	130
Copyright [©] by Chiang Mai Universit	y
All rights reserve	d

LIST OF TABLES

Table 2.1	Seventeen plants selected for preliminary larvicidal screening against	
	the pyrethroid-susceptible (MCM-S) strain of Ae. aegypti	18
Table 3.1	Percentage yield (% Yield), physical characteristics (color, phase,	
	and density) of plant products, including essential oils and	
	ethanolic extracts	35
Table 3.2	Larvicidal activity of plant products, including essential oils	
	and ethanolic extracts, against the pyrethroid susceptible	
	(MCM-S) strain of Ae. aegypti	37
Table 3.3	Larvicidal activity of plant products derived from six selected	
	plant species against the pyrethroid susceptible (MCM-S)	
	strain of Ae aegypti	38
Table 3.4	Larvicidal activity of P. crispum oil against the pyrethroid	
	susceptible (MCM-S) and resistant (PMD-R and UPK-R)	
	strains of Ae. aegypti	40
Table 3.5	Larvicidal activity of temephos, permethrin, and deltamethrin	
	against the pyrethroid susceptible (MCM-S) and resistant	
	(PMD-R and UPK-R) strains of Ae. aegypti	41
Table 3.6	Adulticidal activity of <i>P. crispum</i> oil against the pyrethroid	
	susceptible (MCM-S) and resistant (PMD-R and UPK-R)	
	strains of Ae. aegypti	42

LIST OF TABLES (continued)

Table 3.7	Adulticidal activity of permethrin and deltamethrin against	
	the pyrethroid susceptible (MCM-S) and resistant	
	(PMD-R and UPK-R) strains of Ae. aegypti	43
Table 3.8	Behavioral changes of the pyrethroid susceptible (MCM-S)	
	and resistant (PMD-R and UPK-R) strains of Ae. aegypti	
	larvae after treatment with LC ₉₉ of <i>P. crispum</i> oil	49
Table 3.9	GSTs activity in larvae of Ae. aegypti, pyrethroid-susceptible	
	(MCM-S) and resistant (PMD-R and UPK-R) strains	60
Table 3.10	α -Esterase activity in larvae of Ae. aegypti, pyrethroid-susceptible	
	(MCM-S) and resistant (PMD-R and UPK-R) strains	62
Table 3.11	β -Esterase activity in larvae of <i>Ae. aegypti</i> , pyrethroid-susceptible	
	(MCM-S) and resistant (PMD-R and UPK-R) strains	63
Table 3.12	MFO activity in larvae of Ae. aegypti, pyrethroid-susceptible	
	(MCM-S) and resistant (PMD-R and UPK-R) strains	64
Table 3.13	ACP activity in larvae of Ae. aegypti, pyrethroid-susceptible	
/	(MCM-S) and resistant (PMD-R and UPK-R) strains	66
Table 3.14	ALK activity in larvae of Ae. aegypti, pyrethroid-susceptible	
	(MCM-S) and resistant (PMD-R and UPK-R) strains	67

LIST OF TABLES (continued)

Table 3.15	AChE activity in larvae of Ae. aegypti, pyrethroid-susceptible	
	(MCM-S) and resistant (PMD-R and UPK-R) strains	68
Table 3.16	GSTs activity in adults of Ae. aegypti, pyrethroid-susceptible	
	(MCM-S) and resistant (PMD-R and UPK-R) strains	72
Table 3.17	α -Esterase activity in adults of <i>Ae. aegypti</i> , pyrethroid-susceptible	
	(MCM-S) and resistant (PMD-R and UPK-R) strains	74
Table 3.18	β -Esterase activity in adults of Ae. aegypti, pyrethroid-susceptible	
	(MCM-S) and resistant (PMD-R and UPK-R) strains	75
Table 3.19	MFO activity in adults of Ae. aegypti, pyrethroid-susceptible	
	(MCM-S) and resistant (PMD-R and UPK-R) strains	76
Table 3.20	ACP activity in adults of Ae. aegypti, pyrethroid-susceptible	
	(MCM-S) and resistant (PMD-R and UPK-R) strains	78
Table 3.21	ALK activity in adults of Ae. aegypti, pyrethroid-susceptible	
	(MCM-S) and resistant (PMD-R and UPK-R) strains	79
Table 3.22	AChE activity in adults of Ae. aegypti, pyrethroid-susceptible	
	(MCM-S) and resistant (PMD-R and UPK-R) strains	81
Table 3.23	Chemical constituents of P. crispum fruit oil	82

