DETECTION AND EFFECTS OF METHAMPHETAMINE ON THE LIFECYCLE OF *SARCOPHAGIDAE* IN MALAYSIA

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DETECTION AND EFFECTS OF METHAMPHETAMINE ON THE LIFECYCLE OF *SARCOPHAGIDAE* IN MALAYSIA

by

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requirements for the degree of

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CERTIFICATE

This is to certify that the dissertation entitled 'Detection and Effects of Methamphetamine on the Lifecycle of *Sarcophagidae* in Malaysia' is the bona fide record of research work done by YONG ZI LIAN, 158943 during the period of September 2024 to February 2025 under my supervision. I have read this dissertation and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation to be submitted in partial fulfilment for the degree of Bachelor of Science (Honours) (Forensic Science).

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DECLARATION

I hereby declare that this dissertation is the results of my own investigations, except where otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research and promotional purpose.

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LIST OF ABBREVIATION AND SYMBOLS

ATS	Amphetamine-type stimulants
B. peregrina	*Boettcherisca peregrina*
C. albiceps	*Chrysomya albiceps*
cm	Centimetre
CNS	Central nervous system
C. putoria	*Chrysomya putoria*
R^2	Coefficient of determination
CYP2D	Cytochrome P450 2D
CYP3A	Cytochrome P450 3A
°C	Degree Celsius
et al.	*et alia* (“and others”)
GC	Gas Chromatography
GC-FID	Gas Chromatography-Flame Ionization Detector
HPLC-MS	High-performance Liquid Chromatography-Mass Spectrometry
i.e.	*id est* (“that is”)
kg	Kilogram
L. sericata	*Lucilia sericata*

M	Molarity
MA	Methamphetamine
MET Malaysia	Malaysian Meteorological Department
mg	Milligram
min	Minute
mL	Millilitre
µg	Microgram
µL	Microlitre
µm	Micrometre
mol	Mole
mm	Millimetre
PMI min	Minimum post-mortem interval
ng	Nanogram
N/A	Not available
/	Per
pA	Peak area
pK_a	Acid dissociation constant
PMI	Post-mortem interval

PPSK	*Pusat Pengajian Sains Kesihatan*
RSD	Relative standard deviation
$Å^2$	Square angstrom
SD	Standard deviation
L3	3^{rd} instar larva
US	United State
V	Volume

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DETECTION AND EFFECTS OF METHAMPHETAMINE ON THE LIFECYCLE OF *SARCOPHAGIDAE* IN MALAYSIA

ABSTRAK

Siasatan forensik sering menghadapi cabaran apabila menentukan masa dan punca kematian, terutamanya dalam kes di mana mayat ditemui dalam peringkat penguraian yang lanjut. Entomologi forensik, iaitu kajian serangga yang menjajah mayat yang sedang terurai, telah menjadi alat penting dalam siasatan forensik, memberikan wawasan berharga mengenai selang waktu selepas kematian (PMI) dan punca kematian. Satu bidang yang lebih khusus, entomotoksikologi forensik, memberi tumpuan kepada penggunaan serangga untuk mengesan dadah dan bahan kimia lain dalam tisu yang sedang terurai. Serangga, terutamanya larva, memainkan peranan penting dalam penilaian PMI kerana mereka mengumpul bahan-bahan daripada mayat yang sedang terurai, menawarkan kaedah yang lebih sensitif untuk mengesan toksin dengan gangguan yang lebih sedikit daripada produk sampingan penguraian. Peningkatan penggunaan methamphetamine (MA) telah menyebabkan banyak kematian akibat overdosis, di mana mayat biasanya ditemui selepas beberapa hari penguraian. Kelewatan ini menyukarkan siasatan forensik. Untuk menangani hal ini, analisis entomotoksikologi forensik menggunakan serangga semakin digunakan untuk mengesan kehadiran dadah seperti MA dalam tisu yang sedang terurai. Dalam kajian ini, hati babi dicincang digunakan untuk mensimulasikan proses penguraian dan menarik serangga nekrofag, terutamanya Sarcophagidae. Hati dicincang dengan dua kepekatan MA (5 ng/mg dan 10 ng/mg) untuk mengkaji impaknya terhadap kitaran hidup dan morfologi Sarcophagidae. Sampel diambil pada pelbagai peringkat perkembangan untuk analisis GC-FID bagi mengesan kehadiran MA. Keputusan menunjukkan bahawa pendedahan kepada MA melambatkan kitaran hidup Sarcophagidae, dengan serangga yang terdedah kepada 10 ng/mg MA

mengambil masa 26 hari untuk menyelesaikan kitaran hidup mereka berbanding 25 hari bagi kumpulan yang diberi rawatan 5 ng/mg MA dan kawalan. Selain itu, pendedahan kepada MA menyebabkan peningkatan saiz dan berat larva dan pupa secara berperingkat mengikut dos. Walau bagaimanapun, lalat dewasa dari kumpulan yang dirawat dengan MA sedikit lebih ringan berbanding dengan kumpulan kawalan. MA dikesan dalam larva instar ke-3 pada kedua-dua kepekatan dan dalam lalat dewasa pada kepekatan yang lebih tinggi, mencadangkan bahawa larva instar ke-3 adalah peringkat yang paling sesuai untuk analisis toksikologi.

