ISETC2025

UNIVERSITI TEKNOLOGI MARA

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2 ND INTERNATIONAL SCIENCE, ENGINEERING AND TECHNOLOGY COLLOQUIUM

THE POWER OF SCIENTIFIC INNOVATION

INTELLIGENT SOLUTIONS FOR A SUSTAINABLE WORLD

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PREFACE

Welcome to the 2nd International Science, Engineering and Technology Colloquium 2025 (ISETC 2025)!

It is with great pleasure that we present this year's colloquium, a distinguished gathering of scholars, researchers, and professionals dedicated to the advancement of applied sciences. Held virtually on 9th July 2025, ISETC 2025 serves as a platform to highlight cutting-edge research, foster meaningful networking, encourage collaborations, and explore innovative solutions to global challenges.

This year's theme, "The Power of Scientific Innovation: Intelligent Solutions for a Sustainable World" highlights our shared goal to solve global challenges through applied science. It celebrates innovation as a key to building a more sustainable and resilient future. We're proud to feature keynote speakers and presenters who bring fresh insights, bold ideas, and breakthroughs shaping tomorrow's world.

We would like to extend our heartfelt appreciation to the Faculty of Applied Sciences, Universiti Teknologi MARA, Perak Branch, Tapah Campus, for their outstanding support and assistance throughout the planning and execution of the event. Their dedication has been instrumental to the success of this colloquium. We would like to sincerely thank all participants and researchers for their valuable contributions. We also appreciate the reviewers for their careful evaluations and helpful feedback. Your hard work and support mean a lot to us.

As editors, we are proud to present this collection of proceedings. It is an honour to bring together the interesting and meaningful work of our speakers and authors. We hope the ideas shared will lead to new discoveries, encourage more research, and help solve global challenges through science and innovation.

Warm regards,

Dr. Emi Norzehan Mohamad Mahbob
Dr. Saiyidah Nafisah Hashim
Ms. Lili Widarti Zainuddin
Ms. Nurul Nazwa Mohammad
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ISETC 2025 Proceedings Editors

EFFECT OF GROWTH DEVELOPMENT TEMPERATURE ON CUTICULAR HYDROCARBON (CHC) PROFILES OF *Spiniphora genitalis* 3rd INSTAR LARVAE

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Abstract: In forensic entomology, precise estimation of the postmortem interval (PMI) is important in death investigation, particularly when decomposition exceeds 72 hours. Analysis involving cuticular hydrocarbons (CHCs) is one of promising biochemical approach which have been shown to vary according to insect developmental stage and environmental conditions. This study investigates the effect of growth development temperature on the CHC profiles of *Spiniphora genitalis* 3rd instar larvae using Gas Chromatography-Mass Spectroscopy (GC-MS) and Principal Component Analysis (PCA). Larvae were reared at three different temperatures (21ºC, 23ºC, 25ºC) and CHCs were extracted using hexane and analyzed via GC-MS. The hydrocarbon compound was identified using library search (NIST08), and electron ionization in scan mode was applied for mass spectroscopic detection. The resulting hydrocarbon profiles were subjected to PCA to assess temperature induced variation. The GC-MS studies demonstrated that 30 hydrocarbon were present in cuticle of 3rd instar larvae *Spiniphora genitalis* across different development temperature where 5 of it which is hexadecane, 2,4-Di-tert-butylphenol, Heptadecane, Octadecane and Heneicosane were present in all temperature. At least one hydrocarbon specifically present on each development temperature. This showed that every development temperature can be distinguish based on these unique hydrocarbon that only present on each specific temperature. There is also increase numbers of long hydrocarbon chain specifically straight chain alkane with temperature increase as a response to environmental stressor (temperature) due to responsible of it to minimize water loss thus prevent desiccation. PCA successfully cluster CHC profiles of 3rd instar larvae *Spiniphora genitalis* develop at different temperature. This findings indicate that CHCs could be used as reliable tools for development temperature identification of 3rd instar larvae. For future studies it is recommended to increase the replicates of the sample because PCA is more reliable when involving a large group of data set and must incorporate additional developmental stages and both sexes of *Spiniphora genitalis* to determine whether similar temperature-dependent CHC patterns are observed throughout the life cycle and across genders.

