UNIVERSITI PUTRA MALAYSIA

CHARACTERISTICS OF APIS CERANA (HYMENOPTERA: APIDAE) AND EFFECTS OF DIET VARIABILITY ON BEE VENOM QUALITY AND QUANTITY IN PENINSULAR MALAYSIA

ABUSABBAH, MOHAMMED OMAR A

FP 2014 68

CHARACTERISTICS OF *APIS CERANA* (HYMENOPTERA: APIDAE) AND EFFECTS OF DIET VARIABILITY ON BEE VENOM QUALITY AND QUANTITY IN PENINSULAR MALAYSIA

By

ABUSABBAH, MOHAMMED OMAR A

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

December 2014

DEDICATION

I dedicate this research to my wonderful parents, my beautiful amazing wife and my brilliant kids in appreciation of their love and support.

Abstract of thesis presented to Senate of Universiti Putra Malaysia in fulfilment of the requirements for the Degree of Doctor of Philosophy

CHARACTERISTICS OF *APIS CERANA* (HYMENOPTERA: APIDAE) AND EFFECTS OF DIET VARIABILITY ON BEE VENOM QUALITY AND QUANTITY IN PENINSULAR MALAYSIA

By

ABUSABBAH, MOHAMMED OMAR A

December 2014

Chairman : Professor Dzolkhifli Bin Omar, Ph. D.
Faculty : Agriculture

The bee venom is a complicated chemical and pharmacological product, which has been used for healing several diseases for many centuries by different nations. The components of venom, especially melittin, phospholipase A2 and apamin are the main qualitative factors that affect its chemical properties. Few information on the effect of nutrition on the quality and quantity of the venom produced by bees are available, particularly of Apis cerana. In addition, providing information on the genetic basis of A. cerana in Peninsula Malaysia is highly required. Accordingly, this study aims to investigate the morphometric and phylogenetic of A. cerana, also to investigate the variation in the quality and quantity of the bee venom collected from different sites in Peninsula Malaysia, and the effect of the bee diet on the venom quality. Morphometric study of the A. cerana from different seven localities in Malaysia was performed and the morphometric variables were analysed by multivariate analyses. The analysis of variance (ANOVA) results showed that there were significant differences between means of the different locations, however, the principal component analysis and the discrimination analysis showed that the most important variable to discriminate between A. cerana were body weight and the stinger length. Based on the obtained results, there were no clear clusters observed indicating the high similarity between the A. cerana population in Malaysia. The Phylogenetic showed an acceptable divergence percentage of less than 1% between bee venom samples, implying that the DNA of A. cerana collected from different sites with different types of diets is completely typical, therefore the venom quality and quantity was found to be affected purely by the type of diet.
Investigation the effect of the natural diet on the quality and quantity of bee venom revealed that the source of natural diets (pollen grains) was significantly affected the quality and quantity of bee venom. Bees that foraged on durian were the highest concentrations of the important components melittin, phospholipase A2 and apamin by 677.86±23.68, 477.95±7.75 and 136.10±3.98 µg/µl respectively,

corresponding to the protein content, which was the highest among the seven plants (31.71%). Whereas, the quantity analysis resulted high weight of 7.65±0.25 µg was achieved by star fruit.

The effect of carbohydrate and protein rich mixture was studied. The results showed that the venom produced by bees foraged on supplemented-carbohydrate diets, had melittin, phospholipase A2 and apamin of 535.21±17.73, 374.49±18.94 and 130.36±12.05 µg/µl respectively. The comparison of the alternative diets proved that the protein rich mixture is better than both sucrose diet and the natural diet for yielding venom with high quality. Relocation of the hives from their original locations to the area with different flora led to obvious changes in the quality and quantity of the bee venom. The results showed that the most significant difference was observed in the concentration of the phospholipase A2 between pink power (420.85±13.15µg/µl) and star fruit (360.41±15.74µg/µl).

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

CIRI-CIRI APIS CERANA (HYMENOPTERA APIDAE) DAN KESAN KEPELBAGAIAN DIET KE ATAS QUALITI DAN QUANTY RACUN LEBAH DI SEMENANJUNG MALAYSIA

Oleh

ABUSABBAH, MOHAMMED OMAR A

Disember 2014

Pengerusi : Professor Dzolkhifli bin Omar, PhD
Fakulti : Pertanian

Bisa lebah dihasilkan oleh lebah madu ialah satu produk kimia dan farmakologi yang amat rumit, yang telah digunakan untuk memulihkan beberapa penyakit pada zaman dahulu oleh berbagai negara. Komponen bisa, terutamanya melittin, phospholipase A2 and apamin ialah faktor-faktor kualitatif utama yang menjejaskan sifat kimianya. Beberapa maklumat di kesan pemakanan bagi kualiti dan kuantiti bisa dihasilkan oleh lebah-lebah boleh didapati, terutama *Apis cerana*, serta morfometrik untuk radas bisanya. Sebagai tambahan, menyediakan maklumat di asas genetik *Apis Cerana* di Semenanjung Malaysia amat dikehendaki. Oleh sebab itu ,kajian ini menyasarkan penyiasatan morfometrik dan filogenetik *Apis Cerana.* Selain itu ,kajian juga dijalankan menyiasat variasi bagi kualiti dan kuantiti bisa lebah yang dikutip dari tempat yang berbeza di Semenanjung Malaysia , dan kesan diet lebah bagi kualiti bisa. Ukuran bahagian-bahagian badan serangga telah diusahakan dengan menggunakan sebuah program berasaskan komputer diperuntukkan dengan sebuah kamera digital. Keputusan-keputusan morfometrik menunjukkan bahawa terdapat perbezaan penting bagi panjang sengat antara contoh-contoh lebah dikutip dari berbeza mencari sarang, yang sengatan terpanjang diukur 1.84±0.06 mm di serbuk jambu merah. Saiz ukuran-ukuran pundi bisa lebah menunjukkan perbezaan-perbezaan jelas antara mencari sarang, saiz terbesar telah dicapai oleh lebah dikutip dari Terengganu Paper (letak terkumpul dan memotong sekeliling) kulit kayu 5.35±0.57 mm. Panjang badan lebah dan panjang kepala menunjukkan perbezaan-perbezaan jelas menurut makanan daripada sumber tumbuh-tumbuhan yang di cari. Keputusan menunjukkan badan terpanjang dan kepala terpanjang telah dicapai oleh lebah yang dikutip dari pineapple 11.09±0.77 and 3.13±02 mm masing-masing. Perbezaan hubung kait terhasil antara morfometrik semua ciri-ciri yang telah dirancang.

