

สารออกฤทธิ์ทางชีวภาพที่ฝังงานใช้ในการขับไล่แมด

นางสาวอรรวรรณ ดวงภักดี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต  
สาขาวิทยาศาสตร์ชีวภาพ  
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย  
ปีการศึกษา 2549  
ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

**BIOLOGICAL ACTIVE COMPOUNDS USED BY  
WORKER BEES TO REPEL ANTS**

**Miss Orawan Daungphakdee**

**A Dissertation Submitted in Partial Fulfillment of the Requirements  
for the Degree of Doctor of Philosophy Program in Biological Sciences**

**Faculty of Science**

**Chulalongkorn University**

**Academic Year 2006**


**Copyright of Chulalongkorn University**

**490464**

Thesis Title            BIOLOGICAL ACTIVE COMPOUNDS USED BY  
                                  WORKER BEES TO REPEL ANTS  
By                            Miss Orawan Daungphakdee  
Field of study            Biological Sciences  
Thesis Advisor          Professor Siriwat Wongsiri, Ph.D.  
Thesis Co-advisor      Professor Nikolaus Koeniger, Ph.D.


---


Accepted by the Faculty of Science, Chulalongkorn University in Partial  
Fulfillment of the Requirements for the Doctoral Degree


  
.....Dean of the Faculty of Science  
(Professor Piamsak Menasveta, Ph.D.)


#### THESIS COMMITTEE

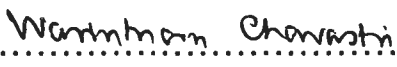
  
.....Chairman  
(Assistant Professor Kumthorn Thirakhupt, Ph.D.)

  
.....Thesis Advisor  
(Professor Siriwat Wongsiri, Ph.D.)

  
.....Thesis Co-advisor  
(Professor Nikolaus Koeniger, Ph. D.)

  
.....Member  
(Assistant Professor Sureerat Deowanish, D.Agr.)

  
.....Member  
(Assistant Professor Pongtharin Lotrakul, Ph. D.)

  
.....Member  
(Assistant Professor Warinthorn Chavasiri, Ph.D.)

  
.....Member  
(Assistant Professor Apichai Daorai, Ph.D.)

อรรวรรณ ดวงภักดี: สารออกฤทธิ์ทางชีวภาพที่ผึ้งงานใช้ในการขับไล่มด

(BIOLOGICAL ACTIVE COMPOUNDS USED BY WORKER BEES TO REPEL ANTS)

อ. ที่ปรึกษา : ศ. ดร. สิริวัฒน์ วงษ์ศิริ, อ. ที่ปรึกษาร่วม : Prof. Dr. Nikolaus Koeniger 153 หน้า.

ผึ้งสังคมใช้ยางไม้หรือพอลิฟีนอลเป็นสารสำคัญในการป้องกันรัง ผึ้งพันธุ์ยุโรป (*Apis mellifera*) ป้องกันการบุกรุกจากศัตรูโดยใช้ยางไม้ในการปิดรอยแตกหรือรอยแยกภายในรัง ผึ้งมี (*Apis florea*) และผึ้งมีเล็ก (*Apis andreniformis*) สร้างแถบเหนียวด้วยยางไม้หุ้มบริเวณกึ่งไม้ที่ทำรังทั้งสองข้าง ส่วนชันโรงใช้ยางไม้ทาบริเวณผิวรังด้านในของปากทางเข้ารังเพื่อป้องกันการเข้าไปรบกวนของมด มีรายงานการวิจัยมากมายนำเสนอว่ายางไม้ที่ใช้ในรังของผึ้งเป็นสารที่มีคุณสมบัติต่อต้านเชื้อโรครภายในรัง เช่น เชื้อแบคทีเรีย เชื้อราและเชื้อไวรัส อย่างไรก็ตามยังไม่มีรายงานเกี่ยวกับคุณสมบัติในด้านการขับไล่มด