***Keywords*:** *Spiniphora genitalis, cuticular hydrocarbon, GC-MS, PCA*

INTRODUCTION

In forensic entomology, postmortem interval (PMI) estimation often relies on the development stages and identification of necrophagous insects, particularly Diptera larvae. Time since death' or post-mortem interval (PMI) is a concept that closely associated with forensic entomology where it is the time that has been elapsed between the death have occurred and the discovery of the body and essential in investigation that involve homicide deaths, suspicious deaths or other untimely deaths as well as natural deaths. After 72 hours of death happen, PMI can be established by studied the life cycle of insect, relationship of the insect to the remains and environmental conditions, and the relationship of the remains to the habitat which they are discovered (Goff, 2009). One of the prominent method to identify the stages of insect in their life cycle by studied the cuticular hydrocarbon profiling of the insect because research showed that insect CHCs varied with each of the development stages in insect lifecycle (Ma et al., 2024). Other than that development temperature can affected the composition of cuticular hydrocarbon (CHC) (Baleba et al., 2024). This profiling is one of the methods that can help accurately to estimate the PMI. *Spiniphora genitalis*, a less studied fly species, may offer potential forensic value through the analysis of its cuticle hydrocarbon (CHC) profile. The objective of this research is to study the effect of growth development temperature on cuticular hydrocarbon profiles 3rd instar larvae of *Spiniphora genitalis* (Phorid) using GC-MS. The reliability of GC-MS as a tool are expected to help separate and identify the complex mix of hydrocarbon compounds on insect's cuticles while gives detailed information on hydrocarbons' carbon chain lengths, saturation, and branching (Srivinas, 2024).

MATERIALS AND METHODS

A total of 10 3rd instar larvae from each different temperature (21ºC, 23 ºC, 25 ºC) were collected and washed with ultra-pure water and gently pat dry with paper towel. The larvae were then placed in a glass tube with 5 ml of hexane ensuring that the larvae were fully submerged. Each sample were then left for 15 min to soaked after which the hexane extract was transferred into a new clean glass test tube. The eluted hexane was dried using gentle stream of nitrogen purging gas until approximately 1 ml left. Each sample were then stored in the 4°C freezer pending analysis. The dried extracts were reconstituted in 40 µL volume of hexane in a vial before being manually injected into the GC–MS (Moore et al., 2014). These steps were repeated for each sample temperature. For each temperature, total of 3 replicates were prepared. The extract was analyzed using GC-MS with spitless injector of Agilent Technologies 5977A Network GC at 250 °C, a HP5-MS capillary column (30 m x 0.25 mm ID, 0.25 µm film thickness) and were coupled to an Agilent 5973 Mass Selective Detector. Elution was performed with helium at 1 mL/min. The oven temperature was set to stay at 50 °C for two minutes, after which it was ramped up to 200 °C at a rate of 25 °C per minute, then from 200 °C to 260 °C at a rate of 3 °C per minute, and finally from 260 °C to 320 °C at a rate of 20 °C per minute, where it was expected to stay for two minutes. The mass spectrometer was run in Electron Ionization mode at 70 eV, scanning at 1.5 scans per second between 40 to 500 amu. The diagnostic fragmented ions, the Kovat indices, and a library search (NIST08) were used to identify hydrocarbons (Moore et al., 2014). Then the data of straight chain hydrocarbon for all the replicates temperature of 21ºC, 23 ºC and 25 ºC were tabulated and analyzed using Principal Component Analysis (PCA). This statistical analysis would display the variance of larva age between each day.

RESULTS AND DISCUSSION

The expected results of this research are for the Gas Chromatography–Mass Spectroscopy (GC-MS) to anticipated to reveal qualitative and quantitative differences in hydrocarbon chains, indicating that temperature influences CHC biosynthesis. Principal Component Analysis (PCA) is expected to effectively discriminate between larvae reared at different temperatures by clustering CHC profiles into temperature specific groups, thus confirming that environmental temperature plays a significant role in modulating the chemical profile of the larval cuticle. This method successfully differentiates cuticular hydrocarbon (CHC) profiling across different variables such as age (Braga et al., 2016) , life cycle (Ma et al., 2024) and species (Moore et al., 2014).

CONCLUSIONS

These temperature dependent CHC variations are crucial because they can serve as biomarkers to estimate the environmental conditions during larval development, which directly impacts PMI estimation. Forensic entomologist may improve the accuracy of PMI estimations, especially in cases where morphological or developmental markers are ambiguous due to fluctuating or unknown temperatures at the crime scene.

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Sekian, terima kasih.

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Setuju.

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