Kajian filogenetik telah dijalankan untuk membuktikan bahawa kewujudan kelainan kualiti dan kuantiti bisa lebah dihasilkan semata-mata oleh faktor pemakanan, dan tidak daripada genetik. Keputusan menunjukkan bahawa peratusan kecapahan yang boleh diterima kurang daripada 1% antara contoh-contoh bisa lebah. Ini menunjukkan bahawa DNA genomik *Apis Cerana* dikutip dari tempat yang berbeza dengan pelbagai jenis diet memang biasa, oleh itu kualiti dan kuantiti bisa didapati terjejas semata-mata oleh jenis diet.

Penentuan pemakanan asli (butir debunga) kesan keatas kualiti dan kuantiti bisa lebah menurut kajian sampingan yang telah dijalankan. Analisis bisa lebah untuk kualiti telah dijalankan menggunakan teknik HPLC dan untuk kualiti ditentukan oleh berat bisa. Keputusan mendedahkan bahawa sumber diet semulajadi (butir debunga) telah menjejaskan kuantiti dan kualiti bisa lebah. Lebah-lebah yang mencari makanan di Durian, mempunyai kepekatan yang lebih tinggi komponen penting melittin, phospholipase A2 and apamin oleh 677.86±23.68, 477.95±7.75 and 136.10±3.98 μg / μl masing-masing. Kajian serupa pada durian, apabila kandungan protein butir debunga dianalisis, peratusan tinggi antara tujuh pokok. Manakala, analisis kuantiti memberi hasil yang berat sebanyak 7.65±0.25 μg telah dicapai oleh belimbing besi di Selangor.

Kesan supplant karbohidrat dan adunan lebihan lemak protein telah dikaji. Keputusan menunjukkan bahawa diantara diet berkarbohidrat diperlengkap kepada sarang lebah, gula maltosa didapati menjadi kualiti terbaik bisa lebah yang memberi penumpuan tertinggi melittin, phospholipase A2 and apamin of 535.21±17.73, 374.49±18.94 dan 130.36±12.05 μg/μl masing-masing. Perbandingan diet alternatif membuktikan bahawa adunan lebihan lemak protein lebih baik daripada diet sukrosa; manakala, tiada perbezaan penting berbanding dengan diet debunga semulajadi dalam menghasilkan bisa berkualiti tinggi , dan penumpuan komponen utama bisa ialah 585.67±12.89, 439.48±63.64 and 120.61±9.01 μg/μl for melittin, phospholipase A2 and apamin, masing-masing. Penempatan semula sarang lebah dari lokasi asal mereka ke kawasan dengan flora berbeza membawa kepada perubahan ketara ke atas kualiti dan kuantiti bisa lebah. Keputusan menunjukkan bahawa paling banyak perbezaan penting telah diperhatikan di tumpuan phospholipase A2 antara kuasa jambu merah dan belimbing besi di (360.41±15.74μg / μl).

ACKNOWLEDGEMENTS

In the name of Allah the most beneficent the most merciful. All praise and glory be to Almighty ALLAH the lord of the world. May the peace and blessings of ALLAH be on our noble prophet Mohammad (SAW), his family, his companions and the generality of believers who sincerely believe in his message until the Day of Judgment (Amen).

My special thanks go to my supervisor Professor Dr Dzolkhifli bin Omar, for his beneficial advice, suggestions and sacrifice of his precious time for this research. The guidance and ideas from him were very useful in order to complete this research. All his contributions and experience during this research were very valuable to me. Without his active support, this research could never have been accomplished.

My profound gratitude goes to my supervisory committee, most especially my co-supervisors Dr Lau Wei Hong, whose help and advice helped tremendously for the success of this research. Also, my sincere thanks to Dr Mohammad bin Muid and all the lecturers and staff at the faculty of Agriculture UPM for their support and guidance.

I would also like to thank my parents for their support both financially, morally and spiritually throughout the period of my study. My warm greeting goes to all the people at the Saudi Arabian Embassy in Malaysia as well as the King Abdullah Scholarship Program board, for their continuous help from the beginning to the end of my research.

Finally, I pray that Almighty ALLAH (SWT) rewards them abundantly (Amen).

I certify that a Thesis Examination Committee has met on 5 Disember 2014 to conduct the final examination of Abusabbah, Mohammed Omar A on his thesis entitled "Characteristics of *Apis cerana* (Hymenoptera: Apidae) and Effects of Diet Variability on Bee Venom Quality and Quantity in Peninsular Malaysia" 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:

Ganesan a/l Vadamalai, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Rita Muhamad Awang @ Rita Suryadi, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Hafidzi bin Mohd Noor, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal Examiner)

De. Omar, Mohamed O.M
Professor
King Saud University
Saudi Arabia
(External Examiner)

ZULKARNAIN ZAINAL, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 15 April 2015

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

Dzolkhifli Bin Omar, PhD
Professor
Faculty of Agriculture
Department of Plant Protection
Universiti Putra Malaysia
(Chairman)

Lau Wei Hong, PhD
Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Mohd Bin Muid, PhD
Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Y.Bhg Dato' Makhdzir Bin Mardan, PhD
Professor
Department of agribusiness
Faculty of Agriculture
Universiti Putra Malaysia
(Member

BUJANG KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work
- quotations, illustrations and citations have been duly referenced
- the thesis has not been submitted previously or comcurrently for any other degree at any institutions
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be owned from supervisor and 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 (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software

Signature**:** _____ Date: _____

Name and Matric No.: Abusabbah, Mohammed Omar A GS22663

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) were adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Dzolkhifli Bin Omar, PhD

Signature: _____
Name of Member of Supervisory Committee: Lau Wei Hong, PhD

Signature: _____
Name of Member of Supervisory Committee: Mohd Bin Muid, PhD

Signature: _____
Name of Member of Supervisory Committee: Y.Bhg Dato' Makhdzir Bin Mardan, PhD

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

HPLC	High performance liquid chromatography
CO1	Cytochrome oxidase subunit 1
CO2	Cytochrome oxidase subunit 2
GPS	Global Positioning System
GIS	Geographical Information System
PCR	Polymerase chain reaction
M	Melittin
P	Phospholipase A_2
A	Apamin
W	Weight of Bee Venom
PCA	Principal Component Analysis
LDA	Linear Discriminant Analysis
PC1	First Principal Components
PC2	Second Principal Component
PC3	Third Principal Component

LIST OF ABBREVIATIONS

HPLC	High performance liquid chromatography
CO1	Cytochrome oxidase subunit 1
CO2	Cytochrome oxidase subunit 2
GPS	Global Positioning System
GIS	Geographical Information System
PCR	Polymerase chain reaction
M	Melittin
P	Phospholipase A_2
A	Apamin
W	Weight of Bee Venom
PCA	Principal Component Analysis
LDA	Linear Discriminant Analysis
PC1	First Principal Components
PC2	Second Principal Component
PC3	Third Principal Component

CHAPTER 1

INTRODUCTION

Honeybees are a group of insects belonging to the order hymenoptera. They play an important role in conserving biodiversity by pollinating flowering plants and increasing the productivity of agricultural crops. This insect comprises an important insect group that provides many products to human beings and serves as a volunteer pollinator to millions of crops worldwide. Ruttner (1988) started the first morphometric analysis of A. *cerana*, which has since been augmented by many researchers (Tanaka et al., 2001; Hepburn et al., 2005 ; Ricketts et al., 2008 and Radloff et al., 2005). Radloff et al., (2003) statistically demarcated the populations of honeybees as morphoclusters.