DETECTION AND EFFECTS OF METHAMPHETAMINE ON THE LIFECYCLE OF *SARCOPHAGIDAE* IN MALAYSIA

ABSTRACT

Forensic investigations often face challenges when determining the time and cause of death, especially in cases where remains are found in advanced decomposition. Forensic entomology, the study of insects that colonize decomposing remains, has become a crucial tool in forensic investigations, providing valuable insights into the post-mortem interval (PMI) and the cause of death. A more specialized field, forensic entomotoxicology, focuses on using insects to detect drugs and other chemical substances in decomposing tissues. Insects, particularly larvae, play a significant role in PMI estimation as they accumulate substances from decomposing bodies, offering a more sensitive method for detecting toxins with fewer interferences from decomposition by-products. The rising prevalence of methamphetamine (MA) abuse has led to numerous overdose deaths, where bodies are typically discovered after several days of decomposition. This delay complicates forensic investigations. To address this, forensic entomotoxicological analysis using insects is increasingly being used to detect the presence of drugs such as MA in decomposing tissues. In this study, pig liver mince was used to simulate the decomposition process and attract necrophagous insects, particularly *Sarcophagidae*. The liver was minced with two concentrations of MA (5 ng/mg and 10 ng/mg) to examine its impact on the lifecycle and morphology of *Sarcophagidae*. Samples were collected at various developmental stages for GC-FID analysis to detect MA presence. The results demonstrated that MA exposure delayed the lifecycle of *Sarcophagidae*, with insects exposed to 10 ng/mg MA taking 26 days to complete their lifecycle compared to 25 days for the 5 ng/mg MA-treated and control groups. Additionally, MA exposure resulted in a dose-dependent increase in the size and weight of larvae and pupae. However, adult

flies from the MA-treated groups were slightly lighter compared to the control group. Notably, MA was detected in 3rd instar larvae at both concentrations and in adult flies at higher concentration, suggesting that the 3rd instar larvae are the most suitable stage for toxicological analysis.

CHAPTER 1: INTRODUCTION

1.1 Background of the Study

Forensic entomology, which involves the study of insects and arthropods like mites for medicocriminal investigations, has become a crucial field in legal medicine (Hall and Huntington, 2009; Magni *et al.*, 2013). Insects play a vital role in ecosystems and are found in almost every habitat. Over 400 insect species have been documented on decaying animal remains, highlighting their significance in decomposition (Payne, 1965). This process makes insects valuable tools in criminal investigations, especially for estimating the postmortem interval (PMI), the time since death (Henßge and Madea, 2004).

Following death, a body undergoes chemical and biological transformations, including autolysis, putrefaction, and changes like algor mortis, livor mortis, and rigor mortis (Amendt, Krettek and Zehner, 2004). These changes are influenced by factors such as ambient temperature and environmental conditions (Pittner *et al.*, 2020), making PMI estimation a complex task. Current PMI estimation methods — such as comparing body and environmental temperatures (Nelson, 2000), analyzing rigor and livor mortis (Madea, 2016), or examining supravital reactions (Cordeiro *et al.*, 2019) — are typically limited by specific conditions and will lose accuracy after 72 – 96 hours (Campobasso, Di Vella and Introna, 2001). Beyond this timeframe, insect colonization provides critical evidence for PMI estimation (Catts and Goff, 1992).

Flesh flies, belonging to the *Sarcophagidae* family (Diptera), are notable for their scavenging behaviour and play a crucial role in forensic investigations. These flies are commonly found colonizing carcasses and cadavers. Various sarcophagid species, particularly adult females, are often pioneers in locating the decomposing bodies and initiating the process of decomposition through a succession of stages (Paseto *et al.*, 2019). Gravid females, attracted to the gases emitted

by decaying bodies, utilize these sites for laying their larvae, which become the developmental environment for their offspring (Denno and Cothran, 1976).

