

CUTICULAR HYDROCARBON OF PUPAL CASES OF *Chrysomya megacephala*
(FABRICIUS) AND ITS POSSIBLE USE FOR ESTIMATING POSTMORTEM
INTERVAL

NURUL NADHIRAH BINTI NASIR

UNIVERSITI TEKNOLOGI MALAYSIA

CUTICULAR HYDROCARBON OF PUPAL CASES OF *Chrysomya megacephala*
(FABRICIUS) AND ITS POSSIBLE USE FOR ESTIMATING POSTMORTEM
INTERVAL

NURUL NADHIRAH BINTI NASIR

A dissertation submitted in partial fulfilment of the
requirements for the award of degree of
Master of Science

Faculty of Science
Universiti Teknologi Malaysia

MARCH 2017

This dissertation is especially dedicated to my late father Nasir bin Jusoh and my beloved mother Narisah binti Mohamed Yusof, siblings, close family members, friends and lecturers.

ACKNOWLEDGEMENTS

All praises to the almighty Allah SWT and blessing be upon to His Prophet SAW whom giving me guidance and patience in completing this study. My sincere gratitude for my supervisor Dr. Naji Arafat bin Mahat with advices, suggestions, patience and encouragement in completing the study. Deepest thanks also to my co-supervisors AP Dr. Jaafariah binti Jaafar and Miss Reena binti Abd. Rashid (UiTM) for their advices and supports.

A special thanks to AP Dr. Umi Kalthom binti Ahmad for all the knowledge and experience during finishing my courses studies. I also like to express my thanks to Puan Fariza, Encik Hairol and late Encik Kadir for the patient and supportive in providing knowledge in analytical laboratory. Not to forget, to Puan Nurul Hajar binti Sapireh for all the helps during my need. Thanks and grateful for my sweet friends: Anis Raihan, Syafianes, Amira, Charlotte, Lynn, Tharsha and Nurul Huda; my junior; Najwa, Fatimah, Asma, Faridah, Aida, Wan Hui and Aidil for all the encouragements, may all of you always in successful journey.

Last but not least, I wish to express my love and gratitude to my late father Nasir bin Jusoh, my mother Narisah binti Mohamed Yusof and my siblings for their understanding and endless love through my studies.

ABSTRACT

Although the pupal cases of *Chrysomya megacephala* have been commonly observed at crime scenes, its utilization for forensic investigation remains nascent. Despite several studies reporting about the use of cuticular hydrocarbons (CHCs) for estimating the age of pupal cases, they were either conducted in laboratory controlled experiments or in environmental conditions peculiar to Malaysia. Hence, this present research that examined the CHCs compositions in the pupal cases of *C. megacephala* exposed to the natural weathering process in Malaysia (up to 14 days) for providing empirical evidence for age estimation deserves forensic consideration. While confirmation of the identity of CHCs was done using Gas Chromatography- Mass Spectrometer (GC-MS), Gas Chromatography- Flame Ionization Detector (GC-FID) was used for quantifying the concentrations. Results revealed that the analysis of the hexane extract of the pupal cases of *C. megacephala* contained a mixture of odd-numbered high molecular weight aliphatic (i.e. heptacosane (*n*-C27) and nonacosane (*n*-C29)) and branched alkanes (i.e. tetradecane, 2, 6, 20-trimethyl- and octadecane, 3-ethyl-5-(2-ethylbutyl)). Significant decrease ($P < 0.05$) in the concentrations of *n*-C27 and *n*-C29 was observed, with the pattern of the decrease being highly correlated ($r > -0.926$) with that of the three subsequent weathering intervals. Such findings had enabled formulation of credible mathematical algorithms for relating the concentrations of *n*-C27 and *n*-C29 in the pupal cases of *C. megacephala* versus the natural weathering intervals, a possible means for estimating the age of such pupal cases. Therefore, the findings have to be duly considered whenever interpretations of the pupal cases need to be relied upon in crime situations with similar environmental conditions.