Bees have received religious testimony by both Christianity and Islam since ancient times (Zumla and Lulat, 1989 and El-Soud and Helmy, 2012). In the Holy Qur'an there is a verse that clarifies the potential healing significance of honeybee products, especially honey (Ali, 1987). In many countries, beekeeping is one of the most practiced economic and social activities that provide additional earnings.

More than 3000 species of bees are morphologically described worldwide and the genetic diversity of the most important ones has been identified using Cytochrome Oxidase subunit 1 (CO1). A. cerana is the most dominant species throughout the tropical, sub-tropical and temperate zones of Asia (Akratanakul, 1986). In Malaysia, A. cerana is distributed all around the country and forages on many plant species with various pollen grains (Kiew and Muid, 1991). Brodschneider and Crailsheim (2010) stated that colonies of bees are endangered by monocultures, poisoning by plants and pesticide residue in nutrients, transgenic products and starvation.

Carbohydrates, proteins, lipids, vitamins and minerals are the essential nutrients that provide hives of bees with energy to accomplish their activities, such as continuity of brood production, as well as longevity and the healthiness of the adults (Naug, 2009 and Oldroyd, 2007). A balanced nutrition is highly important to bee colonies and can be achieved through the supplementation of essential nutrients (Schmidt et al., 1995; Pernal and Currie, 2000; Somerville and Nicol, 2006).

Human and Nicolson (2006) found that the pollen grain of Aloe greatheadii var. davyana (Asphodelaceae) consisted of carbohydrate (35-61% dry weight), crude protein (28-51% dry weight) and lipid content (8-10% dry weight). In addition, they contended that the content of essential amino acids might be more or less than that needed to develop honeybees.

Pollen grains provide honeybees with proteins, vitamins, minerals and fats, which assist in the production of honey and other bee products, especially bee venom. It is generally known that the source of the diet affects the quantity and quality of honey, wax and other brood components. Abusabbah et al., (2012) demonstrated that protein artificial alternative diets increase the brood area and honey production.

Bee venom, which is one of the most necessary products of honeybees used from ancient times worldwide to treat and heal several diseases, is composed of histamine, dopamine, melittin, apamin, mast cell destroying (MCD) – peptide, minimine, and the enzymes phospholipase A2 and hyaluronidase (Ludolph-Hauser et al., 2001). Biló et al., (2005) explained that the quantity of venom produced by bees can be governed by the availability of proteins in the pollen grains of plants.

1.1 Hypothesis

The information regarding factors affecting the quality and quantity of venom of honeybees is contradictory. This study assumes that the bee venom quality characteristic might vary according to the diversity of diets.

1.2 Problem Statement

Bee venom is composed of different major components; the content of these components varies from one venom to another, leading to different venom quality. The concentrations of these components might determine the medical applications of the bee venom, such as the anti-inflammatory effect of the melitin and the anti-oxidant effect of the phospholipase A2 (Markelov and Trushin, 2006). These facts highlight the need to study the effect of nutrition on the quality of the venom produced by honeybees, mainly A. *cerana* (the major group in Malaysia), which is not well understood and needs more elaboration on the scientific data. In addition, the genetic study to exclude the other factors might affect the quality of the venom. Furthermore, the investigation of the effect of the artificial diets on the venom quality is highly required to provide more information about the nutritional effect of supplement diets on the concentration of the venom components, thereby enabling bee venom of a specific quality to be produced for the potential use in medical applications.

1.3 Objectives

This study aims to assess the effects of diet on the quantity and quality of bee venom, and to determine the variation in the concentration of the main venom components according to the source of diet. Specifically, the objectives of this project are to:

1) Determine the morphometrics and DNA-fingerprint of A. *cerana* .
2) Study the effect of diets foraged by A. *cerana* on the quality and quantity of venom.
3) Study the effect of carbohydrate-supplemented diets and protein rich mixture diets on the quality of bee venom.

REFERENCES

Abrol, D. P. and Bhat, A. A. (1990). Studies on Thai sac brood virus' affecting indigenous honeybee *Apis cerana* indica Fab. colonies-prospects and future strategies-I. *Journal of Animal Morphology and Physiology*, **37** (1/2): 101-108.

Abusabbah, M., Mahmoud, M., Mahjoub, M., Omar, D. and Abdelfatah, M. (2012). Promising alternative diets for honey bees to increase hive activities and sustain honey production during dry seasons in Saudi Arabia. *International Journal of AgriScience*, **2** (4): 361-364.

AJA, R. S. and Padmalatha, C. (2004). Ethno-entomological practices in Tirunelveli district, Tamil Nadu. *Indian Journal of Traditional Knowledge*, **3** (4): 442-446.

Akratanakul, P. (1986). *Beekeeping in Asia*. Food & Agriculture Organization., Rome.

Al-Ani, I., Zimmermann, S., Reichling, J. and Wink, M. (2015). Pharmacological synergism of bee venom and melittin with antibiotics and plant secondary metabolites against multi-drug resistant microbial pathogens. *Phytomedicine*, **22** (2): 245-255.

Ali, A. Y. (1987). The Bee (Nahl), surah XVI, verse- 69. In: The Holy Qur'an *2nd edn. American Trust Publications.*

Allen, M. and Ball, B. (1996). The incidence and world distribution of honey bee viruses. *Bee World*, **77** (3).

Alqarni, A. S. (2006). Influence of some protein diets on the longevity and some physiological conditions of honeybee *Apis mellifera* L. workers. *Journal of Biological Science*, **6** (4): 734-737.

Altmann, F., Kubelka, V., Staudacher, E., Uhl, K. and März, L. (1991). Characterization of the isoforms of phospholipase A_2 from honeybee venom. *Insect Biochemistry*, **21** (5): 467-472.

Amssalu, B., Nuru, A., Radloff, S. E. and Hepburn, H. R. (2004). Multivariate morphometric analysis of honeybees (Apis mellifera) in the Ethiopian region. *Apidologie*, **35** (1): 71-82.

Andrade, D. V. and Abe, A. S. (1999). Relationship of venom ontogeny and diet in Bothrops. *Herpetologica*, **55** (2): 200-204.