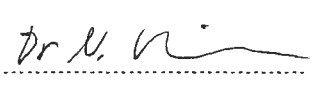
จากการศึกษาพบว่าการนำมดแดงพันธุ์เอเชีย (Asian weaver ants, *Oecophylla smaragdina*) ไล่เข้าไปในรังผึ้งมีมดจะกระตุ้นให้ผึ้งแสดงออกถึงพฤติกรรมการตอบสนองที่เฉพาะเกิดขึ้น โดยหลังจากการปรากฏของมดงานบริเวณด้านบนของรวงรัง จำนวนผึ้งงานในบริเวณแถบเหนียวจะเพิ่มขึ้นอย่างมีนัยสำคัญและยังคงอยู่จนถึงสองชั่วโมงในบริเวณนั้น นอกจากนี้ยังพบว่าปริมาณสารที่ผึ้งใส่เข้าไปในบริเวณแถบเหนียวก็เพิ่มขึ้นเช่นกัน แต่ผึ้งไม่มีการตอบสนองดังกล่าวต่อ ปากติบ ตัวอ่อนหนอนนก (*Tenebrio molitor*) หรือมดอีกชนิดหนึ่งที่สร้างรังบนต้นไม้ คือ *Crematogaster rogenhoferi*

การทดสอบทางชีวภาพได้ถูกพัฒนาเพื่อเปรียบเทียบประสิทธิภาพของสารประเภทยางไม้ที่ใช้ในรังผึ้ง ในการขับไล่มดสองชนิดคือ มดแดงพันธุ์เอเชีย (Asian weaver ant, *O. smaragdina*) และมดพันธุ์ยุโรป (European red wood ant, *Formica polyctena*) โดยเก็บตัวอย่างสารประเภทยางไม้จากรังผึ้ง 3 ชนิดจากสกุล *Apis* และอีก 6 ชนิดจากสกุล *Trigona* โดยทดลองกับมดในสองห้องถิ่นทางภูมิศาสตร์ที่ห่างไกลกัน คือ เอเชียตะวันออกเฉียงใต้ (ไทยและมาเลเซีย) และยุโรป (เยอรมัน) ผลการศึกษาพบว่า ในภูมิภาคเขตร้อน สารจากรังผึ้งพันธุ์เอเชียมีประสิทธิภาพการขับไล่มดแดงพันธุ์เอเชียสูงกว่ามดพันธุ์ยุโรปอย่างมีนัยสำคัญ ในทางกลับกันสารจากรังผึ้งพันธุ์ยุโรปก็มีประสิทธิภาพไล่มดพันธุ์ยุโรปได้ดีกว่าสารจากผึ้งในเอเชีย

ผลการศึกษาพบว่าสารสกัดเพนแทนที่มีส่วนประกอบของสารขับไล่มด จึงได้ทำการแยกต่อด้วยวิธีโครมาโทกราฟีแบบคอลัมน์ จากนั้นได้ทำการทดสอบทางชีวภาพในแต่ละส่วนย่อยที่แยกได้เพื่อหาสารขับไล่มด ผลการวิเคราะห์องค์ประกอบในส่วนย่อยที่แสดงฤทธิ์โดยใช้ GC-MS แล้วเปรียบเทียบกับสเปกตรัมในฐานข้อมูลในเครื่องพบว่าประกอบด้วย สาร 4 กลุ่ม คือ เทอร์ปีนอยด์ ไฮโดรคาร์บอนโซ่ยาว อนุพันธ์ของฟีนอล และอนุพันธ์ของแนพทาเลน และยังมีสารอีกประมาณ 7 ชนิดที่พบในปริมาณมากอย่างมีนัยสำคัญแต่ยังไม่สามารถระบุชนิดได้

สาขาวิชา ..... วิทยาศาสตร์ชีวภาพ ..... ลายมือชื่อนิสิต 

ปีการศึกษา ..... 2549 ..... ลายมือชื่ออาจารย์ที่ปรึกษา 

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม 

# # 457 38520 23: MAJOR BIOLOGICAL SCIENCES

KEY WORDS: BIOLOGICAL ACTIVE COMPOUNDS / WORKER BEES / SOCIAL BEES / RESINOUS MATERIAL / REPEL / ANTS

ORAWAN DUANGPHAKDEE: BIOLOGICAL ACTIVE COMPOUNDS USED BY WORKER BEES TO REPEL ANTS. THESIS ADVISOR: PROF. SIRIWAT WONGSIRI, Ph. D., THESIS CO-ADVISOR: PROF. NIKOLAUS KOENIGER, Ph. D., 153 pp.