LIST OF FIGURES

Figure 1.1	The Aedes mosquito life cycle	5
Figure 1.2	Petroselinum crispum (Mill.) A.W. Hill fruits	15
Figure 3.1	Larvicidal activity of P. crispum oil against the pyrethroid	
	susceptible (MCM-S) and resistant (PMD-R and UPK-R)	
	strains of Ae. aegypti	44
Figure 3.2	Larvicidal activity of temephos, permethrin, and deltamethrin	
	against the pyrethroid susceptible (MCM-S) and resistant	
	(PMD-R and UPK-R) strains of Ae. aegypti	45
Figure 3.3	Adulticidal activity of P. crispum oil against the pyrethroid	
	susceptible (MCM-S) and resistant (PMD-R and UPK-R)	
	strains of Ae. aegypti	46
Figure 3.4	Adulticidal activity of permethrin, and deltamethrin against	
	the pyrethroid susceptible (MCM-S) and resistant	
	(PMD-R and UPK-R) strains of Ae. aegypti	47
Figure 3.5	Light micrograph of body segments and anal gills of	
	Ae. aegypti larvae	50
Figure 3.6	Light micrograph of body segments of Ae. aegypti adult females	51
Figure 3.7	Scanning electron micrograph of body segments and	
	anal gills of Ae. aegypti larvae	53

		Page
Figure 3.8	Scanning electron micrograph of body segments of Ae. aegypti	
	adults after dead for 12 h showing normal appearance in treated	
	adult with compared to those of control	54
Figure 3.9	Scanning electron micrograph of body segments of Ae. aegypti	
	adults after dead for 24 h showing normal appearance in treated	
	adult with compared to those of control	55
Figure 3.10	Scanning electron micrograph of body segments of Ae. aegypti	
	adults after dead for 36 h showing normal appearance in treated	
	adult with compared to those of control	56
Figure 3.11	l Enzyme activity in the whole body homogenates of the fourth	
	instar larvae pyrethroid-susceptible (MCM-S) and resistant	
	(PMD-R and UPK-R) strains (untreated group, 0-h time point)	58
Figure 3.12	2 Determination of threshold time for lethal effect of <i>P. crispum</i> oil	
	on fourth instar larvae with median lethal concentration (LC $_{50}$)	
	against the pyrethroid susceptible (MCM-S) and resistant	
	(PMD-R and UPK-R) strains of Ae. aegypti	59
Eiguro 2 12	3 GSTs activity in the whole body homogenates of the fourth	
Figure 5.13		
	instar larvae pyrethroid-susceptible (MCM-S) and resistant	
	(PMD-R and UPK-R) strains exposed to <i>P. crispum</i> oil (LC ₅₀)	- 1
	after 3, 6, and 24 h	61

Page

Figure 3.14 α -Esterase activity in the whole body homogenates of the fourth	
instar larvae pyrethroid-susceptible (MCM-S) and resistant	
(PMD-R and UPK-R) strains exposed to P. crispum oil (LC ₅₀)	
after 3, 6, and 24 h	62
Figure 3.15 β -Esterase activity in the whole body homogenates of the fourth	
instar larvae pyrethroid-susceptible (MCM-S) and resistant	
(PMD-R and UPK-R) strains exposed to P. crispum oil (LC ₅₀)	
after 3, 6, and 24 h	63
Figure 3.16 MFO activity in the whole body homogenates of the fourth instar	
larvae pyrethroid-susceptible (MCM-S) and resistant (PMD-R and	
UPK-R) strains exposed to <i>P. crispum</i> oil (LC ₅₀) after 3, 6, and 24 h	65
Figure 3.17 ACP activity in the whole body homogenates of the fourth instar	
larvae pyrethroid-susceptible (MCM-S) and resistant (PMD-R and	
UPK-R) strains exposed to P. crispum oil (LC ₅₀) after 3, 6, and 24 h	66
Figure 3.18 ALK activity in the whole body homogenates of the fourth instar	
larvae pyrethroid-susceptible (MCM-S) and resistant (PMD-R and	
UPK-R) strains exposed to <i>P. crispum</i> oil (LC ₅₀) after 3, 6, and 24 h	67
Figure 3.19 AChE activity in the whole body homogenates of the fourth instar	
larvae pyrethroid-susceptible (MCM-S) and resistant (PMD-R and	
UPK-R) strains exposed to P. crispum oil (LC50) after 3, 6, and 24 h	69

t

Page

Figure 3.20 Enzyme activity in the whole body homogenates of the adults	
pyrethroid-susceptible (MCM-S) and resistant (PMD-R and UPK-R)	
strains (untreated group, 0-h time point)	70
Figure 3.21 Determination of threshold time for lethal effect of <i>P. crispum</i> oil	
on adults with median lethal dose (LD_{50}) against the pyrethroid	
susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains	
of Ae. aegypti	71
Figure 3.22 GSTs activity in the whole body homogenates of the adults	
pyrethroid-susceptible (MCM-S) and resistant (PMD-R	
and UPK-R) strains exposed to P. crispum oil (LD ₅₀)	
after 24 and 48 h	73
Figure 3.23 α -Esterase activity in the whole body homogenates of the adult	
pyrethroid-susceptible (MCM-S) and resistant (PMD-R	
and UPK-R) strains exposed to P. crispum oil (LD ₅₀)	
after 24 and 48 h Chiang Mai University	74
Figure 3.24 β -Esterase activity in the whole body homogenates of the adults	
pyrethroid-susceptible (MCM-S) and resistant (PMD-R	
and UPK-R) strains exposed to P. crispum oil (LD ₅₀)	
after 24 and 48 h	75