ABSTRAK

Walaupun kebiasaannya kepompong *Chrysomya megacephala* dicerap di tempat kejadian, penggunaanya dalam penyiasatan forensik masih lagi baru. Meskipun beberapa kajian melaporkan tentang penggunaan kutikel hidrokarbon (CHC) dalam menganggar umur kepompong, ianya dijalankan sama ada dalam kawalan ujikaji makmal ataupun dalam keadaan persekitaran yang berbeza dengan Malaysia. Justeru, pengkajian terhadap komposisi CHC dalam kepompong *C. megacephala* yang terdedah kepada proses luluhawa semulajadi di Malaysia (selama 14 hari) bagi memberikan bukti empirikal untuk menganggar umur dalam kajian ini memerlukan kepentingan forensik. Pengesahan identiti CHC telah dilakukan dengan menggunakan Gas Kromatografi-Spektrometer Jisim (GC-MS) manakala Gas Kromatografi-Pengesan Pengionan Nyala (GC-FID) digunakan untuk menentukan kepekatan. Hasil kajian ini menunjukkan bahawa analisis ekstrak heksana daripada kepompong *C. megacephala* mengandungi campuran alifatik molekul berat yang bernombor ganjil (iaitu heptakosana (*n*-C27) dan nonakosana (*n*-C29)) dan alkana bercabang (iaitu tetradekana, 2, 6, 20-trimetil- dan oktadekana, 3-etil-5-(2-etilbutil)). Kepekatan *n*-C27 dan *n*-C29 serta corak penurunan korelasi yang tinggi ($r > -0,926$) untuk tiga jeda luluhawa berikutnya menunjukkan penurunan yang signifikan ($P < 0,05$). Penemuan tersebut membolehkan perumusan algoritma matematik diterima bagi mengaitkan kepekatan *n*-C27 dan *n*-C29 dalam kepompong *C. megacephala* dengan jeda luluhawa semula jadi untuk menganggarkan usia kepompong tersebut. Oleh itu, penemuan ini perlu dipertimbangkan dengan sewajarnya setiap kali interpretasi tentang kepompong perlu digunapakai dalam situasi jenayah dengan keadaan persekitaran yang sama.

TABLE OF CONTENTS

CHAPTER	TITLE	PAGE
	DECLARATION	ii
	DEDICATION	iii
	ACKNOWLEDGEMENT	iv
	ABSTRACT	v
	ABSTRAK	vi
	TABLE OF CONTENTS	vii
	LIST OF TABLES	x
	LIST OF FIGURES	xi
	LIST OF ABBREVIATIONS	xiii
	LIST OF SYMBOLS	xv
	LIST OF APPENDIX	xvi
1	INTRODUCTION	
	1.1 Background of the Study	1
	1.2 Problem Statement	3
	1.3 Objectives and Hypotheses	3
	1.4 Scope of the Study	4
	1.5 Significance of the Study	5
2	LITERATURE REVIEW	
	2.1 The Use of The Use of <i>C. megacephala</i> as an Indicator for Estimating PMI	6

CHAPTER	TITLE	PAGE
	2.2 Research on Cuticular Hydrocarbon of Insect	9
	2.3 Effect of Weathering Process towards Hydrocarbon Profile	10
	2.4 Analytical Methods to Detect Cuticular Hydrocarbon	11
 MATERIALS AND METHOD		
	3.1 Chemicals and Reagents	13
	3.2 Laboratory Wares	13
	3.3 Instrumentation	14
	3.4 Method	14
	3.4.1 Experimental Design	14
	3.5 Chemical analysis	16
	3.5.1 Preparation of Standard Stock Solution <i>n</i> -C21 – <i>n</i> -C40	16
	3.5.2 Sample pre-treatment	16
	3.5.3 Analytical Instrumentation	17
	3.5.4. Partial Bio-analytical Method Validation	18
	3.6 Data Analysis	18
 4	 RESULTS AND DISCUSSION	
	4.1 Partial Validation of Analytical Method	20
	4.1.1 Calibration Curve, LOD and LLOQ	21
	4.2 Analysis of CHC Compositions in Pupal Cases of <i>C. megacephala</i>	29
	4.2.1 Climatological Conditions	31
	4.2.2 Changes of CHC Profiles in the Pupal Cases over the Stipulated Weathering Intervals	33

CHAPTER	TITLE	PAGE
	4.2.3 Concentration of Two Prevailing Hydrocarbons in the CHC Compositions of <i>C. megacephala</i> Pupal Cases and Its Association with Weathering Intervals	36
5	CONCLUSION AND RECOMMENDATIONS	
	5.1 Conclusion	40
	5.2 Recommendations	41
	REFERENCES	42
	APPENDIX	
	Appendix A	48
	Appendix B	51

LIST OF TABLES

TABLE NO.	TITLE	PAGE
2.1	The prevailing studies in investigating CHCs compositions in various groups of insects with suitable methods	12
3.1	The analytical conditions were used for GC-FID and GC-MS.	17
4.1	Analytical figures of merit, (Coefficient of determination (R^2), LOD and LLOQ for hydrocarbons analysed.	29
4.2	Daily ambient temperature, relative humidity and total rainfall recorded during the observation period (16 th -30 th November 2016)	32
4.3	Mean (\pm SD) relative abundance of CHCs in pupal cases of <i>C. megacephala</i> exposed to three natural weathering intervals.	35
4.4	Concentrations of heptacosane (<i>n</i> -C27) and nonacosane (<i>n</i> -C29) at three different weathering intervals.	37
4.5	Association between the concentrations of <i>n</i> -C27 and <i>n</i> -C29 in the pupal cases of <i>C. megacephala</i> with that of weathering intervals.	39

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE
1.1	Conceptual framework of this research	5
3.1	Location of decomposition and burial site.	15
3.2	Sample of pupal cases of <i>C. megacephala</i> collected from soil surrounding a decomposing beef substrate.	15
3.3	Flow chart of the overall research work.	19
4.1	Chromatogram (GC-MS) obtained for <i>n</i> -C21- <i>n</i> -C40 standard mixture. Heptacosane (<i>n</i> -C27) and nonacosane (<i>n</i> -C29) were used for comparison and regressional studies.	22
4.2	Chromatogram (GC-FID) obtained for <i>n</i> -C21- <i>n</i> -C40 standard mixture. Heptacosane (<i>n</i> -C27) and nonacosane (<i>n</i> -C29) were used for comparison and regressional studies.	23
4.3	Representative chromatogram (GC-MS) of CHC for <i>C. megacephala</i> pupal case at Day-0. Numbers on peaks represent the CHC detected. Heptacosane (<i>n</i> -C27) and nonacosane (<i>n</i> -C29) were used for comparison and regressional studies.	24
4.4	Representative chromatogram (GC-FID) obtained from a sample of <i>C. megacephala</i> pupal case at day-0. Heptacosane (<i>n</i> -C27) and nonacosane (<i>n</i> -C29) were used for comparison and regressional studies.	25

FIGURE NO.	TITLE	PAGE
4.5	Mass spectrum for heptacosane (<i>n</i> -C27) from (a) the NIST library and (b) a sample. As suggested by Miuc <i>et al.</i> (2015), <i>m/z</i> 85 and 380 were used as the major and confirmatory ions, respectively.	26
4.6	Mass spectrum for nonacosane (<i>n</i> -C29) from (a) the NIST library and (b) a sample. As suggested by Miuc <i>et al.</i> (2015), <i>m/z</i> 85 and 408 were used as the major and confirmatory ions, respectively.	27
4.7	Calibration curve for heptacosane (<i>n</i> -C27)	28
4.8	Calibration curve for nonacosane (<i>n</i> -C29)	28

LIST OF ABBREVIATIONS

ANOVA	-	Analysis of Variance
CHC	-	Cuticular Hydrocarbon
cm	-	Centimetre
e.g.	-	Example
FTIR	-	Fourier Transform Infrared
GC-FID	-	Gas Chromatography- Flame Ionization Detector
GC-MS	-	Gas Chromatography- Mass Spectrometer
i.d.	-	Internal diameter
i.e.	-	Such as
LLOQ	-	Lower Limit of Quantitation
LOD	-	Limit of Detection
mg/L	-	Milligram per litre
mins	-	Minutes
mL	-	Millilitre
mm	-	Millimetre
m/z	-	Mass-to-charge ratio
NIST	-	National Institute of Standards and Technology
pH	-	Potential Hydrogen
PMI	-	Postmortem Interval
RSD	-	Relative standard deviation
SD	-	Standard deviation
UK	-	United Kingdom
µL	-	Microlitre
µM	-	Micrometre

$\mu\text{g/mL}$	-	Microgram per millilitre
USA	-	United State America
USFDA	-	US Food and Drug Admnistration
UTM	-	Universiti Teknologi Malaysia

LIST OF SYMBOLS

$^{\circ}\text{C}$	-	Degree Celsius
%	-	Percentage
>	-	More than
\leq		Less than or equal to
<	-	Less than

LIST OF APPENDIX

APPENDIX	TITLE	PAGE
A	Mean (\pm SD) relative abundance of other compounds in pupal cases of <i>C. megacephala</i> exposed to three natural weathering intervals	48
B	SPSS Data Output	51

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Forensic entomology deals with utilization of insect evidence in providing accurate estimation of minimum postmortem interval (PMI) from the developmental patterns of necrophagous insects (Gennard, 2007; Mahat *et al.*, 2014). In Peninsular Malaysia, *Chrysomya megacephala* (Fabricius) has been reported as the first and dominant necrophagus species infesting dead bodies and/or animal models followed by *Chrysomya rufifacies* (Macquart) (Lee *et al.*, 2004; Rajagopal *et al.*, 2013; Mahat and Jayaprakash, 2013). Being the first infesting necrophagous species, estimation of minimum PMI using the developmental pattern of *C. megacephala* may prove as the most appropriate in Malaysia context.

Studies have indicated that the duration for completing the life cycle for *C. megacephala* ranges between 7.25 to 8 days (Lee 1989, Mahat *et al.*, 2009; Mahat and Jayaprakash, 2013); beyond which estimation of PMI has to rely on the developmental patterns of the subsequent necrophagous insects such as *C. rufifacies*. It is pertinent to indicate here that upon completion of the life cycle, pupal cases of *C. megacephala* are commonly found at crime scenes; however, the evidential value

of pupal cases for estimating PMI remains scarcely reported in the body of literature. In this context, developing a means for assessing the age of those pupal cases may be useful for estimating the minimum PMI, considering the fact that *C. megacephala* being the first ovipositing necrophagus insect in corpses/ animal models in many countries including Malaysia. Although the application of cuticular hydrocarbon (CHC) analysis for estimating the age of pupal cases of *C. megacephala* (Zhu *et al.*, 2007; 2013) and larvae of *C. rufifacies* (Zhu *et al.*, 2006) has been suggested, these studies pertained to either a laboratory setting or without adequate indication on the ambient conditions where the field experiments were conducted.

While reporting on the regular changes of CHC composition in the pupal cases of *C. megacephala*, range of the means of daily ambient temperatures (9.4-22.8 °C) and relative humidity, as well as the overall total rainfall for the 90 days of sampling duration in China, Zhu *et al.* (2013) did not report the actual daily ambient data and the total daily rainfall in the field experiment that they conducted. In contrast to the description of the general conditions that they described (Zhu *et al.*, 2013), the ambient temperature in the low land areas of Malaysia has been reported to range between 21.5 °C to 36.3 °C, with rain being a commonplace occurrence (Malaysian Meteorological Department, 2015). Considering the migratory behaviour of the post-feeding larvae of *C. megacephala* and their tendency to bury themselves into the surrounding soil for pupating (Omar *et al.*, 1994), findings reported by Zhu *et al.* (2013) by which the pupal cases 'was placed on the grass land with sandy soil and some shrubs' may not reflect the natural condition of crime scene in Malaysia. In addition, different habitats may have different composition of soil types that may potentially influence the CHC compositions of pupal cases of necrophagous insects.

1.2 Problem Statement

Due to the possible use of CHC profile of the pupal cases of *C. megacephala* in estimating minimum PMI, and since the existing studies (Zhu *et al.*, 2007; 2013) were conducted in environmental temperatures peculiar to Malaysia (i.e. 9.4-22.8°C) with no indication on the daily total rainfall, generalizing the influence of such weathering process on the CHC profiles of pupal cases of *C. megacephala* in Malaysia cannot be assumed. Therefore, this present research aiming at providing the first ever empirical data for exploring the possible use of CHC compositions in the pupal cases of *C. megacephala* exposed to natural weathering conditions prevailing in Malaysia for forensic use, acquires significance.

1.3 Objectives and Hypotheses

This research that investigated the CHC compositions of the pupal cases of *C. megacephala* exposed to the natural weathering process at varying intervals (i.e. the day of discovery (day-0), as well as at day-7 and day-14 of exposure) was designed to:

- 1) Study the changes of CHC profiles over the stipulated weathering intervals.
- 2) Compare the concentrations of the two prevailing hydrocarbons in the CHC compositions i.e. heptacosane (*n*-C27) and nonacosane (*n*-C29) over the stipulated weathering intervals.

- 3) Correlate the concentrations of the two prevailing hydrocarbons i.e. heptacosane (*n*-C₂₇) and nonacosane (*n*-C₂₉) with that of the weathering intervals for providing credible mathematical algorithms for estimating the age of pupal cases.

It was hypothesized that:

- 1) There would be significant differences in the concentrations of the two prevailing hydrocarbons in the pupal cases of *C. megacephala* exposed to the natural weathering process over the three weathering intervals.
- 2) The associations between the two prevailing hydrocarbons versus that of weathering intervals would be statistically significant and highly correlated.

1.4 Scope of the Study

This research utilized pupal cases of the emerged *C. megacephala* tenerals, obtained from decomposing beef substrates in a sunlit habitat within the UTM Johor Bahru Campus during November 2016. Immediately upon the observation of the completion of life cycle (day-0), a sample (8 pupal cases each) was collected and analyzed for its CHC compositions (*n*-C₂₁-*n*-C₄₀). Further collections of the pupal cases in soil surrounding the substrates at day-7 and day-14 of exposure were made and analyzed. While confirmation of the identity of CHC was done using Gas Chromatography-Mass Spectrometer (GC-MS), Gas Chromatography-Flame Ionization Detector (GC-FID) was used for quantitative analysis. Partial validation (calibration curves, limit of detection (LOD) and lower limit of quantitation (LLOQ)) of the analytical figures of merit was also attempted. For facilitating better discussion, data of the ambient temperature, relative humidity as well as total rainfall were recorded daily.

1.5 Significance of the Study

The baseline empirical data of CHC composition in the pupal cases of *C. megacephala* reported here may prove useful for elucidating the real potential of CHC profiling, following the natural weathering process commonplace in Malaysia, for estimating the minimum PMI. The findings of this research would also pave the way to the utilization of pupal cases that are commonly found at crime scenes as valuable pieces of physical evidence during death investigations. Figure 1.1 represents the conceptual framework of this present research.

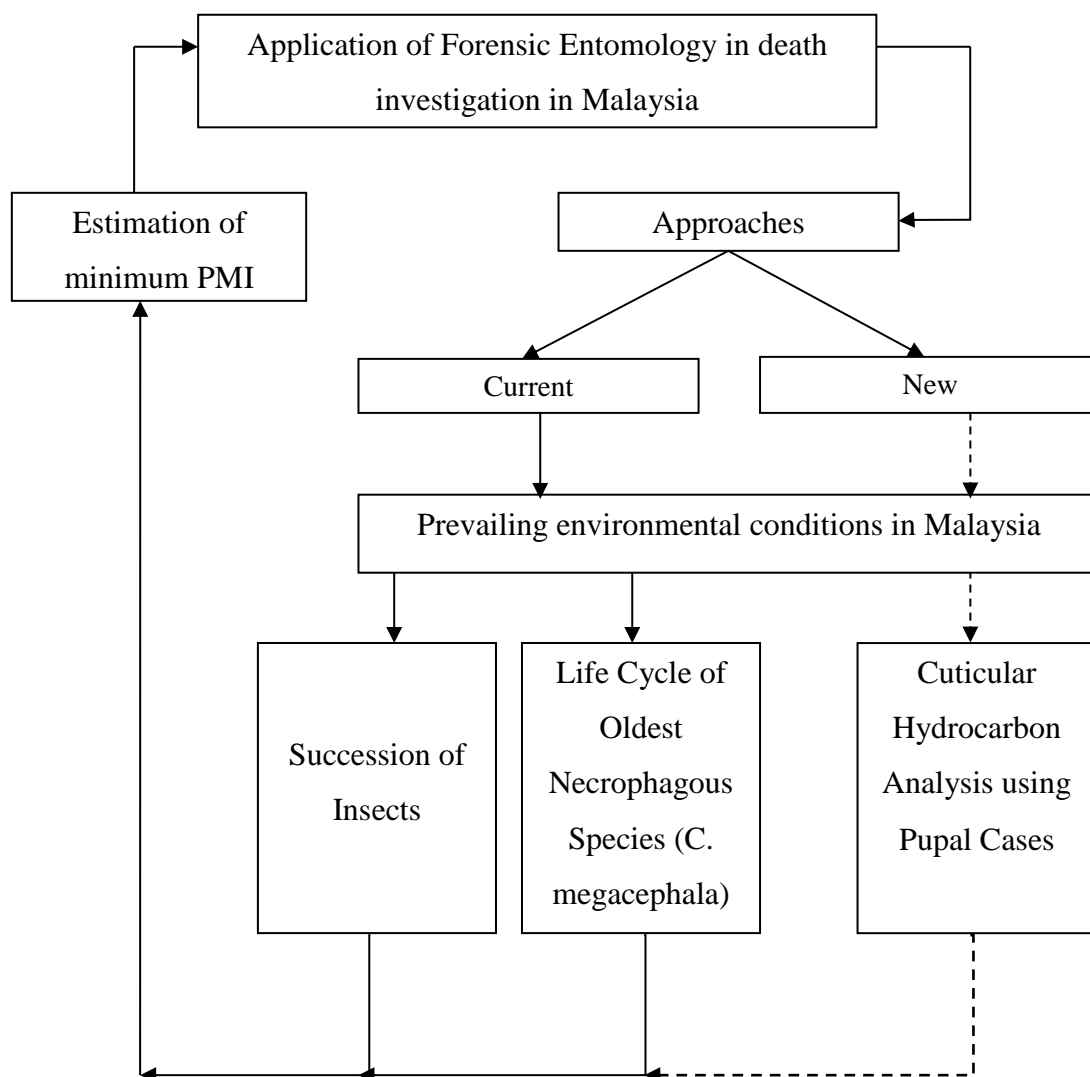


Figure 1.1: Conceptual framework of this research

REFERENCES

- Berthe-Corti, L. and Fetzner, S. (2002). Bacterial Metabolism of n-Alkanes and Ammonia under Oxidic, Suboxic and Anoxic Conditions. *Acta Biotechnologica*. 22(3-4), 299-336.
- Bettelheim, F.A., Brown, W.H., Campbell, M.K., Farrell, S.O., and Torres, O. (2012). *Introduction to General, Organic and Biochemistry*. (10th ed.). USA: Mary Finch.
- Buczowski, G., Kumar, R., Suib, S., and Silverman, J. (2005). Diet-Related Modification of Cuticular Hydrocarbon Profiles of the Argentine Ant, *Linepithema humile*, Diminishes Intercolony Aggression. *Journal of Chemical Ecology*. 31(4), 829-843.
- Boroczky, K., Park, K., Minard, R., Jones, T., Baker, T., and Tumlinson, J. (2008). Differences in cuticular lipid composition of the antennae of *Helicoverpa zea*, *Heliothis virescens*, and *Manduca sexta*. *Journal of Insect Physiology*. 54(10-11), 1385-1391.
- Cruz, A.M. (2006). *Crime Scene Intelligence: An Experiment in Forensic Entomology*. The National Defense Intelligence College Press.
- Dani, F.R. (2006). Cuticular Lipids as Semiochemicals in Paper Wasps and Other Social Insects. *Ann. Zool. Fennici*. 43, 500-514.
- Dibble, J.T. and Bartha, R. (1979). Effect of Environmental Parameters on the Biodegradation of Oil Sludge. *Applied and Environmental Microbiology*. 37(4), 729-739.
- Ferreira-Caliman, M., Nascimento, F., Turatti, I., Mateus, S., Lopes, N., and Zucchi, R. (2010). The Cuticular Hydrocarbons Profiles in the Stingless Bee *Melipona Marginata* Reflect Task-Related Differences. *Journal of Insect Physiology*. 56(7), 800-804.
- Freund, M., Csikos, R., Keszthelyi, R., and Mozes, G.Y. (1982). Chemical, Crystallographical and Physical Properties of Liquid Paraffins and Paraffin Waxes In *Paraffin Products: Properties, Technologies, Applications*. (pp. 91). New York. Elsevier Scientific Pub. Co.

- Frere, B., Suchaud, F., Bernier, G., Cottin, F., Vincent, B., and Dourel, L. (2014). GC-MS Analysis of Cuticular Lipids in Recent and Older Scavenger Insect Puparia. An Approach to Estimate the Postmortem Interval (PMI). *Analytical and Bioanalytical Chemistry*. 406(4), 1081-1088.
- Gennard, D.E. (2007). *Forensic Entomology- An Introduction*. New Jersey: John Wiley & Sons.
- Gibbs, A.G., and Crockett, E.L. (1998). The Biology of Lipids: Integrative and Comparative Perspectives. *Amer Zool*. 38(2), 265-267.
- Greenberg, B., and Kunich, J.C. (2002). *Entomology and the Law-Flies as Forensic Indicators*. Cambridge: Cambridge University Press.
- Haverty, M.I., Page, M., and Blomquist, G.J. (1988). Value of Cuticular Hydrocarbons for Identifying Morphologically Similar Species of Pine Cone Beetles. *Proceedings of the Cone and Insects Working Party Conference*. 26-30 June. Victoria, B.C., Canada, 50-62.
- Hejazi, R.F. and Husain, T. (2004). Landfarm Performance under Arid Conditions: 2. Evaluation of Parameters. *Environ. Sci. Technol*. 38(8), 2457-2469.
- Ingleby, F.C., Hosken, D.J., Flowers, K., Hawkes, M.F., Lane, S.M., Rapkin, J., House, C.M., Sharma, M.D. and Hunt, J. (2014). Environmental Heterogeneity, Multivariate Sexual Selection and Genetic Constraints on Cuticular Hydrocarbons in *Drosophila simulans*. *Journal of Evolutionary Biology*. 27(4), 700-713.
- Ingleby, F. (2015). Insect Cuticular Hydrocarbons as Dynamic Traits in Sexual Communication. *Insects*. 6(3), 732-742.
- Lee, H.L. (1989). Recovery of forensically important entomological specimens from human cadavers in Malaysia-an update. *Malaysian Journal of Pathology*. 11, 33- 36.
- Lee, H.L., Krishnasamy, M., Abdullah, A.G. and Jeffery, J. (2004). Review of Forensically Important Entomological Specimens in the Period of 1972-2002. *Tropical Biomedicine*. 21(2). 69-75.
- Lombaert, G.A. (2002). Methods for the Determination of Deoxynivalenol and other Trichothecenes. In DeVries, J.W., Trucksess, M.W. and Jackson, L.S. (2002). *Mycotoxins and Food Safety*. (pp. 141-154). New York: Kluwer Academic/Plenum Publishers.

- Mahat, N., Zafarina, Z., and Jayaprakash, P.T. (2009). Influence of Rain and Malathion on the Oviposition and Development of Blowflies (Diptera: Calliphoridae) Infesting Rabbit Carcasses in Kelantan, Malaysia. *Forensic Science International*. 192(1-3), 19-28.
- Mahat, N.A., and Jayaprakash, P.T. (2013). Forensic Entomology in Malaysia: A Review. *Malaysian Journal of Forensic Sciences*. 4(1), 1-6.
- Mahat, N.A., Yin, C.L., and Jayaprakash, P.T. (2014). Influence of Paraquat on *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) Infesting Minced-beef Substrates in Kelantan, Malaysia. *Journal of Forensic Sciences*. 59(2), 529-532.
- Martin, S. and Drijfhout, F. (2009). A Review of Ant Cuticular Hydrocarbons. *Journal of Chemical Ecology*. 35, 1151-1161.
- Martin, B. (2010). Beyond Reasonable Doubt. *Conference of Supreme and Federal Court Judges*. 26 January. Canberra, Australia. 225-251.
- Malaysian Meteorological Department (2015, December 3). Retrieved from the Malaysia Meteorological Department website: <http://www.met.gov.my>.
- Miuc, A., Voncina, E. and Lesnik U. (2015). Composition of Organic Compounds Adsorbed on PM10 in the Air Above Maribor. *Acta Chim. Slov.* 62, 834-848.
- Moore, H.E., Adam, C.D. and Drijfhout, F.P. (2014). Identifying 1st Instar Larvae for Three Forensically Important Blowfly Species using “Fingerprint” Cuticular Hydrocarbon Analysis. *Forensic Science International*. 240, 48-53.
- Munro, B.Z. (2005). *Correlation in Statistical Methods for Health Care Research*. (5th ed.). Philadelphia: Lippincott Williams & Wilkins.
- Omar, B., Marwi, M.A., Oothuman, P. and Othman, H.F. (1994). Observations on the Behaviour of Immatures and Adults of Some Malaysian Sarcosaprophagous Flies. *Tropical Biomedicine*. 11: 149-153.
- Pechal, J.L., Moore, H., Drijfhout, F., and Benbow, M.E. (2014). Hydrocarbon Profiles Throughout Adult Calliphoridae Aging: A Promising Tool for Forensic Entomology. *Forensic Science International*. 245, 65-71.
- Rajagopal, K., Wasi, A.N., Tan, T.C., Lee, H.L., and Mohd Sofian, A. (2013). Review of Forensically Important Entomological Specimens Collected from Human Cadavers in Malaysia (2005-2010). *Journal of Forensic and Legal Medicine*. 20, 480-482.

- Rashid, R.A., Zulkifli, N.F., Rashid, R.A., Rosli, S.F., Sulaiman, S.H. and Nazni, W.A. (2012). Effects of Ketum Extract on Blowfly *Chrysomya megacephala* development and detection of mitragynine in larvae sample. *IEEE Symposium on Bussiness, Engineering and Industrial Application (ISBEIA)*. 23-26 September. Bandung, Indonesia, 337-341.
- Rashid, A.R., Siti A.S., Siti, F.R., Reena, A.R., Sharifah, H.S.S., Nurul, F.Z., and Nazni, W.A. (2013). Forensic Implications of Blowfly *Chrysomya rufifacies* (*Calliphoridae: Diptera*) Development Rates Affected by Ketum Extract. *International Journal of Medical, Health, Biomedical, Bioengineering and Pharmaceutical Engineering*. 7(7), 70-74.
- Roux, O., Gers, C., and Legal, L. (2008). Ontogenetic Study of Three Calliphoridae of Forensic Importance Through Cuticular Hydrocarbons Analysis. *Medical and Veterinary Entomology*. 22, 309-317.
- Sarkar, N., Mukherjee, A., and Barik, A. (2013). Long-chain Alkanes: Allelochemicals for Host Location by the Insect Pest, *Epilachna dodecastigma* (Coleoptera: Coccinellidae). *Appl Entomol Zool*.48, 171-179.
- Savarit, F. and Ferveur, J.F. (2002). Temperature affects the Ontogeny of Sexually Dimorphic Cuticular Hydrocarbons in *Drosophila melanogaster*. *The Journal of Experimental Biology*. 205(20), 3241-3249.
- Serrano, A., Gallego, M., Gonzalez, J.L. and Tejada, M. (2008). Natural Attenuation of Diesel Aliphatic Hydrocarbons in Contaminated Agricultural Soil. *Environmental Pollution*. 151, 494-502.
- Sevala, V.L., Bagneres, A.G., Kuenzli, M., Blomquist, G.J., and Schal, C. (2000). Cuticular Hydrocarbons of the Dampwood Termite, *Zootermopsis nevadensis*: Caste Differences and Role of Lipophorin in Transport of Hydrocarbons and Hydrocarbon Metabolites. *Journal of Chemical Ecology*. 26(3), 765-789.
- Sharanowski, B.J., Walker, E.G. and Anderson, G.S. (2008). Insect Succession and Decomposition Patterns on Shaded and Sunlit Carrion in Saskatchewan in Three Different Seasons. *Forensic Science International*. 179, 219-240.
- Sharma, A., Kumar, P. and Rehman, M.B. (2014). Biodegradation of Diesel Hydrocarbon in Soil by Bioaugmentation of *Pseudomonas aeruginosa*: A Laboratory Scale Study. *International Journal of Environmental Bioremediation & Biodegradation*. 2(4), 202-212.

- Sharma, R., Garg, R.K., and Gaur, J.R. (2015). Various Methods for the Estimation of the Postmortem Interval from Calliphoridae: A review. *Egyptian Journal of Forensic Sciences*. 5, 1-12.
- Shrivastava, A. and Gupta, V.B. (2011). Methods of the Determination of Limit of Detection and Limit of Quantitation of the Analytical Methods. *Chron Young Sci*. 2(1), 21-25.
- Smith, K.G.V. (1986). *A Manual of Forensic Entomology*. New York: British Museum (Natural History) and Cornell University Press.
- Tiwari, G. and Tiwari, R. (2010). Bioanalytical Method Validation: An Updated Review. *Pharmaceutical Methods*. 1(1), 25-38.
- Tregenza, T., Buckley, S.H., Pritchard, V.L. and Butlin, R.K. (2000). Inter- and Intra-population Effects of Sex and Age on Epicuticular Composition of Meadow Grasshopper, *Chorthippus parallelus*. *Journal of Chemical Ecology*. 26(1), 257-278.
- Trewin, B. (2014). *The climates of the Tropics and How They are Changing*. In Harding, S., McComiskie, R., Wolff, M., Trewin, D., and Hunter, S. (Eds.). State of the Tropics 2014 Report (pp. 39-51). Australia, James Cook University.
- USFDA (2001). *Guidance for Industry: Bioanalytical Method Validation*. U.S. Department of Health and Human Services Food and Drug Administration-Center for Drug Evaluation and Research (CDER)-Center for Veterinary Medicine (CVM).
- Vincent, J.F.V. and Wegst, U.G.K. (2004). Design and Mechanical Properties of Insect Cuticle. *Arthropod Structure & Development*. 33, 187-199.
- Wagoner, K.M., Lehmann, T., Huestis, D., Ehrmann, B.M., Cech, N.B. and Wasserberg, G. (2014). Identification of Morphological and Chemical Markers of Dry- and Wet-season Conditions in Female *Anopheles gambiae* mosquitoes. *Parasites & Vectors*. 7(294), 1-13.
- Wagner, D., Tissot, M. and Gordon, D. (2001) Task-related Environment Alters the Cuticular Hydrocarbon Composition of Harvester Ants. *Journal of Chemical Ecology*. 27(9), 1805-1819.
- Wang, Z., Fingas, M., Lambert, P., Zeng, G., Yang, C., and Hollebone, B. (2004). Characterization and Identification of the Detroit River Mystery Oil Spill. *J. Chromatogr. A*. 1038, 201-214.

- Ye, G., Li, K., Zhu, J.Y., Zhu, G.H. and Hu, G. (2007). Cuticular Hydrocarbon Composition in Pupal Exuviae for Taxonomic Differentiation of Six Necrophagous Flies. *Journal of Medical Entomology*. 44(3), 451-456.
- Young, H.P., Larabee, J.K., Gibbs, A.G. and Schall, C. (2000). Relationship between Tissue-specific Hydrocarbon Profiles and Lipid Melting Temperatures in the Cockroach *Blattella germanica*. *Journal of Chemical Ecology*. 26(5), 1245-1263.
- Zhu, G.H., Ye, G.Y., Hu, C., Xu, X.H. and Li, K. (2006). Development Changes of Cuticular Hydrocarbons in *Chrysomya rufifacies* Larvae: Potential for Determining Larval Age. *Medical and Veterinary Entomology*. 20, 438-444.
- Zhu, G.H., Xu, X.H., Yu, X.J., Zhang, Y., and Wang, J.F. (2007). Puparial Case Hydrocarbons of *Chrysomya megacephala* as an indicator of the Postmortem Interval. *Forensic Science International*. 169(1), 1-5.
- Zhu, G.H., Yu, X.J., Xie, L.X., Luo, H., Wang, D., Lv, J.Y. and Xu, X.H. (2013). Time of Death Revealed by Hydrocarbons of Empty Puparia of *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae): A Field Experiment. *PLoS ONE*. 8(9), 1-7.