The use of resin or propolis forms an important part of the social bees's nest defense systems. Cracks and other small openings are sealed by propolis in the hive of the European honey bees, *Apis mellifera*, to prevent intruders. The dwarf honey bee, *Apis florea*, and the small dwarf honey bee, *Apis andreniformis*, build sticky resin rings around the branch at each side of the nest. The stingless bees apply this resin to the inner surface of the entrance tube to prevent invasion ants. There are substantive number of publications indicating that bees use propolis in defense against diseases cause agent as it has antibiotic, antifungal and antiviral properties. However, its effectiveness as a class of ant repellent is questionable, and has not been reported yet.

This study showed that the introduction of the Asian weaver ant, *Oecophylla smaragdina*, on the comb of *A. florea* induces a specific behavioral response of the worker bees. After worker ant exposure at the top of the comb, the number of worker bees on the sticky band zone increased significantly and remained at higher level for 2 hours. Additionally, more sticky material was deposited by the bees after ant exposure. This behavior was not observed after exposure of an empty forceps, a *Tenebrio molitor* larva or an another arboreal predatory ant, *Crematogaster rogenhoferi*.

A biotest was developed to compare the reaction of the Asian weaver ants, *O. smaragdina* and of the European red wood ant, *Formica polyctena* to the repellent compounds of resin material from various social bee species (three species from the genus *Apis*, six species from the genus *Trigona*). Tests were performed in two different geographic regions, in Southeast Asia (Thailand, Malaysia) and in Europe (Germany). In the tropical region, the repellent efficacy of the Asian bee material on Asian weaver ants was significantly stronger than that observed on the European red wood ant, *F. polyctena*, and *visa versa*. That is, the material from nest of the European honey bee, *A. mellifera* showed a higher repellent effect upon the European red wood ant, than did the material; from the nests of the Asian bees.

Pentane extracts of the resin were found to contain ant repellent compounds and these were further analyzed by partial purification using column chromatography. Fractions from column chromatography was assayed in the above biotest for ant repellent activity. Positive fractions for this activity were then characterized by GC-MS. Their mass spectra were taken and compared with an existant spectrum library. As a result, four chemical groups were identified: terpenoids, long chained hydrocarbons, phenol derivatives and naphthalene derivatives. Moreover, about seven compounds which were found in significant quantities in the positive fractions could not be identified by comparison to the spectra contained within the reference library.

Field of study ..... Biological Sciences ..... Student's signature ..... *Orawan Duangphakdee*  
 Academic year ..... 2006 ..... Advisor's signature ..... *Siriwat Wongsiri*  
 Co - advisor's signature ..... *Nikolaus Koeniger*

## Acknowledgements

I specifically designed to note my appreciation to those people who stand out most notably in my mind, my advisor Prof. Dr. Siriwat Wongsiri and my co-advisor Prof. Dr. Nikolaus Koeniger, for their kind help, guidance, support and encouragement throughout my study.

I expressed my gratitude to Asst. Prof. Dr. Kumthorn Thirakhupt, Asst. Prof. Dr. Sureerat Deowanish, Asst. Prof. Dr. Apichai Daorai, Asst. Prof. Dr. Warinthorn Chavasiri and Asst. Prof. Dr. Pongtharin Lotrakul for being a member of the dissertation committee.

I have the invaluable contribution and support from many institutions and member who encouraged my effort since the beginning. My truly thanks to Assoc. Prof. Chariya Lekprayoon, Asst. Prof. Dr. Sureerat Deowanish, Mr. Marut Fuangarworn, Ms. Ezra Mongkolchaichana from Center of Excellence in Entomology: Bee Biology, Biodiversity of Insects and Mites, Chulalongkorn University for their collaboration and significant assistance. I had great time in Institut für Bienenkunde (Polytechnische Gesellschaft), Fachbereich Biowissenschaften der J.W.Goethe-Universität Frankfurt am Main, Germany. Dr. Gudrun Koeniger is greatly acknowledged for her proofreading assistance and support to the research during the staying at institute. I sincerely thanks Prof. Dr. Ulrich Maschwitz and Prof. Dr. Stefan Fuchs for valuable suggestions and improvements of earlier versions of the manuscript. I wish to particularly thanks to Assist. Prof. Dr. Warinthorn Chavasiri from Natural Products Research Unit (NPRU), Department of Chemistry, Chulalongkorn University for providing facilities and technical advice in chemical separation. My appreciation is also extended to Dr. Volker Witte from Division of Evolutionary Ecology, Department Biology II, Ludwig-Maximilians-Universität München, Germany who has contributed his time and effort in assisting me in the chemical identification (GC-MS). Thanks to Mr. Salim Tingek and his collaborators from Agricultural Research Station, Lagud Seberang, Sabah, Malaysia for supported me generously and kindly co-operation about experiments at Tenom. Agricultural Research Station, Chantaburi, Thailand is also be acknowledged for sharing the facilities during several weeks of experiments. Thanks also the members from those institutions for their collaboration and significant assistance.

