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

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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.

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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.

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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

µg/mL	-	Microgram per millilitre
USA	-	United State America
USFDA	-	US Food and Drug Admnistration
UTM	-	Universiti Teknologi Malaysia

LIST OF SYMBOLS

°C	-	Degree Celsius
%	-	Percentage
>	-	More than
≤		Less than or equal to
<	-	Less than

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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*-C_{21}-*n*-C_{40}). 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.

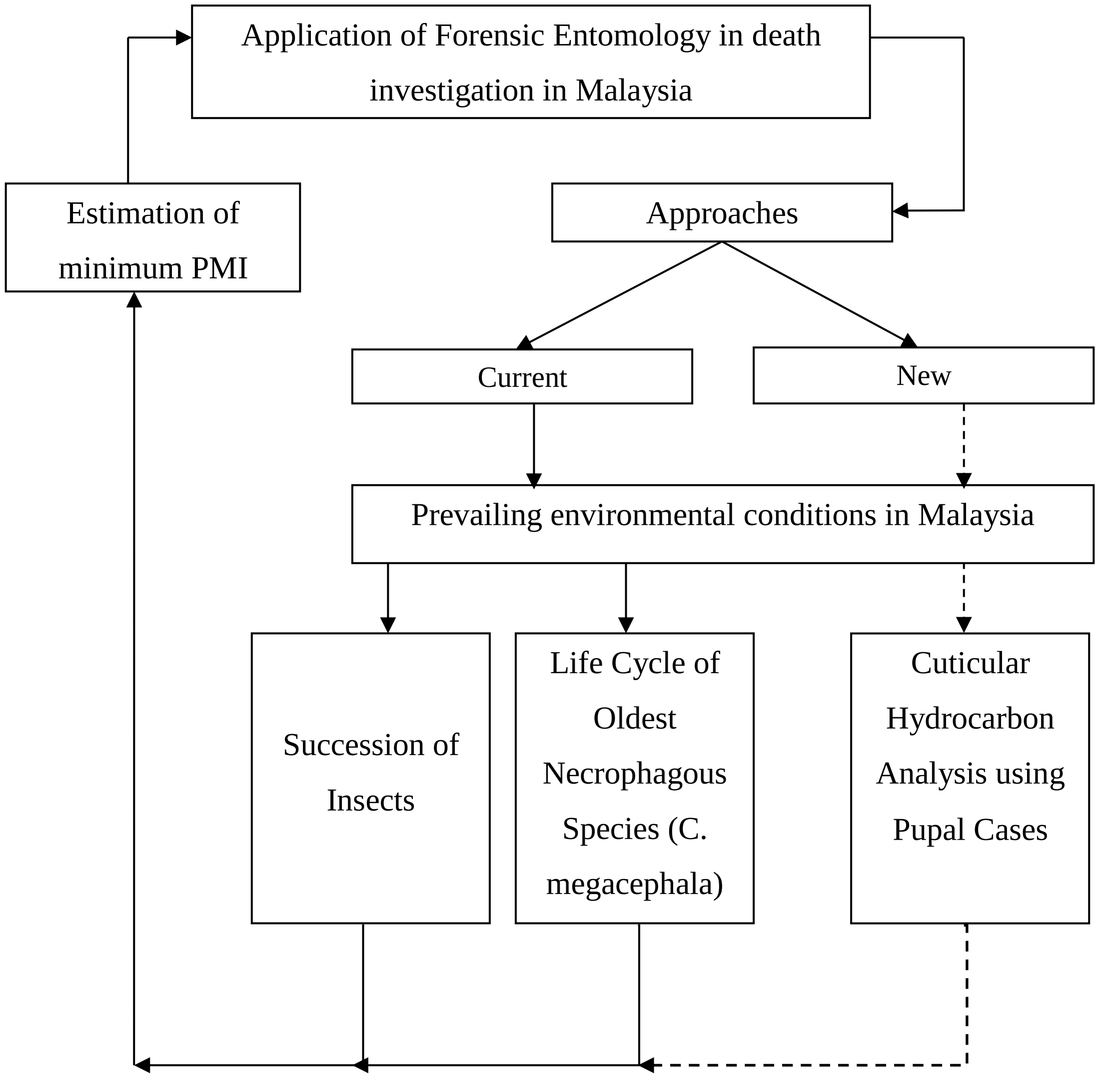

Figure 1.1: Conceptual framework of this research

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