AOAC. (1990). Official methods of analysis. *Association of Official Analytical Chemists, Arlington, VA.*(14th edn).

Araújo, E., Costa, M., Chaud-Netto, J. and Fowler, H. G. (2004). Body size and flight distance in stingless bees (Hymenoptera: Meliponini): inference of flight range and possible ecological implications. *Brazilian Journal of Biology*, 64 (3B): 563-568.

Arias, M. C. and Sheppard, W. S. (2005). Phylogenetic relationships of honey bees (Hymenoptera: Apinae: Apini) inferred from nuclear and mitochondrial DNA sequence data. *Molecular Phylogenetics and Evolution*, **37** (1): 25-35.

Attalla, K. M., Owayss, A. A. and Mohanny, K. M. (2007). Antibacterial activities of bee venom, propolis and royal jelly produce by three honey bee, *Apis mellifera* L., hybrids reared in the same environmental conditions. *Annals of Agricultural Sciences* **45** (2): 895-902.

Bachmayer, H., Kreil, G. and Suchanek, G. (1972). Synthesis of promelittin and melittin in the venom gland of queen and worker bees: patterns observed during maturation. *Journal of Insect Physiology*, **18** (8): 1515-1521.

Bath, P. K. and Singh, N. (1999). A comparison between *Helianthus annuus* and *Eucalyptus lanceolatus* honey. *Food Chemistry*, **67** (4): 389-397.

Benton, A. W., Morse, R. A. and Stewart, J. D. (1963). Venom collection from honey bees. *Science*, **142** (3589): 228-230.

Biló, B., Rueff, F., Mosbech, H., Bonifazi, F. and Oude-Elberink, J. (2005). Diagnosis of Hymenoptera venom allergy. *Allergy*, **60** (11): 1339-1349.

Bishop, H. (2005). *Robbing the Bees: A Biography of Honey, the Sweet Liquid Gold that Seduced the World*. Simon and Schuster, New York.

Boehme, P., Amendt, J. and Zehner, R. (2012). The use of COI barcodes for molecular identification of forensically important fly species in Germany. *Parasitology Research*, **110** (6): 2325-2332.

Bohonak, A. J. and Jenkins, D. G. (2003). Ecological and evolutionary significance of dispersal by freshwater invertebrates. *Ecology letters*, **6** (8): 783-796.

Bosch, J. and Vicens, N. (2006). Relationship between body size, provisioning rate, longevity and reproductive success in females of the solitary bee Osmia cornuta. *Behavioral Ecology and Sociobiology*, **60** (1): 26-33.

Box, G. E., Hunter, J. S. and Hunter, W. G. (2005). *Statistics for Experimenters: Design, Innovation, and Discovery*. Wiley Online Library.

Brodschneider, R. and Crailsheim, K. (2010). Nutrition and health in honey bees. *Apidologie*, **41** (3): 278-294.

Buchmann, S. L. and O'rourke, M. K. (1991). Importance of pollen grain volumes for calculating bee diets. *Grana*, **30** (3-4): 591-595.

Chalapathy, C. V., Puttaraju, H. and Sivaram, V. (2014). A pilot study on genetic diversity in Indian honeybees-*Apis cerana* of Karnataka populations. *Journal of Entomology and Zoology Studies*, **2** (3): 7-13.

Clarke, A. R., Armstrong, K. F., Carmichael, A. E., Milne, J. R., Raghu, S., Roderick, G. K. and Yeates, D. K. (2005). Invasive phytophagous pests arising through a recent tropical evolutionary radiation: the *Bactrocera dorsalis* complex of fruit flies. *Annual Review of Entomology*, **50**: 293-319.

Crailsheim, K. (1990). The protein balance of the honey bee worker. *Apidologie*, **21** (5): 417-429.

Crailsheim, K., Schneider, L., Hrassnigg, N., Bühlmann, G., Brosch, U., Gmeinbauer, R. and Schöffmann, B. (1992). Pollen consumption and utilization in worker honeybees (*Apis mellifera carnica*): Dependence on individual age and function. *Journal of Insect Physiology*, **38** (6): 409-419.

Crane, E. (1990). *Bees and Beekeeping: Science, Practice and World Resources*. Comstock Pub. Associates, Minnesota.

Crozier, R. and Crozier, Y. (1993). The mitochondrial genome of the honeybee *Apis mellifera*: complete sequence and genome organization. *Genetics*, **133** (1): 97-117.

Damus, M., S. and Otis, G., W. (1997). A morphometric analysis of Apis cerana F and Apis nigrocincta Smith populations from Southeast Asia. *Apidologie*, **28** (5): 309-323.

De Abreu, R. M. M., Silva de Moraes, R. L. M. and Camargo-Mathias, M. I. (2010). Biochemical and cytochemical studies of the enzymatic activity of the venom glands of workers of honey bee *Apis mellifera* L.(Hymenoptera, Apidae). *Micron*, **41** (2): 172-175.

De Abreu, R. M. M., Silva de Moreas, R. L. M. and Malaspina, O. (2000). Histological aspects and protein content of *Apis mellifera* L. worker venom glands: the effect of electrical shocks in summer and winter. *Journal of Venomous Animals and Toxins*, **6** (1): 87-98.

De la Rúa, P., Simon, U., Tilde, A., Moritz, R. and Fuchs, S. (2000). MtDNA variation in *Apis cerana* populations from the Philippines. *Heredity*, **84** (1): 124-130.

de Lello, E. (1971). Adnexal glands of the sting apparatus of bees: Anatomy and histology, I (Hymenoptera: Colletidae and Andrenidae). *Journal of the Kansas Entomological Society*: **44** (1): 5-13.

Decourtye, A., Mader, E. and Desneux, N. (2010). Landscape enhancement of floral resources for honey bees in agro-ecosystems. *Apidologie*, **41** (3): 264-277.

El-Soud, A. and Helmy, N. (2012). Honey between traditional uses and recent medicine. *Macedonian Journal of Medical Sciences*, **5** (2): 205-214.

Engel, M. S. (1998). Fossil honey bees and evolution in the genus *Apis* (Hymenoptera: Apidae). *Apidologie*, **29** (3): 265-281.

Felke, M. and Langenbruch, G.-A. (2005). Auswirkungen des Pollens von transgenem Bt-Mais auf ausgewählte Schmetterlingslarven. *Schweizerische Bienen-Zeitung*, **157**: 143.

Fry, B. G., Wickramaratna, J. C., Hodgson, W. C., Alewood, P. F., Kini, R., Ho, H. and Wüster, W. (2002). Electrospray liquid chromatography/mass spectrometry fingerprinting of Acanthophis (death adder) venoms: taxonomic and toxinological implications. *Rapid Communications in Mass Spectrometry*, **16** (6): 600-608.

Gajski, G. and Garaj-Vrhovac, V. (2013). Melittin: A lytic peptide with anticancer properties. *Environmental Toxicology and Pharmacology*, **36** (2): 697-705.

Garnery, L., CORNUET, J. M. and Solignac, M. (1992). Evolutionary history of the honey bee *Apis mellifera* inferred from mitochondrial DNA analysis. *Molecular Ecology*, **1** (3): 145-154.

Gasparich, G. E., Silva, J. G., Han, H.-Y., McPheron, B. A., Steck, G. J. and Sheppard, W. S. (1997). Population genetic structure of Mediterranean fruit fly (Diptera: Tephritidae) and implications for worldwide colonization patterns. *Annals of the Entomological Society of America*, **90** (6): 790-797.

Gatesy, J., DeSalle, R. and Wheeler, W. (1993). Alignment-ambiguous nucleotide sites and the exclusion of systematic data. *Molecular Pylogenetics and Eolution*, **2** (2): 152-157.

Genersch, E., Yue, C., Fries, I. and de Miranda, J. R. (2006). Detection of *Deformed wing virus*, a honey bee viral pathogen, in bumble bees (*Bombus terrestris* and *Bombus pascuorum*) with wing deformities. *Journal of Invertebrate Pathology*, **91** (1): 61-63.

Gilliam, M. (1997). Identification and roles of non-pathogenic microflora associated with honey bees1. *FEMS Microbiology Letters*, **155** (1): 1-10.

Graham, J. M. (1992). *The Hive and the Honey Bee*. Dadant & Sons, Minnesota.

Hájek, J. and Fikáček, M. (2008). A review of the genus Satonius (Coleoptera: Myxophaga: Torridincolidae): taxonomic revision, larval morphology, notes on wing polymorphism, and phylogenetic implications. *Acta Entomologica Musei Nationalis Pragae*, **48** (2): 655-676.

Harvey, M. L., Dadour, I. R. and Gaudieri, S. (2003). Mitochondrial DNA cytochrome oxidase I gene: potential for distinction between immature stages of some forensically important fly species (Diptera) in western Australia. *Forensic Science International*, **131** (2): 134-139.

Hauser, R. A., Daguio, M., Wester, D., Hauser, M., Kirchman, A. and Skinkis, C. (2001). Bee-venom therapy for treating multiple sclerosis: a clinical trial. *Alternative and Complementary Therapies*, **7** (1): 37-45.

Haydak, M. H. (1970). Honey bee nutrition. *Annual Review of Entomology*, **15** (1): 143-156.

Hepburn, H. R., Radloff, S. E., Verma, S. and Verma, L. R. (2001). Morphometric analysis of Apis cerana populations in the southern Himalayan region. *Apidologie*, **32** (5): 435-447.

Hepburn, H. R., Radloff, S. E., Otis, G. W., Fuchs, S., Verma, L., Ken, T., Chaiyawong, T., Tahmasebi, G., Ebadi, R. and Wongsiri, S. (2005). *Apis florea*: morphometrics, classification and biogeography. *Apidologie*, **36** (3): 359.

Hepburn, R., Duangphakdee, O., Phiancharoen, M. and Radloff, S. (2010). Comb wax salvage by the red dwarf honeybee, *Apis florea* F. *Journal of Insect Behavior*, **23** (2): 159-164.

Herbert, E., Sylvester, H., Vandenberg, J. and Shimanuki, H. (1988). Influence of nutritional stress and the age of adults on the morphometrics of honey bees *Apis Mellifera*. *Apidologie*, **19** (3): 221-230.

Herbert Jr, E. W., Shimanuki, H. and CARON, D. (1977). Optimum protein levels required by honey bees (Hymenoptera, Apidae) to initiate and maintain brood rearing. *Apidologie*, **8** (2): 141-146.

Hider, R. C. (1988). Honeybee venom: A rich source of pharmacologically active peptides. *Endeavour*, **12** (2): 60-65.

Higes, M., Martín, R. and Meana, A. (2006). *Nosema ceranae*, a new microsporidian parasite in honeybees in Europe. *Journal of Invertebrate Pathology*, **92** (2): 93-95.

Holle, L., Song, W., Holle, E., Wei, Y., Wagner, T. and Yu, X. (2003). A matrix metalloproteinase 2 cleavable melittin/avidin conjugate specifically targets tumor cells in vitro and in vivo. *International Journal of Oncology*, **22** (1): 93-98.

Hrassnigg, N., Brodschneider, R., Fleischmann, P. H. and Crailsheim, K. (2005). Unlike nectar foragers, honeybee drones (*Apis mellifera*) are not able to utilize starch as fuel for flight. *Apidologie*, **36** (4): 547-557.

Human, H., Nicolson, S., Strauss, K., Pirk, C. and Dietemann, V. (2007). Influence of pollen quality on ovarian development in honeybee workers (*Apis mellifera scutellata*). *Journal of Insect Physiology*, **53** (7): 649-655.

Human, H. and Nicolson, S. W. (2006). Nutritional content of fresh, bee-collected and stored pollen of Aloe greatheadii var. davyana (Asphodelaceae). *Phytochemistry* **67** (14): 1486-1492.

Hunt, G. J., Guzmán-Novoa, E., Fondrk, M. K. and Page, R. E. (1998). Quantitative trait loci for honey bee stinging behavior and body size. *Genetics*, **148** (3): 1203-1213.

Inouye, D. W. (1980). The effect of proboscis and corolla tube lengths on patterns and rates of flower visitation by bumblebees. *Oecologia*, **45** (2): 197-201.

Jang, J. P., Yılız, M. A., Fakhri, B. and Nobakht, A. (2011). A Study of the diversity in COI-COII intergenic region of mitochondrial DNA in different Persian honeybee (*A. Mellifera Meda*). *Journal of Basic and Applied Scientific Research*, **1** (11): 2150-2154.

Jean-Prost, P. and Médori, P. (1994). *Apiculture: Know The Bee, Manage the Apiary*. Intercept Limited, Andover.

Jordaens, K., Sonet, G., Richet, R., Dupont, E., Braet, Y. and Desmyter, S. (2013). Identification of forensically important *Sarcophaga* species (Diptera: Sarcophagidae) using the mitochondrial COI gene. *International Journal of Legal Medicine*, **127** (2): 491-504.

Kaya, H. K., Marston, J. M., Lindegren, J. E. and Peng, Y. (1982). Low susceptibility of the honey bee, *Apis mellifera* L.(Hymenoptera: Apidae), to the entomogenous nematode, Neoaplectana carpocapsae Weiser. *Environmental Entomology*, **11** (4): 920-924.