I would like to deeply thank Prof. Dr. Charles D. Michener who assisted with species identification of *Trigona* spp. And also thanks to Mr. Yhon Nonchan for collecting *Apis florea* and *Oecophylla smaragdina* nests. Mr. Sawang Piyaphichat is also acknowledged for collecting the propolis from *A. mellifera* in Thailand.

Thanks as well as the funding provided by Financial support the Thailand Research Fund through Royal Golden Jubilee project (No. PHD/0222/2545) and Chulalongkorn University Graduate Scholarship Commemorate The 72<sup>nd</sup> Anniversary of H.M. King Rama IX for the financial support at the beginning of my study.

There are many people behind the scenes who have encouraged and supported my work, and I wish to thank them for their warmth, friendship and kindly supports. They are Dr. Mananya Phiancharoen, Pratak Sawatpon, Pannarai Wuthipanyarattanakun, Atsalek Rattanawanee, Jasna Kralj, Sittipong Wongvilas, Christian Fierber, Orawan Kongpet, Teernud Yatkrotok and Tobias Eckrich and his parents, Friedrich & Reuate Eckrich. Without their care and consideration, this thesis would likely not have matured.

It is regretful that I could not mention and do justice to all people who reflected the contributions of this thesis. Therefore, their roles at least be acknowledged here.

There is one main in my life that urged me on by way of their untiring support and seemingly unlimited belief in me. Thank you my parents, my sister and my brother.

## Table of Contents

	<b>Page</b>
Thai Abstract.....	iv
English Abstract.....	v
Acknowledgements.....	vi
Table of Contents.....	vii
List of Tables.....	ix
List of Figures.....	x
CHAPTER I Introduction.....	1
CHAPTER II Literature review.....	5
CHAPTER III Social Defense of the dwarf honeybee ( <i>Apis florea</i> ) released by the weaver ant ( <i>Oecophylla smaragdina</i> ).....	27
3.1 Introduction.....	28
3.2 Materials and methods.....	29
3.2.1 The bees.....	29
3.2.2 The ants.....	30
3.2.3 Presentation of different objects to the <i>Apis florea</i> colony.....	30
3.2.4 Number of bees in sticky band zone.....	31
3.2.5 Deposition of material in sticky band zone.....	31
3.3 Results.....	32
3.3.1 Behavior of bees towards different objects presented on the upper side of the comb.....	32
3.3.2 Number of bees at the sticky band zone.....	33
3.3.3 Behavior at sticky band after presentation of <i>Oecophylla smaragdina</i> .....	35
3.3.4 Depositions in sticky band zone.....	35
3.4 Discussion.....	36
CHAPTER IV Biological activity of bee material against ants.....	39
4.1 Introduction.....	40
4.2 The Bioassay, repellent index and statistical analysis	
4.2.1 The bioassay .....	41

	<b>Page</b>
4.2.2 The general procedure to keep the colony of red wood ants, <i>F. polycтена</i> .....	43
4.2.3 The index.....	44
4.2.4 Statistical analysis.....	45
4.3 Material and methods.....	46
4.3.1 The ants and locations of experiments.....	46
4.3.2 Samples collected from bee nests.....	48
Samples from nests of Genus <i>Apis</i> .....	48
Nest entrance of <i>Trigona</i> .....	50
4.3.3 Preparation of the experimental samples.....	56
4.3.4 Isolation of active fraction.....	57
4.3.5 Characterization of compounds by Gas chromatography–mass spectrometry (GC-MS).....	62
4.4 Results.....	64
4.4.1 Effect of material used by bees at the nest entrance on <i>O. smaragdina</i> .....	64
The biological activity of raw material.....	64
Tests of fractions on repellent activity.....	80
A. The extracts from Soxhlet.....	80
B. Chromatographed fractions .....	84
4.4.2 Repellent activity of bee material on red wood ants, <i>F. polycтена</i> .....	92
The biological activity of raw material.....	92
Tests of fractions on repellent activity.....	101
A. Extracts from Soxhlet.....	101
B. Chromatographed fractions.....	103
4.4.3 The constituents in active fractions.....	107
4.5 Discussion.....	118
CHAPTER V Conclusions.....	134
References.....	138
Biography.....	153