However, factors like burial depth, concealment, and location influence insect activity. For example, most Dipterans cannot access bodies buried deeper than 30 cm, although some species, such as Phoridae, may colonize deeply buried remains (Campobasso and Introna, 2001). Geographical region, season, and local ecology also affect insect succession on a corpse. Because these variables vary widely, PMI estimations must be adapted to specific conditions at the death scene. Insects can establish a timeline from death to discovery, often providing minimum PMI (PMImin) estimates based on larval development stages (Campobasso, Di Vella and Introna, 2001).

Entomotoxicology, a branch of forensic entomology, examines toxicants in carrion-feeding insects to assess drug effects on their lifecycle (Campobasso and Introna, 2001). In decomposed remains where traditional toxicological samples are unavailable, maggots and pupae offer alternative substrates for analysis. Studies have shown that substances like morphine (Bourel *et al.*, 2001), heroin (Goff *et al.*, 1991), cocaine (Alves JR MJ, et al., 2007; Wood, Pyper and Casali, 2022), and amphetamines (Gagliano-Candela and Aventaggiato, 2001) accumulate in larvae, affecting their development and potentially altering PMI estimates. Techniques such as gas chromatography-mass spectrometry (GC-MS) and immunohistochemistry allow the detection of drugs within insect tissues. However, the absence of a drug in larvae does not confirm its absence in the body due to varying rates of metabolism and absorption (De Carvalho, 2010).

Forensic entomology not only aids PMI estimation but also helps determine trauma sites, movement of remains (Sadek and Khan, 2018), and toxicological profiles. It provides insights into the circumstances of death, including location, time of decapitation, and child neglect cases (Wayne D. Lord and William C. Rodriguez, 1989; Goff, Charbonneau and Sullivan, 1991).

Nevertheless, despite its broad applications, this field remains underutilized in Malaysia. Limited attention to forensic entomotoxicology restricts progress in understanding how substances like methamphetamine affect the lifecycle of *Sarcophagidae*. As methamphetamine abuse continues to be a significant issue in Malaysia, hence, this study will focus on the detection and effects of methamphetamine on the lifecycle of *Sarcophagidae*.

1.2 Problem Statement

Methamphetamine (MA), a Schedule II drug in the United States, is widely abused due to its strong central nervous system (CNS) stimulant properties, particularly the dextro-methamphetamine (D-methamphetamine), which enhances the release of dopamine, serotonin, and norepinephrine in the brain (Abbruscato and Trippier, 2018). While approved for limited medical use, its illicit recreational use has become a significant public health concern, with an estimated 35 million users worldwide (Messina *et al.*, 2014). The rise of MA abuse has been particularly alarming in Southeast Asia, where amphetamine-type stimulants (ATS) have overtaken opiates as the primary drugs of abuse. In Malaysia, the National Anti-Drugs Agency (NADA) reported 19,523 ATS addiction cases (National Anti-Drugs Agency (NADA), 2024), and the United Nations Office on Drugs and Crime (UNODC) recorded a staggering 190 tons of MA seized in East and Southeast Asia in 2023 (United Nations Office on Drugs and Crime (UNODC), 2024), highlighting the growing prevalence of ATS-related crimes in the region. This surge in ATS consumption has been partly attributed to Malaysia's porous borders with the Golden Triangle.

Forensic entomotoxicology has emerged as a vital field for investigating drug-related deaths, particularly when traditional tissues are no longer available due to decomposition or skeletonisation (Basilicata *et al.*, 2019). This field studies the effects of drugs or toxins on the development of necrophagous insects, such as *Calliphoridae* (blowflies) and *Sarcophagidae* (flesh flies), which feed on decaying remains. MA has been shown to significantly alter the development of blowfly species, including accelerated larval growth, increased size across all life stages, delayed pupation, and reduced pupal weight (Mullany *et al.*, 2014; Wang *et al.*, 2020). Such alterations impair the precision of PMI estimations, which are crucial for forensic investigations.

However, there is limited research on MA effects on *Sarcophagidae* in Malaysia. Previous studies have investigated the effects of other drugs on *Sarcophagidae*, but the impact of MA has yet to be explored. Moreover, most of these studies were conducted in other countries, making their findings not directly applicable to the conditions in Malaysia. This gap in knowledge poses a challenge for forensic entomologists, increasing the risk of inaccuracies in PMI estimations and misinterpretation of entomological evidence in drug-influenced environments.

Hence, this study seeks to address these gaps by examining the effects of MA on the lifecycle of *Sarcophagidae* in Malaysia and identifying the most suitable developmental stage for toxicological analysis. By understanding how methamphetamine impacts these insects' development, this research aims to enhance the reliability of PMI estimations in cases involving drug-related deaths, thereby contributing to the advancement of forensic entomology and improving the accuracy of legal and investigative processes.