Ken, T., Fuchs, S., Koeniger, N. and Ruiguang, Z. (2003). Morphological characterization of *Apis cerana* in the Yunnan province of China. *Apidologie*, **34** (6): 553-561.

Kiew, R. and Muid, M. (1991). *Beekeeping in Malaysia: pollen atlas*. Malaysian Beekeeping Research and Development Team., Kuala Lumpur

Kim, J., Lee, H. Y., Kim, M. H., Han, T. S., Cho, K. R., Kim, G. and Choi, S. H. (2007). Antinociceptive efficacy of Korean bee venom in the abdominal pain of the mouse. *Journal of Veterinary Clinics-Seoul* **24** (3): 320-324.

Konper, H. M., El-Alfy, S. H., Mansour, H. A., Barakat, E. M. and Salama, M. S. (2010). Protein analysis of venom from Carniolan, Italian and hybrid honeybees (*Apis mellifera*: Hymenoptera) by electrophoresis *African Journal of Biological Science*, **6** (1): 117-124.

Kozmus, P., Stevanović, J., Stanimirović, Z., Stojić, V., Kulišić, Z. and Meglič, V. (2007). Analysis of mitochondrial DNA in honey bees (*Apis mellifera*) from Serbia. *Acta Veterinaria*, **57** (5-6): 465-476.

Krell, R. (1996). *Value-added Products Froom Beekeeping*. Food and Agriculture Orgnaization, Rome.

Lallement, G., Fosbraey, P., Baille-Le-Crom, V., Tattersall, J. E. H., Blanchet, G., Wetherell, J. R., Rice, P., Passingham, S. L. and Sentenac-Roumanou, H. (1995). Compared toxicity of the potassium channel blockers, apamin and dendrotoxin. *Toxicology*, **104** (1–3): 47-52.

Lambert, I. H. and Hansen, D. B. (2011). Regulation of taurine transport systems by protein kinase CK2 in mammalian cells. *Cellular Physiology and Biochemistry*, **28** (6): 1099-1110.

Larkin, M., Blackshields, G., Brown, N., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A. and Lopez, R. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics*, **23** (21): 2947-2948.

Lauterwein, J., Bösch, C., Brown, L. R. and Wüthrich, K. (1979). Physicochemical studies of the protein-lipid interactions in melittin-containing micelles. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, **556** (2): 244-264.

Ludolph-Hauser, D., Ruëff, F., Fries, C., Schöpf, P. and Przybilla, B. (2001). Constitutively raised serum concentrations of mast-cell tryptase and severe anaphylactic reactions to *Hymenoptera* stings. *The Lancet*, **357** (9253): 361-362.

Lunt, D., Zhang, D. X., Szymura, J. and Hewltt, O. (1996). The insect cytochrome oxidase I gene: evolutionary patterns and conserved primers for phylogenetic studies. *Insect Molecular Biology*, **5** (3): 153-165.

Mahmood, R., Wagchoure, E. S. and Sarwar, G. (2013). Influence of supplemental diets on Apis mellifera L. colonies for honey production. *Pakistan Journal of Agriclture Research. Vol*, **26** (4): 290-294.

Marcucci, M. C. (1995). Propolis: chemical composition, biological properties and therapeutic activity. *Apidologie*, **26** (2): 83-99.

Markelov, V. V. and Trushin, M. V. (2006). Bee venom therapy and low dose naltrexone for treatment of multiple sclerosis. *Nepal Journal of Neuroscience*, **3**: 71-77.

Marz, R., Mollay, C., Kreil, G. and Zelger, J. (1981). Queen bee venom contains much less phospholipase than worker bee venom. *Insect Biochemistry*, **11** (6): 685-690.

Matysiak, J., Schmelzer, C. E., Neubert, R. H. and Kokot, Z. J. (2011). Characterization of honeybee venom by MALDI-TOF and nanoESI-QqTOF mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, **54** (2): 273-278.

Meixner, M., Leta, M., Koeniger, N. and Fuchs, S. (2011). The honey bees of Ethiopia represent a new subspecies of Apis mellifera—Apis mellifera simensis n. ssp. *Apidologie*, **42** (3): 425-437.

Mirza, Z., Pillai, V. G. and Zhong, W.-Z. (2014). Structure of N-terminal sequence Asp-Ala-Glu-Phe-Arg-His-Asp-Ser of Aβ-peptide with phospholipase A2 from venom of Andaman Cobra sub-species Naja naja sagittifera at 2.0 Å resolution. *International journal of molecular sciences*, **15** (3): 4221-4236.

Morse, D. H. (1978). Size-related foraging differences of bumble bee workers. *Ecological Entomology*, **3** (3): 189-192.

Naug, D. (2009). Nutritional stress due to habitat loss may explain recent honeybee colony collapses. *Biological Conservation*, **142** (10): 2369-2372.

Neupane, K. and Thapa, R. (2005). Alternative to off-season sugar supplement feeding of honeybees. *Journal of the Institute of Agriculture and Animal Science*, **26**: 77-81.

O'Neill, K. M., Pearce, A. M., O'Neill, R. P. and Miller, R. S. (2010). Offspring size and sex ratio variation in a feral population of alfalfa leafcutting bees (Hymenoptera: Megachilidae). *Annals of the Entomological Society of America*, **103** (5): 775-784.

O'Toole, C. and Raw, A. (1991). *Bees of the World*. Blandford Press, Maryland.

Okamoto, I., Taniguchi, Y., Kunikata, T., Kohno, K., Iwaki, K., Ikeda, M. and Kurimoto, M. (2003). Major royal jelly protein 3 modulates immune responses in vitro and in vivo. *Life Sciences*, **73** (16): 2029-2045.

Oldroyd, B., Reddy, M., Chapman, N., Thompson, G. and Beekman, M. (2006). Evidence for reproductive isolation between two colour morphs of cavity nesting honey bees (*Apis*) in South India. *Insectes Sociaux*, **53** (4): 428-434.

Oldroyd, B. P. (2007). What's killing American honey bees? *PLoS Biology*, **5** (6): e168.

Owen, M. D. (1978). Venom replenishment, as indicated by histamine, in honey bee (*Apis mellifera*) venom. *Journal of Insect Physiology*, **24** (5): 433-437.

Owen, M. D. and Braidwood, J. L. (1974). A quantitative and temporal study of histamine and histidine in honey bee (*Apis mellifera L.*) venom. *Canadian Journal of Zoology*, **52** (3): 387-392.

Owen, M. D. and Bridges, A. R. (1976). Aging in the venom glands of queen and worker honey bees (*Apis mellifera L.*): some morphological and chemical observations. *Toxicon*, **14** (1): 1-5.

Owen, M. D. and Bridges, A. R. (1982). Catecholamines in honey bee (*Apis mellifera L.*) and various vespid (Hymenoptera) venoms. *Toxicon*, **20** (6): 1075-1084.

Owen, M. D., Pfaff, L. A., Reisman, R. E. and Wypych, J. (1990). Phospholipase A_2 in venom extracts from honey bees (*Apis mellifera* L.) of different ages. *Toxicon*, **28** (7): 813-820.

Palma, M. S. (2006). *Insect Venom Peptides*. Academic Press: San Diego.

Palma, M. S. and Brochetto-Braga, M. R. (1993). Biochemical variability between venoms from different honey-bee (*Apis mellifera*) races. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, **106** (2): 423-427.

Paramás, A. M. G., Bárez, J., Marcos, C. C., García-Villanova, R. J. and Sánchez, J. S. (2006). HPLC-fluorimetric method for analysis of amino acids in products of the hive (honey and bee-pollen). *Food Chemistry*, **95** (1): 148-156.

Peng, Y., Nasr, M. and Locke, S. (1989). Geographical races of *Apis cerana* Fabricius in China and their distribution. Review of recent Chinese

publications and a preliminary statistical analysis. *Apidologie*, **20** (1): 9-20.

Peng, Z. and Pan, J. (1994). Protein content in honey and its effects on precipitation of beverage. *Food Science*, **12**: 6-8.

Pernal, S. F. and Currie, R. W. (2000). Pollen quality of fresh and 1-year-old single pollen diets for worker honey bees (*Apis mellifera L.*). *Apidologie*, **31** (3): 387-410.

Piek, T. (2013). *Venoms of the Hymenoptera: biochemical, pharmacological and behavioural aspects*. Elsevier, London

Pirk, C. W., Boodhoo, C., Human, H. and Nicolson, S. W. (2010). The importance of protein type and protein to carbohydrate ratio for survival and ovarian activation of caged honeybees (*Apis mellifera scutellata*). *Apidologie*, **41** (1): 62-72.

Radloff, S. E., Hepburn, C., Hepburn, H. R., Fuchs, S., Hadisoesilo, S., Tan, K., Engel, M. S. and Kuznetsov, V. (2010). Population structure and classification of *Apis cerana*. *Apidologie*, **41** (6): 589-601.

Radloff, S. E., Hepburn, H. R., Hepburn, C., Fuchs, S., Otis, G. W., Sein, M., Aung, H., Pham, H., Tam, D. and Nuru, A. (2005). Multivariate morphometric analysis of *Apis cerana* of southern mainland Asia. *Apidologie*, **36** (1): 127-139.

Radloff, S. E., Hepburn, R. and Fuchs, S. (2005). The morphometric affinities of Apis cerana of the Hindu Kush and Himalayan regions of western Asia. *Apidologie*, **36** (1): 25-30.

Radloff, S. E., Hepburn, H. R., Fuchs, S., Otis, G. W., Hadisoesilo, S., Hepburn, C. and Ken, T. (2005). Multivariate morphometric analysis of the Apis cerana populations of oceanic Asia. *Apidologie*, **36** (3): 475-492.

Radloff, S. E., Hepburn, R. and Bangay, L. J. (2003). Quantitative analysis of intracolonial and intercolonial morphometric variance in honeybees, *Apis mellifera* and *Apis cerana*. *Apidologie*, **34** (4): 339-352.

Remigio, E. A. and Hebert, P. D. (2003). Testing the utility of partial COI sequences for phylogenetic estimates of gastropod relationships. *Molecular Phylogenetics and Evolution*, **29** (3): 641-647.

Ricketts, T. H., Regetz, J., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., Bogdanski, A., Gemmill-Herren, B., Greenleaf, S. S., Klein, A. M. and Mayfield, M. M. (2008). Landscape effects on crop pollination services: are there general patterns? *Ecology Letters*, **11** (5): 499-515.

Roat, T. C., Nocelli, R. C. F. and da Cruz Landim, C. (2006). The venom gland of queens of *Apis mellifera* (Hymenoptera, Apidae): morphology and secretory cycle. *Micron*, **37** (8): 717-723.

Rodríguez, G., Sulbarán de Ferrer, B., Ferrer, A. and Rodríguez, B. (2004). Characterization of honey produced in Venezuela. *Food Chemistry*, **84** (4): 499-502.

Roehrdanz, R. (1993). An improved primer for PCR amplification of mitochondrial DNA in a variety of insect species. *Insect Molecular Biology*, **2** (2): 89-91.

Rortais, A., Arnold, G., Halm, M.-P. and Touffet-Briens, F. (2005). Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. *Apidologie*, **36** (1): 71-83.

Roubik, D. W., Sakagami, S. F. and Kudo, I. (1985). A note on distribution and nesting of the Himalayan honey bee *Apis laboriosa* Smith (Hymenoptera: Apidae). *Journal of the Kansas Entomological Society*, **58** (4): 746-749.

Roulston, T. A. H. and Cane, J. H. (2000). The effect of diet breadth and nesting ecology on body size variation in bees (Apiformes). *Journal of the Kansas Entomological Society*, **73** (3): 129-142.

Ruttner, F. (1988a). *Biogeography and Taxonomy of Honeybees*. Springer-Verlag, California.

Ruttner, F. (1988b). *Variation and Speciation (Biogeography and Taxonomy of Honeybees)*. Springer-Verlag, California.

Rybak-Chmielewska, H. and Szczêsna, T. (2004). HPLC study of chemical composition of honeybee (*Apis mellifera* L.) venom. *Journal of Apicultural Science* **48** (2): 103-109.

Saldarriaga, M. M., Otero, R., Núñez, V., Toro, M. F., Díaz, A. and Gutiérrez, J. M. (2003). Ontogenetic variability of *Bothrops atrox* and *Bothrops asper* snake venoms from Colombia. *Toxicon*, **42** (4): 405-411.

Scheffer, S. J. and Lewis, M. L. (2001). Two nuclear genes confirm mitochondrial evidence of cryptic species within *Liriomyza huidobrensis* (Diptera: Agromyzidae). *Annals of the Entomological Society of America*, **94** (5): 648-653.

Schmidt, J. O. and Thoenes, S. C. (1990). Honey bee (Hymenoptera: Apidae) preferences among artificial nest cavities. *Annals of the Entomological Society of America*, **83** (2): 271-274.

Schmidt, J. O., Thoenes, S. C. and Levin, M. (1987). Survival of honey bees, *Apis mellifera* (Hymenoptera: Apidae), fed various pollen sources. *Annals of the Entomological Society of America*, **80** (2): 176-183.

Schmidt, L. S., Schmidt, J. O., Rao, H., Wang, W. and Xu, L. (1995). Feeding preference and survival of young worker honey bees (Hymenoptera: Apidae) fed rape, sesame, and sunflower pollen. *Journal of Eonomic Eomology*, **88** (6): 1591-1595.

Seeley, T. D. (2009). *The Wisdom of the Hive: The Social Physiology of Honey bee Colonies*. Harvard University Press, USA.

Severson, D. and Erickson, E. (1984). Honey bee (Hymenoptera: Apidae) colony performance in relation to supplemental carbohydrates. *Journal of Economic Entomology*, **77** (6): 1473-1478.

Shipolini, R., Bradbyry, A., Callewaert, G. and Vernon, C. (1967). The structure of apamin. *Chemical Communications* (14): 679-680.

Slessor, K., Winston, M. and Le Conte, Y. (2005). Pheromone Communication in the Honeybee (*Apis mellifera L.*). *Journal of Chemical Ecology*, **31** (11): 2731-2745.

Smith, D. R. and Hagen, R. H. (1996). The biogeography of *Apis cerana* as revealed by mitochondrial DNA sequence data. *Journal of the Kansas Entomological Society*, **69** (4): 294-310.

Smith, D. R., Taylor, O. R. and Brown, W. M. (1989). Neotropical Africanized honey bees have African mitochondrial DNA. *Nature*, **339** (6221): 213-215.

Smith, D. R., Villafuerte, L., Otis, G. and Palmer, M. R. (2000). Biogeography of *Apis cerana* F. and *Apis nigrocincta* Smith: insights from mtDNA studies. *Apidologie*, **31** (2): 265-280.

Somerville, D. (2006). Lipid content of honey bee-collected pollen from south-east Australia. *Animal Production Science*, **45** (12): 1659-1661.

Somerville, D. and Nicol, H. (2006). Crude protein and amino acid composition of honey bee-collected pollen pellets from South east Australia and a note on laboratory disparity. *Animal Production Science*, **46** (1): 141-149.

Sperling, F. A. and Hickey, D. A. (1994). Mitochondrial DNA sequence variation in the spruce budworm species complex (Choristoneura: Lepidoptera). *Molecular Biology and Evolution*, **11** (4): 656-665.

Surendra, N. S., Jayaram, G. N., Reddy, M., Reddy, S. and Ravikumar, H. (2013). Comparative morphometric studies of the sting apparatus of the worker bees of four different *Apis* species (*Apis dorsata*, *Apis mellifera*, *Apis cerana* and *Apis florea*). *Journal of Apicultural Research*, **52** (2): 74-80.

Szczêsna, T. (2006). Protein content and amino acid composition of bee-collected pollen from selected botanical origins. *Journal of Apicultural Science*, **50** (2): 81-90.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, **30** (12): 2725-2729.

Tan, K., Latty, T., Hu, Z., Wang, Z., Yang, S., Chen, W. and Oldroyd, B. P. (2014). Preferences and tradeoffs in nectar temperature and nectar concentration in the Asian hive bee *Apis cerana*. *Behavioral Ecology and Sociobiology*, **68** (1): 13-20.

Tanaka, H., Roubik, D., Kato, M., Liew, F. and Gunsalam, G. (2001). Phylogenetic position of *Apis nuluensis* of northern Borneo and phylogeography of *A. cerana* as inferred from mitochondrial DNA sequences. *Insectes Sociaux*, **48** (1): 44-51.

Terwilliger, T. C., Weissman, L. and Eisenberg, D. (1982). The structure of melittin in the form I crystals and its implication for melittin's lytic and surface activities. *Biophysical Journal*, **37** (1): 353-361.

Tumrasvin, W., Kurahashi, H. and Kano, R. (1979). Studies on medically important flies in Thailand VII. Report on 42 species of Calliphorid flies, including the taxonomic keys (Diptera: Calliphoridae). *The Bulletin of Tokyo Medical and Dental University*, **26** (4): 243-272.

Wallman, J. F. and Donnellan, S. C. (2001). The utility of mitochondrial DNA sequences for the identification of forensically important blowflies (Diptera: Calliphoridae) in southeastern Australia. *Forensic Science International*, **120** (1): 60-67.

Wenner, A. M. and Johnson, D. L. (1966). Simple conditioning in honey bees. *Animal Behaviour*, **14** (1): 149-155.

Wille, A. (1983). Biology of the stingless bees. *Annual Review of Entomology*, **28** (1): 41-64.

Willis, L. G., Winston, M. L. and Honda, B. M. (1992). Phylogenetic relationships in the honeybee (Genus *Apis*) as determined by the sequence of the cytochrome oxidase II region of mitochondrial DNA. *Molecular Phylogenetics and Evolution*, **1** (3): 169-178.

Winston, M. L. (1991). *The Biology of the Honey Bee*. Harvard University Press, USA.

WuÈster, W., Daltry, J. C. and Thorpe, R. S. (1999). Can diet explain intraspecific venom variation? Reply to Sasa. *Toxicon*, **37**: 253-258.

Wykes, G. (1952). The preferences of honeybees for solutions of various sugars which occur in nectar. *Journal of Experimental Biology*, **29** (4): 511-519.

Yoirish, N. (2001). *Curative Properties of Honey and Bee venom*. University Press of the Pacific, California.

Yu, L., Ramaswamy, H. S. and Boye, J. (2013). Protein rich extruded products prepared from soy protein isolate-corn flour blends. *LWT - Food Science and Technology*, **50** (1): 279-289.

Zalat, S., Abouzeid, A., Ibrahim, A. and Abd El-Aal, M. (2002). Protein pattern of the honeybee venoms of Egypt. *Egyptian Journal of Biology*, **4**: 42-46.

Zalat, S., Nabil, Z., Hussein, A. and Rakha, M. (2004). Biochemical and haematological studies of some solitary and social bee venoms. *Egyptian Journal of Biology*, **1** (1): 57-71.

Zehner, R., Amendt, J., Schütt, S., Sauer, J., Krettek, R. and Povolný, D. (2004). Genetic identification of forensically important flesh flies (Diptera: Sarcophagidae). *International Journal of Legal Medicine*, **118** (4): 245-247.

Zheng, B., Wu, Z. and Xu, B. (2014). The Effects of Dietary Protein Levels on the Population Growth, Performance, and Physiology of Honey Bee Workers During Early Spring. *Journal of Insect Science*, **14** (1): 1-7.

Zumla, A. and Lulat, A. (1989). Honey--a remedy rediscovered. *Journal of the Royal Society of Medicine*, **82** (7): 384-385

Zheng, B., Wu, Z. and Xu, B. (2014). The Effects of Dietary Protein Levels on the Population Growth, Performance, and Physiology of Honey Bee Workers During Early Spring. *Journal of Insect Science*, **14** (1).