## List of Tables

	Page
<b>Table 2.1</b> Compounds analyzed from resinous material collected from nests of honeybee, <i>A. mellifera</i> and stingless bee, <i>Frieseomelitta</i> spp., and of different regions.....	24
<b>Table 4.1</b> The definition of Repellent index (R) value.....	45
<b>Table 4.2</b> Test ant nest description and experimental locations.....	47
<b>Table 4.3</b> The list of bee materials in experiments.....	56
<b>Table 4.4</b> The eluent of the separation in each species (MeOH=methanol, CH <sub>2</sub> CL <sub>2</sub> = dichloromethane, EtOAc= ethyl acetate).....	62
<b>Table 4.5</b> The active fractions identified in this study.....	63
<b>Table 4.6</b> The comparison of repellent indices within species and geographic region (Thailand, Malaysia).....	79
<b>Table 4.7</b> The repellent indices of bee material. The Location of experiments were conducted at Thailand (THA) and Malaysia (MAS).....	81
<b>Table 4.8</b> The comparison of repellent indices from different extracts within species (Kruskal Wallis-tested).....	84
<b>Table 4.9</b> The repellent indices resulted from the different chromatographed fractions.....	91
<b>Table 4.10</b> The comparison of repellent indices within species (Kruskal Wallis-tested)....	100
<b>Table 4.11</b> The repellent indices of bee material.....	101
<b>Table 4.12</b> Characterized compounds from fraction exhibited repellent activity separated from samples collected from bee nests.....	117
<b>Table 4.13</b> Comparative study of social bee's defensive strategies.....	120

## List of Figures

	Page
<b>Figure 1.1</b> Weaver ant workers carried the honey bee worker, <i>A. florea</i> .....	1
<b>Figure 2.1</b> The weaver ant, <i>O. smaragdina</i> , nest.....	6
<b>Figure 2.2</b> The red wood ant, <i>F. polyctena</i> , nest.....	8
<b>Figure 2.3</b> The <i>A. florea</i> nest.....	13
<b>Figure 2.4</b> The <i>A. andreniformis</i> nest.....	14
<b>Figure 2.5</b> The <i>A. mellifera</i> colony.....	15
<b>Figure 2.6</b> Composite cladogram showing relationships of taxa of Aculeata which should be recognized at the family level (Brothers, 1999).....	16
<b>Figure 2.7</b> A schematic of the evolution of ants (Formicidae) at the subfamily level (Wilson and Hölldobler, 2005).....	17
<b>Figure 2.8</b> Phylogeny of the genus <i>Apis</i> (Oldroyd and Wongsiri, 2006).....	20
<b>Figure 3.1</b> The weaver ant worker stuck on the sticky band of <i>A. florea</i> .....	29
<b>Figure 3.2</b> The weaver ant worker was held by the forceps and presented on the top of <i>A. florea</i> 's colony.....	31
<b>Figure 3.3</b> The double layers of plastic band were fixed on the sticky band zone.....	32
<b>Figure 3.4</b> Increase of bees counted at the sticky band zone after presentation of weaver ant worker.....	34
<b>Figure 3.5</b> Increase of bees counted at the sticky band zone after presentation of different stimuli.....	34
<b>Figure 3.6</b> Deposition of material in sticky band zone.....	36
<b>Figure 4.1</b> The tests ants were trained to the stick and cross over the bridge to collect food offered on feeding arena.....	41
<b>Figure 4.2</b> Sequences of the assay.....	43
<b>Figure 4.3</b> The 1.5 cm gap between stick and the bridge in purpose to prevent the ant form multiple chains over the material bridge in the experiment with <i>O. smaragdina</i> .....	43
<b>Figure 4.4</b> The locations of tested weaver ant nests in Thailand .....	47
<b>Figure 4.5</b> The sticky band of <i>A. florea</i> .....	49
<b>Figure 4.6</b> The propolis from <i>A. mellifera</i> .....	49
<b>Figure 4.7</b> Nest entrance of <i>T. apicalis</i> in Uthaitani, Thailand .....	52
<b>Figure 4.8</b> Nest entrance of <i>T. collina</i> in Kanchanaburi, Thailand .....	52
<b>Figure 4.9</b> Nest entrance of <i>T. terminata</i> in Chantaburi, Thailand.....	53

	<b>Page</b>
<b>Figure 4.10</b> Nest entrance of <i>T. terminata</i> in Tenom, Malaysia .....	53
<b>Figure 4.11</b> Nest entrance of <i>T. melanocephala</i> in Tenom, Malaysia .....	54
<b>Figure 4.12</b> Nest entrance of <i>T. laeviceps</i> in Tenom, Malaysia .....	54
<b>Figure 4.13</b> Nest entrance of <i>T. minor</i> in Chantaburi, Thailand .....	55
<b>Figure 4.14</b> The bridges covered with the resinous material from <i>T. terminata</i> collected from Thailand (A) and <i>T. terminata</i> collected from Malaysia.....	56
<b>Figure 4.15</b> The bridges from <i>A. mellifera</i> (A), <i>T. colina</i> (B) and <i>T. minor</i> (C).....	57
<b>Figure 4.16</b> Soxhlet extractor.....	58
<b>Figure 4.17</b> The repellent indices of sticky band raw material of 40 colonies of <i>A.</i> <i>florea</i> . Samples collected in Thailand.....	65
<b>Figure 4.18</b> The repellent indices of sticky band raw material from 10 colonies of <i>A.</i> <i>andreniformis</i> . Samples collected from Tenom, Malaysia and Thailand.....	66
<b>Figure 4.19</b> The repellent indices of sticky band raw material from 5 colonies of <i>A.</i> <i>andreniformis</i> . Samples collected from Tenom, Malaysia.....	66
<b>Figure 4.20</b> The repellent indices of sticky band raw materials of <i>A. andreniformis</i> . Experiments were conducted in Thailand and Tenom, Malaysia.....	67
<b>Figure 4.21</b> The repellent indices of raw materials from 12 colonies of <i>A. mellifera</i> . Samples collected in Thailand.....	68
<b>Figure 4.22</b> The repellent indices of raw materials from 12 colonies of <i>A. mellifera</i> . Samples collected in Germany.....	69
<b>Figure 4.23</b> The repellent indices of raw material of <i>A. mellifera</i> . Samples collected in Thailand (12 colonies) and Germany (12 colonies).....	70
<b>Figure 4.24</b> The repellent indices of raw materials from 12 colonies of <i>T. apicalis</i> . Experiments conducted in Thailand.....	71
<b>Figure 4.25</b> The repellent indices of raw material from 14 colonies of <i>T. apicalis</i> Samples were collected in Thailand.....	71
<b>Figure 4.26</b> The repellent indices of raw materials from 14 colonies of <i>T. collina</i> .....	72
<b>Figure 4.27</b> The repellent indices of raw material from 14 colonies of <i>T. collina</i> . Samples collected in Thailand.....	73
<b>Figure 4.28</b> The repellent indices of raw material from 13 colonies of <i>T. terminata</i> .....	74
<b>Figure 4.29</b> The repellent indices of raw material from 13 colonies of <i>T. terminata</i> . Samples collected in Thailand.....	74

	<b>Page</b>
<b>Figure 4.30</b> The repellent indices of raw material of 8 colonies of <i>T. terminata</i> in Thailand. Samples collected in Tenom, Malaysia.....	75
<b>Figure 4.31</b> The repellent indices of raw material from 5 colonies of <i>T. terminata</i> in Tenom, Malaysia. Samples collected in Tenom, Malaysia.....	76
<b>Figure 4.32</b> The repellent indices of raw material of <i>T. terminata</i> . Experiments were conducted in Thailand and Tenom, Malaysia.....	76
<b>Figure 4.33</b> The repellent indices of raw materials from 3 colonies of <i>T. melanocephala</i> . Sample collected in Tenom, Malaysia.....	77
<b>Figure 4.34</b> The repellent indices of raw material from 3 colonies of <i>T. melanocephala</i> . Samples collected in Tenom, Malaysia.....	77
<b>Figure 4.35</b> The repellent indices of raw material of <i>T. laeviceps</i> . Sample collected in Tenom, Malaysia.....	78
<b>Figure 4.36</b> The repellent indices of raw material from <i>T. minor</i> . Sample collected from Tenom, Malaysia.....	79
<b>Figure 4.37</b> The repellent indices of raw material. Samples from 140 colonies of 10 species of honey bees and stingless bees.....	80
<b>Figure 4.38</b> The repellent indices of pentane extracts. Samples were collected from 9 species of social bees.....	82
<b>Figure 4.39</b> The repellent indices of acetone extracts. Samples were collected from 9 species of social bees.....	82
<b>Figure 4.40</b> The repellent indices of methanol extracts. The experiments were done in Thailand. Samples were collected from 7 species of social bees.....	83
<b>Figure 4.41</b> The repellent indices of samples collected from nests of 10 species of bees. The results show in different extract types from soxhlet extraction.....	83
<b>Figure 4.42</b> The repellent indices of the pentane extract of <i>A. mellifera</i> collected from Thailand after quick column chromatography (solvent system: hexane/ethyl acetate).....	85
<b>Figure 4.43</b> The repellent index of the second chromatographed fraction of pentane extract (1 <sup>st</sup> chromatographed fractions were the mixture of 20% and 40% ethyl acetate in hexane) of <i>A. mellifera</i> collected from Thailand.....	85
<b>Figure 4.44</b> The repellent indices of the pentane extract of <i>A. mellifera</i> collected from Germany after quick column chromatography (solvent system: hexane/ethyl acetate).....	87

	<b>Page</b>
<b>Figure 4.45</b> The repellent indices of the pentane extract of <i>T. apicalis</i> collected from Tenom after quick column chromatography (solvent system: hexane/ethyl acetate).....	88
<b>Figure 4.46</b> The repellent indices of the second step of separation of fractions of pentane extract (1 <sup>st</sup> step of elution: 20% and 40% ethyl acetate in hexane) of <i>T. apicalis</i> collected from Thailand.....	88
<b>Figure 4.47</b> The repellent indices of the pentane extract of <i>T. colina</i> collected from Tenom after quick column chromatography (solvent system: hexane/ethyl acetate).....	89
<b>Figure 4.48</b> The repellent indices of the pentane extract of <i>T. terminata</i> collected from Tenom after quick column chromatography (solvent system: hexane/ethyl acetate).....	90
<b>Figure 4.49</b> The repellent indices of sticky band raw material from 7 colonies of <i>A. florea</i> . Samples collected in Thailand.....	92
<b>Figure 4.50</b> The repellent indices of raw materials from 7 colonies of <i>A. mellifera</i> . Samples collected in Thailand.....	93
<b>Figure 4.51</b> The repellent indices of raw material from 7 colonies of <i>A. mellifera</i> Samples collected in Germany.....	94
<b>Figure 4.52</b> The repellent indices of raw material from 9 colonies of <i>T. apicalis</i> .....	95
<b>Figure 4.53</b> The repellent indices of raw material from 7 colonies of <i>T. collina</i> .....	96
<b>Figure 4.54</b> The repellent indices of raw material from 7 colonies of <i>T. terminata</i> .....	97
<b>Figure 4.55</b> The repellent indices of raw material from 6 colonies of <i>T. terminata</i> , samples collected from Tenom, Malaysia.....	98
<b>Figure 4.56</b> The repellent indices of raw materials from 2 colonies of <i>T. minor</i> .....	99
<b>Figure 4.57</b> The repellent indices of raw material. Samples were collected from 8 species of social bees.....	100
<b>Figure 4.58</b> The repellent indices of pentane extracts. Samples were collected from 8 species of social bees.....	101
<b>Figure 4.59</b> The repellent indices of acetone extracts. Samples were collected from 6 species of social bees.....	102
<b>Figure 4.60</b> The repellent indices of pentane extracts of <i>A. mellifera</i> collected from Thailand after quick column chromatography (solvent system: hexane/ethyl acetate).....	103

	<b>Page</b>
<b>Figure 4.61</b> The repellent indices of pentane extracts of <i>A. mellifera</i> collected from Germany after quick column chromatography (solvent system: hexane/ethyl acetate).....	104
<b>Figure 4.62</b> The repellent indices of the pentane extract of <i>T. apicalis</i> after quick column chromatography (solvent system: hexane/ethyl acetate).....	105
<b>Figure 4.63</b> The repellent indices of the pentane extract of <i>T. terminata</i> , samples collected from Tenom, Malaysia, after quick column chromatography (solvent system: hexane/ethyl acetate).....	106
<b>Figure 4.64</b> The separation chart of the repellent fraction from samples of <i>A. florea</i> .....	107
<b>Figure 4.65</b> Chromatograms of the pentane raw extracts of the sticky band material collected at <i>A. florea</i> nests, from Nakorn Sawan province.....	107
<b>Figure 4.66</b> The separation chart of the repellent fraction from samples of <i>A. mellifera</i> in Thailand.....	108
<b>Figure 4.67</b> Chromatograms of fraction 1, which was chromatographed from propolis collected from nest of <i>A. mellifera</i> in Thailand.....	108
<b>Figure 4.68</b> Chromatograms of fraction 3, which was chromatographed from propolis collected from nest of <i>A. mellifera</i> in Thailand.....	109
<b>Figure 4.69</b> Chromatograms of fraction 4, which was chromatographed from propolis collected from nest of <i>A. mellifera</i> in Thailand.....	109
<b>Figure 4.70</b> Chromatograms of fraction 9, which was chromatographed from propolis collected from nest of <i>A. mellifera</i> in Thailand.....	110
<b>Figure 4.71</b> The separation chart of the repellent fractions from samples of in <i>A. mellifera</i> in Germany.....	110
<b>Figure 4.72</b> Chromatograms of fraction 2 (eluent =5% ethyl acetate in hexane), which was chromatographed from propolis collected from nest of <i>A. mellifera</i> in Germany.....	111
<b>Figure 4.73</b> Chromatograms of fraction 5 (eluent =40% ethyl acetate in hexane), which was chromatographed from propolis collected from nest of <i>A. mellifera</i> in Germany.....	111
<b>Figure 4.74</b> The separation chart of the repellent fraction from samples of <i>T. apicalis</i> .....	112

	<b>Page</b>
<b>Figure 4.75</b> Chromatograms of fraction 3, which was chromatographed from entrances tubes from nest of <i>T. apicalis</i> in Thailand.....	112
<b>Figure 4.76</b> The separation chart of the repellent fraction of samples from <i>T. collina</i> .....	113
<b>Figure 4.77</b> Chromatograms of fraction 5 (eluent =40% ethyl acetate in hexane), which was chromatographed from entrance tube from nest of <i>T. colina</i> in Thailand.....	113
<b>Figure 4.78</b> The separation chart of the repellent fractions of samples from in <i>T. terminata</i> in Tenom, Malaysia.....	114
<b>Figure 4.79</b> Chromatograms of fraction 2 (eluent= 5% ethyl acetate in hexane) which was chromatographed from entrance tube collected from nest of <i>T. terminata</i> in Tenom, Malaysia.....	114
<b>Figure 4.80</b> Chromatograms of fraction 5 (eluent= 40% ethyl acetate in hexane) which was chromatographed from entrance tube collected from nest of <i>T. terminata</i> in Tenom, Malaysia.....	115
<b>Figure 4.81</b> Comparison the worker size of honey bees.....	121
<b>Figure 4.82</b> <i>A. dorsata</i> nest has no sticky band on the nesting branches .....	122
<b>Figure 4.83</b> The weaver ants attacked the <i>A. koschevnikovi</i> colony by waiting near the nest entrances and captured the bees flying in and out .....	122
<b>Figure 4.84</b> The Assassin bug (Hemiptera: Reduviidae) at the nest entrance of <i>T. collina</i> await for the arrival bees close to the entrance in order to capture them.....	124
<b>Figure 4.85</b> The nest entrance of <i>A. cerana</i> . No obvious resinous material barrier between the nest and out side.....	125
<b>Figure 4.86</b> The model of recruitment pattern (Beekman <i>et al.</i> , 2001).....	129
<b>Figure 4.87</b> The increase in the number of ants walking to the feeder along the trail in relation to colony size .....	130