Page

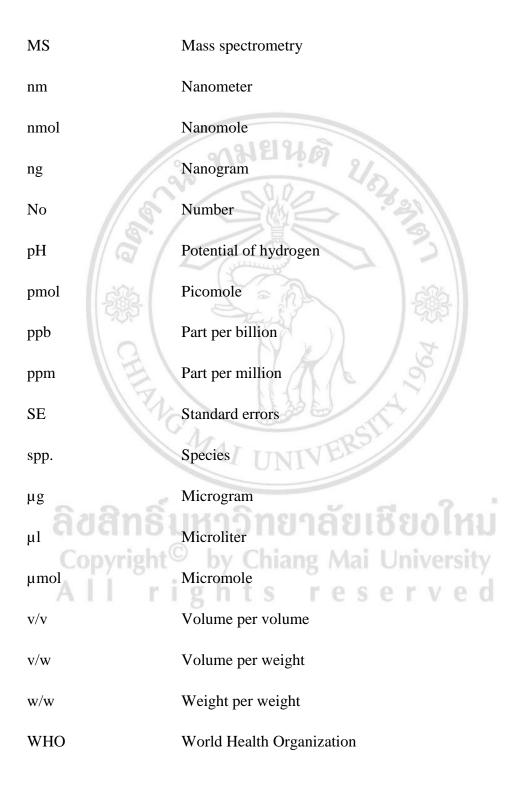
Figure 3.25 MFO activity in the whole body homogenates of the adults pyrethroid-	
susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains	
exposed to P. crispum oil (LD ₅₀) after 24 and 48 h	77
Figure 3.26 ACP activity in the whole body homogenates of the adult pyrethroid-	
susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains	
exposed to P. crispum oil (LD ₅₀) after 24 and 48 h	79
Figure 3.27 ALK activity in the whole body homogenates of the adult pyrethroid-	
susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains	
exposed to P. crispum oil (LD ₅₀) after 24 and 48 h	80
Figure 3.28 AChE activity in the whole body homogenates of the adult pyrethroid-	
susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains	
exposed to P. crispum oil (LD ₅₀) after 24 and 48 h	81
Figure 3.29 GC/MS total ion chromatograms of <i>P. crispum</i> fruit oil	83
ลิขสิทธิ์มหาวิทยาลัยเชียงไหม	
Copyright [©] by Chiang Mai University	
All rights reserved	

v

LIST OF ABBREVIATIONS

cm	Centimeter
CMU	Chiang Mai University
e.g.	For example
et al.	And others et cetera
etc.	et cetera
GC	Gas chromatography
h See	Hour
L	Liter
LC ₅₀	Median lethal concentration
LD ₅₀	Median lethal dose
М	Mole UNIVERSIT
mg	Milligram
adansı	Minute
	⁹ by Chiang Mai University ^{Milliliter} gnts reserved
mm	Millimeter
mmol	Millimole
mM	Millimolar

LIST OF ABBREVIATIONS (continued)



LIST OF SYMBOLS



ข้อความแห่งการริเริ่ม

 วิทยานิพนธ์นี้ได้รายงานครั้งแรกถึงฤทธิ์ฆ่ายุงของน้ำมันหอมระเหยจากเทียนเยาวพาณี (*Petroselinum crispum*) ต่อยุงลายสายพันธุ์ที่ไวต่อไพรีทรอยด์ (MCM-S) และดื้อต่อไพรีทรอยด์ (PMD-R และ UPK-R)

 วิทยานิพนธ์นี้ได้ด้นพบน้ำมันหอมระเหยเทียนเยาวพาณีที่มีประสิทธิภาพในการฆ่ายุงในระยะ ลูกน้ำและตัวเต็มวัยในยุงสายพันธุ์ที่ไวและดื้อต่อไพรีทรอยด์

 วิทยานิพนธ์นี้ได้รายงานบทบาทของน้ำมันหอมระเหยเทียนเยาวพาณีต่อการเปลี่ยนแปลงทั้ง ทางด้านพฤติกรรม สัณฐานวิทยาและชีวเกมีในยุงลายสายพันธุ์ที่ไวและดื้อต่อไพรีทรอยด์



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved

STATEMENTS OF ORIGINALITY

1. In this thesis, the mosquitocidal potential of *Petroselinum crispum* oil was reported for the first time against the pyrethroid susceptible (MCM-S) and resistant (PMD-R and UPK-R) strains of *Aedes aegypti*.

2. In this thesis, *P. crispum* oil was found to be effective in killing mosquitoes, larvae and adults against the pyrethroid susceptible and resistant strains of *Ae. aegypti*.

3. In this thesis, *P. crispum* oil was reported for behavioral response, physical performance, and biochemical constituents of target insects in the pyrethroid susceptible and resistant strains of *Ae. aegypti*.



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved