DEVELOPMENT, CHARACTERISATION AND EVALUATION OF *Backhousia citriodora* ESSENTIAL OIL NANOEMULSIONS FOR LARVICIDAL ACTIVITY AND GUT MICROBIOME MODULATION IN *Aedes aegypti* (L.)

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PEMBANGUNAN, PENCIRIAN DAN PENILAIAN NANOEMULSI MINYAK PATI *Backhousia citriodora* UNTUK AKTIVITI LARVISIDAL DAN MODULASI MIKROBIOM USUS DALAM *Aedes aegypti* (L.)

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DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged.

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ABSTRAK

Kerintangan racun serangga yang semakin meningkat dalam *Aedes aegypti* telah mengurangkan keberkesanan strategi kawalan vektor semasa. Minyak pati (EOs) menjanjikan alternatif semula jadi berbanding penggunaan insektisid sintetik. Namun begitu, sifat hidrofobik dan ketidakstabilan EOs mengehadkan aplikasinya sebagai agen larvisidal. Kajian ini bertujuan untuk membangunkan dan mencirikan nanoemulsi *Backhousia citriodora* EO (BCEONs) serta menilai kesannya terhadap aktiviti larvisidal dan mikrobiom usus larva *Ae. aegypti.* Sebatian fitokimia dalam BCEO dikenal pasti melalui kaedah kromatografi gas-spektrometri jisim (GC-MS). BCEONs telah dihasilkan menggunakan teknik pengemulsian ultrasonik dengan kepekatan tetap BCEO (10% w/w) serta minyak jagung (5% w/w), dan gabungan variasi air ultra tulen (50-80% w/w) serta Tween 80 (5-35% w/w) bagi mengoptimumkan formulasi. BCEONs yang optimum dicirikan untuk menentukan morfologi, saiz titisan, potensi zeta, dan kestabilan. Aktiviti larvisid terhadap *Ae. aegypti* dijalankan mengikut protokol piawai WHO, termasuk analisis morfologi dan morfometrik. Komposisi dan potensi fungsi mikrobiom usus larva telah dinilai menggunakan penjujukan gen 16S rRNA. Analisis GC-MS menunjukkan BCEO mengandungi 86.46% sitral. Formulasi BCEON yang optimum (NE1) terdiri daripada 10% BCEO, 5% minyak jagung, 5% Tween 80, dan 80% w/w air ultra tulen, yang menunjukkan kestabilan yang baik pada 4ºC, 25ºC, and 37°C untuk tempoh 28 hari, dengan saiz titisan <123 nm, indeks polidispersiti <0.2, potensi zeta antara -4.74 to -11.55 mV, dan indeks bias 1.3639-1.3652. Pemerhatian melalui mikroskop elektron transmisi (TEM) mengesahkan bentuk titisan berupa sfera. NE1 menunjukkan aktiviti larvisid yang tinggi secara signifikan (ANOVA sehala, $P<0.05$) berbanding BCEO, dengan nilai LC_{50} dan LC_{90} masing-masing menunjukkan 136.78 dan 237.71 ppm pada 24 hours, dan 118.55 dan 198.31 ppm pada 48 jam, manakala BCEO mencatatkan nilai LC_{50} dan LC_{90} masing-masing, iaitu 242.66 dan 344.03 ppm pada 24 jam, dan 237.51 dan 332.37 ppm pada 48 jam. Larva yang dirawat dengan NE1 menunjukkan kecacatan morfologi dan perubahan morfometrik yang ketara. Profil bakteria usus larva menunjukkan perbezaan dalam komposisi dan kelimpahan relatif antara kumpulan rawatan dan kawalan. Analisis menunjukkan rawatan NE1 mengurangkan kelimpahan relatif filum Actinobacteriota, serta genus *Microbacterium_A* secara signifikan (ANOVA sehala, $P<0.05$), sementara meningkatkan kelimpahan relatif *Bacillus_A* secara signifikan (ANOVA sehala, $P<0.05$). Ramalan fungsi berdasarkan laluan KEGG menunjukkan bahawa NE1 memberi kesan signifikan (ujian-t Welch dua hala, $P<0.01$) terhadap laluan yang terlibat dalam biosintesis amino asid, metabolisme karbohidrat dan tenaga, pemprosesan informasi genetik, biosintesis dan metabolisme glikan, metabolisme lipid, metabolisme ko-faktor dan vitamin, metabolisme terpenoid dan poliketida, dan metabolisme dan biodegradasi xenobiotik. Secara keseluruhan, NE1 menunjukkan kestabilan dan keberkesanan larvisidal yang tinggi, serta modulasi yang ketara terhadap komposisi dan fungsi mikrobiom usus, sekaligus menyokong potensinya sebagai larvisid yang mampan.

ABSTRACT

The increasing insecticide resistance of *Aedes aegypti* has reduced the effectiveness of current vector control strategies. Essential oils (EOs) are promising natural alternatives to synthetic insecticides. However, their hydrophobicity and instability limit their larvicidal application. This study aimed to develop and characterise *Backhousia citriodora* EO nanoemulsions (BCEONs) and evaluate their effects on larvicidal activity and the gut microbiome of *Ae. aegypti* larvae. Phytochemical compounds in BCEO were identified through gas chromatography-mass spectrometry (GC-MS). The BCEONs were developed by ultrasonic emulsification with fixed BCEO (10% w/w) and corn oil (5% w/w) and varying ultrapure water (50-80% w/w) and Tween 80 (5-35% w/w) to optimise the formulation. The optimised BCEONs were characterised to determine their morphology, droplet size, zeta potential, and stability. The larvicidal activity against *Ae. aegypti* was conducted following the WHO-standard bioassay protocol, including morphological and morphometric analysis. The gut microbiome composition and functional potential of larvae were assessed using 16S rRNA gene sequencing. GC-MS analysis revealed that BCEO was composed of 86.46% citral. The optimised BCEON formulation (NE1) contained 10% BCEO, 5% corn oil, 5% Tween 80 and 80% w/w ultrapure water, exhibiting good stability at 4ºC, 25ºC, and 37°C over 28 days, with droplet size <123 nm, polydispersity index <0.2, zeta potential ranging from -4.74 to -11.55 mV, and refractive index of 1.3639-1.3652. Transmission electron microscopy (TEM) confirmed spherical-shaped droplets. NE1 exhibited significantly higher larvicidal activity (one-way ANOVA, $P < 0.05$) compared to BCEO, with LC_{50} and LC_{90} values of 136.78 and 237.71 ppm at 24 hours, and 118.55 and 198.31 ppm at 48 hours, whereas BCEO showed LC_{50} and LC_{90} values of 242.66 and 344.03 ppm at 24 hours, and 237.51 and 332.37 ppm at 48 hours, respectively. Larvae treated with NE1 displayed severe morphological deformities and morphometric changes. The gut bacterial profiles of larvae differed in composition and relative abundance across the treatment and control groups. The analysis indicated that NE1 treatment significantly (one-way ANOVA, $P<0.05$) reduced the relative abundance of the phylum Actinobacteriota, and the genera *Microbacterium_A*, while significantly (one-way ANOVA, $P<0.05$) increasing the abundance of *Bacillus_A*. Functional predictions based on KEGG pathways revealed that NE1 significantly (two-sided Welch's t-test, $P<0.01$) affected pathways involved in amino acid metabolism, carbohydrate and energy metabolism, genetic information processing, glycan biosynthesis and metabolism, lipid metabolism, metabolism of cofactors and vitamins, metabolism of terpenoids and polyketides, and xenobiotic biodegradation and metabolism. Overall, NE1 demonstrated superior stability, larvicidal efficacy, and pronounced modulation of gut microbiome composition and function, supporting its potential as a sustainable larvicide.

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LIST OF SYMBOLS

%	Percent
<	Less than
>	More than
Δ	Change
≥	More or equal to
°C	Degree Celsius
µg/mL	Microgram per millilitre
µL	Microlitre
µL/cm^2	Microlitre per cubic centimetre
µL/mL	Microlitre per millilitre
µm	Micrometre
cm	Centimetre
g	Gram
i.d	Internal diameter
kHz	Kilohertz
m	Metre
mg/L	Milligram per litre
mg/ml	Milligram per millilitre
min	Minutes
mL	Millilitre
mL/min	Millilitre per minute
mm	Millimetre
MPa	Megapascal
mV	Millivolt
nm	Nanometre
º	Degree
P	Pressure

ppm	Parts per million
r	Droplet radius
rpm	Revolutions per minute
s	Seconds
v/v	Volume per volume
W	Watt
α	Alpha
β	Beta
σ	Surface tension
χ2	Chi-square

LIST OF ABBREVIATIONS

10% sVB	10% Sucrose Solution Supplemented with Vitamin B
16S rRNA	Smal Subunit of Ribosomal RNA
Abd	Abdomen
ACE	Abundance-based Coverage Estimator
AChE	Acetylcholinesterase
ADE	Antibody-dependent Enhancement
A_{min}	Surface Area Per Molecule
ANOSIM	Analysis of Similarities
ANOVA	Analysis of Variance
ASEAN	Association of Southeast Asian Nations
ASV	Amplicon Sequence Variants
ATP	Adenosine Triphosphate
BC	*Backhousia citriodora*
BCEO	*Backhousia citriodora* Essential Oil
BCEON	*Backhousia citriodora* Essential Oil Nanoemulsion
Bti	*Bacillus thuringiensis* svar. *israelensis*
CarEs	Carboxylesterases
CD	Cephalic Diameter
CHIKV	Chikungunya Virus
COVID19	Coronavirus Disease 2019
CP	Cuticle Protein
CPCoA	Constrained Principal Coordinate Analysis
CRIM	Centre for Research and Instrumentation Management
CTmix	Sonicated mixture of 5% (w/w) corn oil, 5% (w/w) Tween 80, and 9% (w/w) ultrapure water
CYD-TDV	Chimeric Yellow Fever-Dengue-Tetravalent Dengue Vaccine
CYP	Cytochrome P450
DDT	Dichlorodiphenyltrichloroethane

DEET	N, N-diethyl-m-toluamide
DENV	Dengue Virus
df	Degree of Freedom
DLS	Dynamic Light Scattering
DNA	Deoxyribonucleic Acid
e.g.	Exemplarities
ECSA	East/Central/South Africa
EO-NEs	Essential oil-based Nanoemulsions
EOs	Essential Oils
EPA	United States Environmental Protection Agency
EYE	Eliminate Yellow Fever Epidemics
FDA	Food and Drug Administration
FIFRA	US EPA's Federal Insecticide, Fungicide, and Rodenticide Act
GABA	Gamma-aminobutyric Acid
GC-MS	Gas Chromatography-Mass Spectrometry
gDNA	Genomic DNA
GRAS	Generally Recognised as Safe
GST	Glutathione-S-transferase
H	Head
HLB	Hydrophilic-lipophilic Balance
HPH	High-pressure Homogenisation
IMG	Integrated Microbial Genomes and Microbiomes
IOL	Indian Ocean lineage
IRAC	Insecticide Resistance Action Committee
IRM	Insecticide Resistance Management
IRS	Indoor-residual Spray
ITNs	Insecticide-Treated Nets
IVM	Integrated Vector Management
kdr	Knockdown Resistance

KEGG	Kyoto Enclyopedia of Genes and Genomes
LC_{50}	Lethal Concentration at 50% population
LC_{90}	Lethal Concentration at 90% population
LCL	Lower Confidence Limit
LCTs	Long-chain Triglycerides
LDA	Linear Discriminant Analysis
LEfSE	LDA Effect Size
LL	Larval Length
LRI	Linear Retention Indices
MAPs	Medicinal and Aromatic Plants
MARDI	Malaysian Agricultural Research and Development Institute
MBL	Metallo β-lactamase
MCTs	Medium-chain Triglycerides
MDE	Metabolic Detoxification Enzymes
MSD	Mass Selective Detector
NAD	Nicotinamide Adenine Dinucleotide
N/A	Not Available
NCBI	National Center for Biotechnology Information
NEs	Nanoemulsions
NHPs	Non-human Primates
NIST	National Institute of Standards and Technology
NMDS	Non-metric Multidimensional Scaling
O/W	Oil-in-water Nanoemulsion
OPAA	Organophosphorus Acid Anhydrolases
OTU	Operational Taxonomic Unit
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
PDF	Pigment-Dispersing Factors
PDI	Polydispersity Index

PHEIC	Public Health Emergency of International Concern
PIC	Phase Inversion Composition
PICRUST	Phylogenetic Investigation of Communities by Reconstruction of Unobserved States
PIT	Phase Inversion Temperature
POE	Polyoxyethylene
PTEs	Phosphotriesterases
QIIME	Quantitative Insights into Microbial Ecology
qPCR	Quantitative Polymerase Chain Reaction
RDL	Resistance to Dieldrien
rHLB	Required Hydrophilic-lipophilic Balance
RI	Refractive Index
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
RR	Resistance Ratio
Rt	Retention Times
S	Siphon
SAM	S-adenosyl-L-methionine
ScV-L-A	*Saccharomyces cerevisiae* virus L-A
SD	Standard Deviation
SDG	Sustainable Development Goal
SFE	Supercritical Fluid Extraction
SFME	Solvent-Free Microwave Extraction
SL	Siphon Length
S_{mix}	Surfactant Mixture
STAMP	Statistical Analysis of Metagenomic Profiles
SWE	Subcritical Water Extraction
T	Thorax
TCA	Tricarboxylic Acid

TEM	Transmission Electron Microscope
TSV	Tab-separated values
UCL	Upper Confidence Limit
UKM	Universiti Kebangsaan Malaysia
UKMB	Universiti Kebangsaan Malaysia, Bangi
ULV	Ultra-low Volume
US	United States
UUF	Unweighted UniFrac
Vssc	Voltage-sensitive Sodium Channel
W/O	Water-in-oil Nanoemulsion
w/w	Weight Per Weight
WHO	World Health Organisation
WUF	Weighted UniFrac
YFV	Yellow Fever Virus
ZIKV	Zika Virus

LIST OF NOMENCLATURE

$C_{10}H_{16}$	β-Myrcene
$C_{10}H_{16}O$	2,6-Octadienal, 3,7-dimethyl-, (E)- (Geranial)
$C_{10}H_{16}O$	Citral
$C_{10}H_{16}O$	Limonene oxide, cis-
$C_{10}H_{16}O_2$	Oxiranecarboxaldehyde, 3-methyl-3-(4-methyl-3-pentenyl)-
$C_{10}H_{17}Br$	1-Bromo-3,7-dimethyl-2,6-octadiene
$C_{10}H_{18}O$	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)- (nerol)
$C_{10}H_{18}O$	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)- (nerol)
$C_{10}H_{18}O$	Citronellal
$C_{10}H_{18}O$	Geraniol
$C_{10}H_{18}O$	p-Menth-1(7)-en-9-ol
$C_{10}H_{18}O$	1,6-Octadien-3-ol, 3,7-dimethyl-
$C_{10}H_{1}O$	2,6-Octadienal, 3,7-dimethyl-, (Z)- (neral)
$C_{15}H_{24}$	Caryophyllene
$C_{17}H_{28}O_2$	Cyclopropanecarboxyllic acid, tridec-2-ynyl ester
$C_5H_8O_2$	2-Butenoic acid, methyl ester, (E)-
C_7H_{10}	1,3-Cycloheptadiene
$C_8 H_{12}O$	2,5-Dimethylhex-5-en-3-yn-2-ol
C_8H_{14}	Cyclohexane, ethenyl-
CO_2	Carbon dioxide
H_2O	Water

CHAPTER I

INTRODUCTION

1.1 Background

Mosquito-borne viral infections remain one of the top global health issues, particularly impacting both tropical and subtropical regions, including in travellers. *Aedes aegypti*, a prominent mosquito species that competently carries multiple types of viruses, leading to dengue fever, yellow fever, Zika fever and chikungunya. According to the World Health Organization (WHO), although the incidence of Zika virus infections has declined since 2017 and the TAK-426 Zika vaccine has demonstrated immune persistence for up to two years, while yellow fever can be effectively prevented with a highly efficacious single-dose vaccine, dengue continues to pose a major global public health threat (De Aguiar et al. 2026; WHO 2022; WHO 2025a; 2025b) . An estimated 100–400 million dengue infections occur annually worldwide, and despite the recent development and updating of dengue vaccines, disease control remains only partially effective (WHO 2025a). The first licensed dengue vaccine, CYD-TDV (Dengvaxia®), a tetravalent live attenuated vaccine utilising a yellow fever vaccine backbone, was licensed in 2015 but can only be given to seropositive individuals (those previously infected by dengue) to ensure optimal efficacy and safety on the vaccinees (Wilder-Smith 2020). In seronegative recipients, Dengvaxia® may mimic a primary infection, increasing the risk of Antibody-Dependent Enhancement (ADE), which can lead to severe dengue upon subsequent natural infection (Shukla et al. 2020). Therefore, dengue treatment mainly focuses on relieving symptoms and providing supportive care, highlighting the significance of intensive vector control programs for disease prevention, as the spread of the dengue virus is caused primarily by the bite of infected female *Ae. aegypti*. Consequently, chemical-based vector control strategies, particularly the application of synthetic insecticides, remain as the most extensively used method to supress *Ae. aegypti* populations and reduce dengue transmission, followed by habitat management, non-chemical larviciding, population replacement methods, and genetic techniques (Van Den Berg et al. 2021; Weeratunga et al. 2017).

Synthetic insecticides, including pyrethroids, organophosphates, and carbamates, have historically been used for mosquito control programs. These chemicals are extensively used to target both adult and larval stages of mosquitoes. Targeting the mosquito larvae can effectively reduce the emergence of adult mosquitoes, hence restricting the transmission. Moreover, the confined space inhabited by the larvae facilitates their eradication, as it allows insecticides to access them more readily than the flying adults. However, these synthetic chemicals pose risks to non-target organisms, including beneficial insect and aquatic life, such as algal components and plankton, as well as harming the water quality and fish diversity, which leads to ecosystem imbalances and the possibility of food chain contamination (Barathi et al. 2024; Kumar et al. 2023). Synthetic insecticides can also pose significant hazards to the environment and public health, causing acute toxicity through accidental inhalation, ingestion or direct contact, and prolonged exposure can lead to chronic toxicity (Ahmad et al. 2024). Furthermore, the rampant use of synthetic insecticides has resulted in the rapid emergence of insecticide resistance, diminishing their effectiveness, particularly organophosphate and pyrethroids (Silalahi et al. 2022; Wang et al. 2023).

Insecticide resistance has become a global risk to agriculture, pest management, and public health, with the microbiota increasingly recognised as an important contributing factor (Ul Malook et al. 2025). Mosquito gut microbiota shows complex community structure with rich species diversity, serving profound roles in growth, development, survival, and reproduction (Liu et al. 2024). However, recent breakthroughs have identified the multiple mechanisms through which the gut microbiota facilitate insecticide resistance. The microbiota can induce insecticide resistance in their host through the isolation and degradation of insecticidal compounds or by modulating host gene expression, linking specific taxa to increased tolerance (Liu et al. 2024; Sato et al. 2021). For instance, *Serratia oryzae* isolated from the adult *Ae. albopictus* demonstrated the capacity to enhance deltamethrin resistance, both *in vivo* and *in vitro,* by stimulating metabolic detoxification genes and autocrine metabolic enzymes (Wang et al. 2022). Furthermore, permethrin-selected strains of *Ae. aegypti* exhibited higher microbial richness relative to their parental strain, suggesting a possible correlation between gut microbiota composition and the emergence of insecticide resistance (Muturi et al. 2021). *Serratia marcescens,* an efficient bacteria capable of colonising mosquitoes, demonstrating enhanced antimicrobial activity and virulence through prodigiosin and other secondary metabolites (Heu et al. 2021). Additionally, *Bacillus thuringiensis svar. israelensis* (Bti) infection rapidly altered the larval gut microbiome of *Ae. aegypti*, with tolerant larvae exhibiting reduced bacterial diversity but greater inter-individual variability, alongside a lower Bti load in host tissue compared to susceptible larvae, indicating an active-host medicated control of infection (Tetreau et al. 2018). While *Serratia* and Bti are known to induce gut dysbiosis and protease-related damage, the specific contribution of plant-derived secondary metabolites (terpenoids, polyphenols, and alkaloids) to these changes remain underexplored. Furthermore, these findings indicate that the gut microbiome may play a significant role in modulating insecticide resistance within mosquito populations. This could challenge existing vector control strategies, which primarily target mosquito genetics and insecticide susceptibility, by introducing an additional microbiome-mediated mechanism that may reduce insecticide effectiveness and influence field-level control outcomes.

To mitigate the rising issue of insecticide resistance, some biological control methods have been implemented by introducing a safer and effective alternative through the application of bacterial toxin as larvicide by using Bti against *Ae. aegypti* (Rajchanuwong et al. 2025; Silva-Filha et al. 2021). Furthermore, there is also the application of predatory biology to control the larvae of *Aedes* spp. such as *Gambusia affinis* or *Poecilia sphenops* (Syifa et al. 2024; Zubaidah et al. 2024). However, these biological control techniques to eradicate mosquito populations have several disadvantages. These methods require a specific and optimal environmental area for it to be carried out successfully, as it is vulnerable to environmental changes, hence limiting the sustained control in all settings (Hamed et al. 2022). The larvicidal result from biological control is slower compared to the application of synthetic insecticides. Furthermore, this method requires high cooperation from the nearby community and enforcement from the relevant authority for it to be executed to its maximum potential. These limitations highlight the urgent need for innovative, sustainable and eco-friendly solutions for mosquito control, with particular emphasis on safe and effective larvicidal formulations.

Given these challenges, there has been increasing interest in developing eco-friendly and biodegradable alternatives, such as plant-derived essential oils (EOs) as promising natural insecticides. These EOs exhibit a diverse range of biological activities, including antibacterial, antibiofilm, antifungal, nematocidal, repellent, and insecticidal properties (Bai et al. 2023; Lim et al. 2022; Rehman et al. 2024; Salam et al. 2025; Wu et al. 2024). The EOs are also regarded as environmentally friendly, biodegradable, and have less risk of inducing resistance in target species, making them attractive alternatives to conventional insecticides. Some of the common EOs used in pesticide formulations are cinnamon, citronella, clove, lemon grass, and rosemary. Most of EOs are considered safe for consumers to use and have been granted Generally Recognised as Safe (GRAS) status by the Food and Drug Administration (FDA) (Jackson-Davis et al. 2023). The GRAS status of EOs supports their application as an environmentally sustainable substitute for conventional insecticides. This signifies that EOs are safe for humans, animals, and the environment when utilised appropriately. Their natural biodegradability and minimal toxicity are appealing factors as suitable options for the formulation of sustainable insect control. Despite these advantages, the practical application of EOs is hindered by their volatility, poor water solubility, and vulnerability to oxidation and photodegradation, which reduce the effectiveness of their bioactive compounds to act on the target organism.

Recent breakthroughs in nanotechnology have offered a viable solution to these EOs' limitations. Nanoemulsion-based delivery system enhances the stability of active ingredients, bioavailability, solubility, and permeability of EOs (Barradas & De Holanda E Silva 2021; Preeti et al. 2023). Nanoemulsions are a kinetically stable colloidal dispersion system, characterised by droplet sizes ranging between 20 and 200 nm (Xiong et al. 2025). The nanosized droplets and extensive surface area of nanoemulsion provide greater compound stability, enhanced solubility of hydrophobic chemicals, and increased biological efficacy. These features of nanoemulsion have resulted in extensive uses across multiple sectors, including pharmaceuticals, food and beverages, cosmeceuticals, and agriculture, highlighting their potential as an efficient and adaptable delivery system (Abu - Huwaij et al. 2022; Jadhav et al. 2024; Khairan et al. 2025; Xiong et al. 2025). Nanoemulsion formulation generally requires three main components: oil, surfactant, and water. The ratio of each component is optimised to achieve the desired physicochemical properties. The addition of other chemicals, such as co-surfactants, can be considered based on the requirements identified during the optimisation process.

In this study, *Backhousia citriodora* essential oil (BCEO), also referred to as lemon myrtle essential oil, was utilised for the development of a nanoemulsion system. The primary bioactive compound in BCEO is citral (a mixture of geranial and neral) with proven effectiveness as an antimicrobial, antibiofilm, and insecticidal agent (Apolinário et al. 2025; Lim et al. 2022; Southwell 2021) According to the United States Food and Drug Administration, citral is also considered GRAS, supporting its suitability for a wider application range (Dosoky & Setzer 2021). *B. citriodora* is emerging as a new crop in Malaysia, with its current application oriented towards the application for food, beverages, food flavourings, and body care products (Ab Kahar Sandrang 2013). Previously, Greive et al. (2010) utilised BCEO as one of the components mixed with Melaleuca oil for repellent activity against *Ae. aegypti*. Therefore, we proposed a comprehensive study on the development of optimised *Backhousia citriodora* essential oil nanoemulsions (BCEONs), followed by their characterisation and evaluation of larvicidal efficacy of BCEONs, as well as elucidating their impact on the gut microbiota modulation in *Ae. aegypti* larvae, relative to the crude BCEO treatment.

The phytochemical constituents of BCEO were first identified using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Then, the BCEONs were formulated via the ultrasonication emulsification method using fixed concentrations of BCEO (10% w/w) and corn oil (5% w/w), while varying the proportions of ultrapure water (50–80% w/w) and Tween 80 (5–35% w/w) to optimise the nanoemulsion system. The optimised BCEONs were then characterised in terms of morphology, droplet size, zeta potential, refractive index, physical stability, and transmission electron microscope (TEM) imaging. Larvicidal activity against *Ae. aegypti* was evaluated following WHO standard bioassay protocols, including assessments of larval morphological and morphometric changes. In addition, the effects of BCEONs on the gut microbiome composition and functional potential of *Ae. aegypti* larvae were investigated using 16S rRNA gene sequencing. This study provided new insights into the mechanisms underlying the larvicidal activity of BCEONs, supporting their potential application as an effective green larvicide through morphological disruption and modulation of the larval gut microbiome.

1.2 Problem Statement

The *Aedes* mosquito-borne diseases, especially dengue, continue to pose a significant health threat in Malaysia, and insecticide-based intervention remains the essential measure in the dengue control strategy (Van Den Berg et al. 2021; Weeratunga et al. 2017). However, the rapid emergence of insecticide resistance has hampered the effectiveness of the existing dengue control efforts. Resistance to chemical insecticides has been documented in *Ae. aegypti*, including those in Malaysia (Rasli et al. 2021). Additionally, the extensive use of synthetic insecticides raises concerns regarding environmental persistence, non-target organism toxicity, and human health risks, highlighting the urgent need for safer, sustainable and more effective alternatives for vector control management.

In recent years, the insect gut microbiota has gained recognition for its potential role in modulating susceptibility or resistance to insecticides in mosquitoes (Liu et al. 2024; Muturi et al. 2021; Sato et al. 2021).Variations in gut microbial communities may alter larval metabolic processes, detoxification pathways, and immune responses, thereby affecting their sensitivity or resistance to the larvicidal compound. Despite growing evidence of microbiota-mediated insecticide responses, limited studies have explored how plant-based larvicides influence the gut microbiome of mosquito larvae, particularly at the nanoformulation level.

One of the alternative approaches is by utilising plant EOs due to their abundance of bioactive compounds, low toxicity and strong insecticidal efficacy (Şengül Demirak & Canpolat 2022). Nevertheless, their hydrophobic nature and high volatility limit their practical application, highlighting the need for the development of an efficient and effective delivery strategy to optimise their insecticidal potency against *Ae. aegypti* larvae. The encapsulation of EOs into nanoemulsions has become an effective approach to overcome these limitations by improving stability, dispersion, solubility, and bioavailability.

B. citriodora (lemon myrtle) plant is an emerging crop in Malaysia, valued for its high citral content, antibacterial, and insecticidal properties. Although the bioactivity of BCEO has been reported (Greive et al. 2010; Lim et al. 2022; Southwell 2021), its nanoemulsified form remains underexplored for mosquito larval control, particularly in terms of its interaction with larval gut microbiota. To date, the larvicidal efficacy of BCEONs and their impact on the gut microbiota of *Ae. aegypti* larvae have not been reported and remain poorly understood. Bridging this knowledge gap is vital, as elucidating the microbial community affected upon exposure to phytochemical-based nanoformulations can provide mechanistic insights into larvicidal action and support the development of microbiome-informed vector control strategies.

Therefore, this study aimed to develop and characterise BCEONs, evaluate their larvicidal activity, and elucidate their impact on the gut microbiota of *Ae. aegypti* larvae, in comparison with BCEO, to verify bioefficacy and support the advancement of green larvicides for sustainable dengue vector control.

1.3 Research Objectives

1.3.1 General objective

To characterise the physicochemical properties of *Backhousia citriodora* essential oil nanoemulsions (BCEONs) and, evaluate their larvicidal efficacy, and gut microbiota modulation in *Ae. aegypti* larvae

1.3.2 Specific objectives

i. To identify the phytochemical compositions of *Backhousia citriodora* essential oil (BCEO) using gas chromatography-mass spectrometry (GC-MS)
ii. To develop and characterise seven formulations of BCEON and determine the best two formulations in terms of physicochemical properties and stability
iii. To determine the larvicidal activity of BCEON (NE1) against *Ae. aegypti* in comparison with BCEO
iv. To evaluate the effects of BCEON (NE1) on the composition and functional potential of the gut microbiota *of Ae. aegypti* larvae

1.4 Research Questions

i. What are the major phytochemical constituents present in BCEO?
ii. Can stable BCEONs be successfully developed, and what are their physicochemical properties, including droplet size, morphology, zeta potential, and stability?
iii. How effective are BCEONs in causing larval mortality in *Ae. aegypti* compared to BCEO, and how does larvicidal activity vary with concentration?
iv. How does exposure to BCEONs affect the composition and functional potential of the gut microbiota in *Ae. aegypti* larvae?

1.5 Research Hypotheses

i. BCEO contains a range of bioactive phytochemical constituents, which may contribute to its biological activities.
ii. BCEONs can be successfully developed and will exhibit stable physicochemical properties, including uniform droplet size, appropriate zeta potential, and good structural stability.
iii. BCEONs exhibit higher larvicidal activity against *Ae. aegypti* larvae than BCEO, and larval mortality increases in a concentration-dependent manner.
iv. Exposure to BCEONs alters the gut microbiota composition and functional potential of *Ae. aegypti* larvae, potentially contributing to the observed larvicidal effects.

1.6 Research Significance

The primary scientific significance of this research is that it addresses the limitations of conventional vector control agents. In Malaysia, the management of *Ae. aegypti* larvae have relied on synthetic insecticides, such as organophosphates (temephos). The extensive use of these single-molecule chemicals has exerted strong selective pressure on the mosquito population, which increases the emergence of insecticide resistance. The widespread insecticide resistance of *Ae. aegypti* has also been documented across Malaysia, particularly in urban hotspots, such as Selangor, Kuala Lumpur, Penang, Kelantan, and Sarawak (Akhir et al. 2022; Ishak et al. 2017; Nurul-Nastasea et al. 2023). Furthermore, the Ministry of Health Malaysia (2024) reported the rising incidence of local dengue cases, with an 86.3% increase in cases during 2023 (123 133 cases) compared to 2022 (66 102 cases), highlighting the evolving challenges in managing *Ae. aegypti* vector within the urbanised population.

This research introduces the utilisation of BCEO, as EOs contain complex bioactive compounds, such as phenols and terpenoids, which can act on multiple biological targets, such as inhibition of acetylcholinesterase (AChE) or modulation of GABA receptors, making it more difficult for mosquitoes to develop metabolic resistance compared to single-active synthetic chemicals (Jankowska et al. 2017; Li et al. 2023; Rants'o et al. 2022). By encapsulating EOs in nanoemulsions (NEs) technology, this study addresses the limitations of crude EOs to be applied as larvicide, due to their high volatility and hydrophobicity. The nanoscale droplet size of NE enhances the bioavailability and penetration of the EOs into the larval cuticle, due to the higher surface area of bioactive compounds approaching the target organism.

The significance of this study also closely aligns with the National Strategic Plan for Dengue Prevention and Control (NSPDPC) 2022-2026, published by the Ministry of Health Malaysia (2022), emphasising the need for innovative, sustainable, and eco-friendly vector control among research institutes and universities. Therefore, this research directly supports the NSPDPC strategies on research and innovation by offering scientifically proven and effective EO-based NEs. Moreover, our study corresponds to the integrated vector management (IVM) rationale in encouraging a decision-making approach that promotes efficient, cost-effective, reduced chemical reliance, and provides sustainable resources for vector control (Tiffin et al. 2025). Thus, the findings of this research provide fundamental data to enable the transition of EO-based larvicides for vector control. On the global scale, the development of eco-friendly NEs contributes to the Sustainable Development Goal (SDG) 3: Good Health and Well-being by reducing the transmission of neglected tropical diseases, including dengue spread by *Aedes* mosquito. Moreover, this study also adheres to SDG 12: Responsible Consumption and Production by mitigating the environmental chemical residue through biodegradable pesticide alternatives, as this research advances the environmentally friendly approach through the development of EO-based NEs.

Overall, this research utilised EO encapsulated with NEs technology, overcoming the limitations of synthetic insecticide resistance and adhering to Malaysia's national health policies, and global sustainable development objectives. The study provides a comprehensive framework for the application of safe, effective, and sustainable interventions for future vector control. Furthermore, it emphasizes the practical utilisation of nanotechnology in public health, indicating scientific advancement. Therefore, the establishment of EO-based NEs as sustainable, effective, and optimised resource usage can be used to control *Ae. aegypti* in a safer and more resilient vector prevention strategies.

1.7 Conceptual Framework

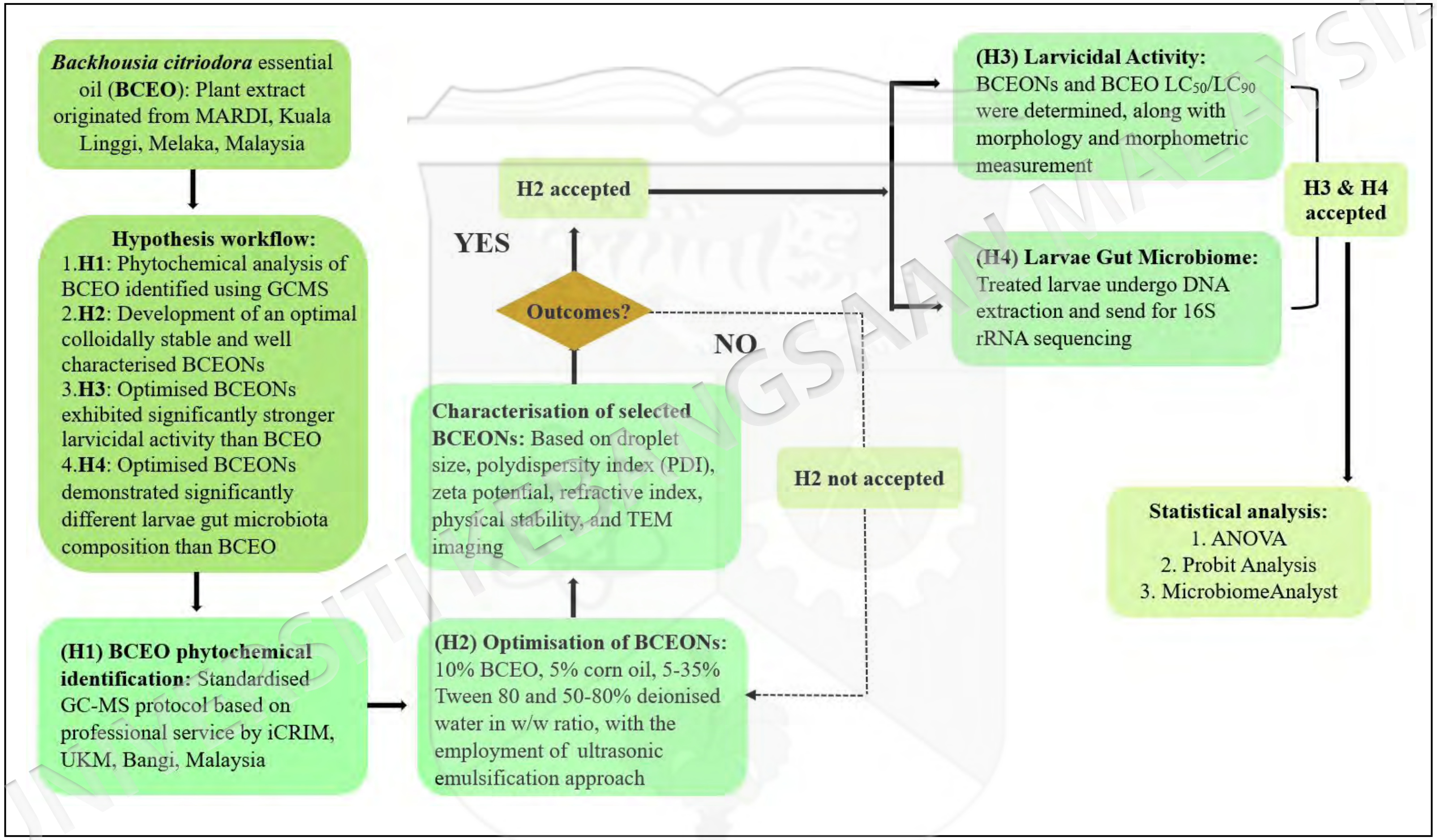

Figure 1.1 Conceptual framework.

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APPENDIX A

Backhousia citriodora Plant Specimen Voucher

UNIVERSITI KEBANGSAAN MALAYSIA
The National University of Malaysia

HERBARIUM UNIVERSITI KEBANGSAAN MALAYSIA (UKMB), FAKULTI SAINS DAN TEKNOLOGI

Rujukan/Reference: UKMB01/2024-01
Tarikh/Date: 05 FEB 2024

Shirley Tang Gee Hoon
Fakulti Sains Kesihatan, UKM

UNIVERSITI KEBANGSAAN MALAYSIA HERBARIUM
Telah disahkan pada 05.02.2024

YBhg. Tan Sri/Dato'/Datin/Tuan/Puan,

PENGESAHAN SPESIES/ CONFORMATION SPECIES

Dengan segala hormatnya perkara di atas dirujuk

2. Dimaklumkan bahawa spesimen tanaman yang diserahkan untuk pengenalan adalah di bawah:

Nama Saintifik	Keluarga	Nama yang selalu digunakan	Tag	Nombor baucar
Backhousia citriodora	Myrtaceae	Lemon myrtle	Dr. Shirley	ID001/2024

3. Harus diingat bahawa kami tidak terlibat dengan apa-apa hasil penyelidikan yang dilakukan oleh pihak YBhg. Tan Sri/Dato'/Datin/Tuan/Puan.

Terima kasih.

Yang Benar,

DR. SHAMSUL BIN KHAMIS
Ahli Botani/Botanist
Herbarium Universiti Kebangsaan Malaysia/

CERTIFIED TO ISO 9001:2015 CERT. NO. QMS 01100

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HERBARIUM UNIVERSITI KEBANGSAAN MALAYSIA *
Telah disahkan pada
05.02.2024

s.k - Kurator

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Lampiran

Nota: Ada bau seakan limau/lemon apabila daun di koyakkan

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APPENDIX B

Two-way Repeated Measure ANOVA for BCEON Physicochemical Characterisations

Table 1 Two-way repeated measure ANOVA of storage days and temperature effects on droplet size of BCEON formulations (NE1 and NE2)

NE1 Droplet Size	Sum of Square	*df*	Mean Square	*F*-value	*p*-value
Days	411.68	1.64	251.00	4.30	0.051
Temperatures	346.50	2	173.25	4.39	0.067
Days * Temperatures	998.49	3.28	304.38	5.21	0.019
Error (days)	575.03	9.84	58.43	N/A	N/A
NE2 Droplet Size	**Sum of Square**	***df***	**Mean Square**	***F*-value**	***p*-value**
Days	34183.55	2.05	16700.25	145.07	<0.001
Temperatures	95601.93	2	47800.97	97.98	<0.001
Days * Temperatures	44626.58	4.09	10901.08	94.69	<0.001
Error (days)	1413.85	12.28	115.12	N/A	N/A

Table 2 Two-way repeated measure ANOVA of storage days and temperature effects on polydispersity index of BCEON formulations (NE1 and NE2)

NE1 Polydispersity Index	Sum of Square	*df*	Mean Square	*F*-value	*p*-value
Days	0.01	4	0.00	3.44	0.023
Temperatures	0.01	2	0.01	10.17	0.012
Days * Temperatures	0.01	8	0.00	3.53	0.008
Error (days)	0.01	24	0.00	N/A	N/A
NE2 Polydispersity Index	**Sum of Square**	***df***	**Mean Square**	***F*-value**	***p*-value**
Days	0.03	4	0.01	14.62	<0.001
Temperatures	0.08	2	0.04	23.55	0.001
Days * Temperatures	0.02	8	0.00	4.62	0.002
Error (days)	0.01	24	0.00	N/A	N/A

Table 3 Two-way repeated measure ANOVA of storage days and temperature effects on zeta potential of BCEON formulations (NE1 and NE2)

NE1 Zeta Potential	**Sum of Square**	***df***	**Mean Square**	***F*-value**	***p*-value**
Days	98.31	4	24.58	14.92	<0.001
Temperatures	15.50	2	7.75	4.67	0.060
Days * Temperatures	88.81	8	11.10	6.74	<0.001
Error (days)	39.54	24	1.65	N/A	N/A
NE2 Zeta Potential	**Sum of Square**	***df***	**Mean Square**	***F*-value**	***p*-value**
Days	4.64	4	1.16	3.26	0.029
Temperatures	13.25	2	6.63	17.56	0.003
Days * Temperatures	18.86	8	2.36	6.62	<0.001
Error (days)	8.55	24	0.36	N/A	N/A

Table 4 Two-way repeated measure ANOVA of storage days and temperature effects on refractive index of BCEON formulations (NE1 and NE2)

NE1 Refractive Index	**Sum of Square**	***df***	**Mean Square**	***F*-value**	***p*-value**
Days	1.18 E^{-6}	4	2.94 E^{-7}	4.37	0.009
Temperatures	1.54 E^{-6}	2	7.72 E^{-7}	4.42	0.066
Days * Temperatures	1.64 E^{-6}	8	2.05 E^{-7}	3.05	0.016
Error (days)	1.61 E^{-6}	24	6.72 E^{-8}	N/A	N/A
NE2 Refractive Index	**Sum of Square**	***df***	**Mean Square**	***F*-value**	***p*-value**
Days	0.00	1.15	0.00	27.88	<0.001
Temperatures	3.54 E^{-6}	2	1.77 E^{-6}	1.15	0.378
Days * Temperatures	0.00	2.30	0.00	30.67	<0.001
Error (days)	2.50 E^{-5}	6.91	3.62 E^{-6}	N/A	N/A

APPENDIX C

Predicted Pathways for Larvae Gut Microbiome Based on STAMP Analysis

Table 1 Predicted pathways of larvae gut microbiome treated with NE_LC_{50} and water

Regulation Direction	Functional Cluster	Pathway Count	Percentage (%)	Pathway Affected
Supressed by NE_LC_{50} (24 pathways:72.7%)	Metabolism of Cofactors and Vitamins	7	21.2	mono-trans, poly-cis decaprenyl phosphate biosynthesis
				cob(II)yrinate a,c-diamide biosynthesis I (early cobalt insertion)
				mycothiol biosynthesis
				adenosylcobalamin biosynthesis from cobyrinate a,c-diamide I
				adenosylcobalamin salvage from cobinamide II
				adenosylcobalamin salvage from cobinamide I
				6-hydroxymethyl-dihydropterin diphosphate biosynthesis III (*Chlamydia*)
	Carbohydrate and Energy Metabolism	5	15.2	2-methylcitrate cycle II
				isopropanol biosynthesis
				formaldehyde assimilation II (RuMP Cycle)
				formaldehyde oxidation I
				succinate fermentation to butanoate
	Metabolism of Terpenoids and Polyketides	4	12.1	superpathway of geranylgeranyldiphosphate biosynthesis I (via mevalonate)
				mevalonate pathway I
				isoprene biosynthesis II (engineered)
				superpathway of geranylgeranyl diphosphate biosynthesis II (via MEP)

	Amino Acid Metabolism	4	12.1	L-glutamate and L-glutamine biosynthesis
				L-lysine biosynthesis III
				L-lysine biosynthesis VI
				L-tryptophan biosynthesis
	Genetic Information Processing	3	9.1	guanosine ribonucleotides de novo biosynthesis
				superpathway of guanosine nucleotides de novo biosynthesis II
				adenosine ribonucleotides de novo biosynthesis
	Lipid Metabolism	1	3.0	methyl ketone biosynthesis
Upregulated by NE_LC$_{50}$ (9 pathways:27.3%)	Amino Acid Metabolism	4	12.1	L-methionine biosynthesis I
				superpathway of L-methionine biosynthesis (transsulfuration)
				superpathway of S-adenosyl-L-methionine biosynthesis
				L-histidine degradation I
	Metabolism of Cofactors and Vitamins	3	9.1	1,4-dihydroxy-2-naphthoate biosynthesis I
				superpathway of menaquinol-8 biosynthesis I
				superpathway of phylloquinol biosynthesis
	Energy Metabolism	2	6.1	sulfate reduction I (assimilatory)
				superpathway of sulfate assimilation and cysteine biosynthesis
	Total	**33**	**100**	

Table 2 Predicted pathways of larvae gut microbiome treated with NE_LC_{90} and water

Regulation Direction	Functional Cluster	Pathway Count	Percentage (%)	Pathway Affected
Supressed by NE_LC_{90} (21 pathways:60%)	Genetic Information Processing	6	17.1	adenosine ribonucleotides de novo biosynthesis
				guanosine ribonucleotides de novo biosynthesis
				superpathway of guanosine nucleotides de novo biosynthesis I
				superpathway of adenosine nucleotides de novo biosynthesis I
				superpathway of adenosine nucleotides de novo biosynthesis II
				superpathway of pyrimidine deoxyribonucleotides de novo biosynthesis (*E. coli*)
	Amino Acid Metabolism	5	14.3	L-glutamate and L-glutamine biosynthesis
				L-isoleucine biosynthesis II
				superpathway of branched amino acid biosynthesis
				L-lysine biosynthesis III
				L-lysine biosynthesis I
	Carbohydrate and Energy Metabolism	4	11.4	isopropanol biosynthesis
				formaldehyde assimilation II (RuMP Cycle)
				formaldehyde oxidation I
				pyruvate fermentation to butanoate
	Metabolism of Cofactors and Vitamins	3	8.6	cob(II)yrinate a,c-diamide biosynthesis I (early cobalt insertion)
				mono-trans, poly-cis decaprenyl phosphate biosynthesis
				tetrapyrrole biosynthesis I (from glutamate)

	Metabolism of Terpenoids and Polyketides	2	5.7	superpathway of geranylgeranyldiphosphate biosynthesis I (via mevalonate)
				mevalonate pathway I
	Lipid Metabolism	1	2.9	methyl ketone biosynthesis
Upregulated by NE_LC$_{90}$ **(14 pathways:40%)**	Metabolism of Cofactors and Vitamins	7	20.0	superpathway of menaquinol-8 biosynthesis I
				superpathway of demethylmenaquinol-8 biosynthesis
				superpathway of menaquinol-11 biosynthesis
				superpathway of menaquinol-12 biosynthesis
				superpathway of menaquinol-13 biosynthesis
				superpathway of menaquinol-7 biosynthesis
				superpathway of phylloquinol biosynthesis
	Amino Acid Metabolism	5	14.3	arginine, ornithine and proline interconversion
				superpathway of polyamine biosynthesis I
				L-methionine biosynthesis I
				superpathway of S-adenosyl-L-methionine biosynthesis
				L-arginine biosynthesis III (via N-acetyl-L-citrulline)
	Lipid Metabolism	1	2.9	fatty acid elongation -- saturated
	Carbohydrate Metabolism	1	2.9	pentose phosphate pathway
	Total	**35**	**100**	

Table 3 Predicted pathways of larvae gut microbiome treated with NE_LC_{50} and 0.02 ppm temephos

Regulation Direction	Functional Cluster	Pathway Count	Percentage (%)	Pathway Affected
Supressed by NE_ LC_{50} (17 pathways:54.8%)	Metabolism of Cofactors and Vitamins	7	22.6	cob(II)yrinate a,c-diamide biosynthesis I (early cobalt insertion)
				adenosylcobalamin salvage from cobinamide I
				adenosylcobalamin biosynthesis from cobyrinate a,c-diamide I
				adenosylcobalamin salvage from cobinamide II
				thiamin salvage II
				NAD biosynthesis II (from tryptophan)
				superpathway of heme biosynthesis from glutamate
	Carbohydrate and Energy Metabolism	4	12.9	pyruvate fermentation to butanoate
				superpathway of *Clostridium acetobutylicum* acidogenic fermentation
				Calvin-Benson-Bassham cycle
				pyruvate fermentation to acetone
	Genetic Information Processing	3	9.7	tRNA charging
				pyrimidine deoxyribonucleotides de novo biosynthesis II
				superpathway of pyrimidine deoxyribonucleotides de novo biosynthesis (*E. coli*)
	Amino Acid Metabolism	2	6.5	superpathway of L-threonine metabolism
				L-isoleucine biosynthesis IV

	Glycan Biosynthesis and Metabolism	1	3.2	superpathway of UDP-N-acetylglucosamine-derived O-antigen building blocks biosynthesis
Supressed by 0.02 ppm Temephos (14 pathways: 45.2%)	Carbohydrate and Energy Metabolism	7	22.6	TCA cycle VIII (helicobacter)
				TCA cycle VII (acetate-producers)
				superpathway of glyoxylate bypass and TCA
				TCA cycle IV (2-oxoglutarate decarboxylase)
				TCA cycle I (prokaryotic)
				superpathway of sulfate assimilation and cysteine biosynthesis
				sulfate reduction I (assimilatory)
	Metabolism of Cofactors and Vitamins	5	16.1	1,4-dihydroxy-2-naphthoate biosynthesis I
				superpathway of menaquinol-11 biosynthesis
				superpathway of menaquinol-12 biosynthesis
				superpathway of menaquinol-13 biosynthesis
				superpathway of phylloquinol biosynthesis
	Amino Acid Metabolism	2	6.5	superpathway of L-serine and glycine biosynthesis I
				L-histidine degradation I
Total		**31**	**100**	

Table 4 Predicted pathways of larvae gut microbiome treated with NE_LC_{90} and 0.02 ppm temephos

Regulation Direction	Functional Cluster	Pathway Counts	Percentage (%)	Pathway Affected
Supressed by NE_LC_{90} (44 pathways: 84.6%)	Genetic Information Processing	21	46.7	tRNA charging
				adenosine ribonucleotides de novo biosynthesis
				adenosine deoxyribonucleotides de novo biosynthesis II
				guanosine deoxyribonucleotides de novo biosynthesis II
				superpathway of pyrimidine ribonucleotides de novo biosynthesis
				inosine-5'-phosphate biosynthesis I
				UMP biosynthesis
				pyrimidine deoxyribonucleotide phosphorylation
				superpathway of guanosine nucleotides de novo biosynthesis II
				superpathway of guanosine nucleotides de novo biosynthesis I
				superpathway of purine nucleotides de novo biosynthesis II
				guanosine ribonucleotides de novo biosynthesis
				superpathway of adenosine nucleotides de novo biosynthesis II
				superpathway of adenosine nucleotides de novo biosynthesis I
				5-aminoimidazole ribonucleotide biosynthesis II
				superpathway of 5-aminoimidazole ribonucleotide biosynthesis
				superpathway of pyrimidine deoxyribonucleotides de novo biosynthesis (*E. coli*)

				pyrimidine deoxyribonucleotides de novo biosynthesis II
				superpathway of purine nucleotides de novo biosynthesis I
				pyrimidine deoxyribonucleotides de novo biosynthesis I
				superpathway of pyrimidine ribonucleosides salvage
	Amino Acid Metabolism	5	11.1	L-histidine biosynthesis
				L-tryptophan biosynthesis
				L-isoleucine biosynthesis II
				superpathway of L-threonine biosynthesis
				superpathway of L-isoleucine biosynthesis I
	Metabolism of Cofactors and Vitamins	4	8.9	cob(II)yrinate a,c-diamide biosynthesis I (early cobalt insertion)
				coenzyme A biosynthesis I
				flavin biosynthesis I (bacteria and plants)
				thiamin salvage II
	Glycan Biosynthesis and Metabolism	2	4.4	UDP-N-acetyl-D-glucosamine biosynthesis I
				O-antigen building blocks biosynthesis (*E. coli*)
	Metabolism of Terpenoids and Polyketides	2	4.4	methylerythritol phosphate pathway I
				methylerythritol phosphate pathway II
	Lipid Metabolism	2	4.4	phosphatidylglycerol biosynthesis I (plastidic)
				phosphatidylglycerol biosynthesis II (non-plastidic)
	Carbohydrate Metabolism	1	2.2	homolactic fermentation
	Metabolism of Cofactors and	4	8.9	superpathway of menaquinol-8 biosynthesis I

Supressed by 0.02 pp Temephos **(8 pathways: 15.4%)**	Vitamins			superpathway of demethylmenaquinol-8 biosynthesis
				biotin biosynthesis I
				superpathway of heme biosynthesis from uroporphyrinogen-III
	Carbohydrate and Energy Metabolism	3	6.7	TCA cycle IV (2-oxoglutarate decarboxylase)
				glyoxylate cycle
				pentose phosphate pathway
	Amino Acid Metabolism	1	2.2	superpathway of polyamine biosynthesis I
	Total	**45**	**100**	

Table 5 Predicted pathways of larvae gut microbiome treated with NE_LC_{50} and EO_LC_{50}

Suppression Agent	Functional Cluster	Pathway Count	Percentage (%)	Pathway Affected
NE_LC_{50} (14 pathways: 100%)	Carbohydrate and Energy Metabolism	7	50.0	Bifidobacterium shunt
				heterolactic fermentation
				ethylmalonyl-CoA pathway
				pyruvate fermentation to propanoate I
				formaldehyde assimilation II (RuMP Cycle)
				formaldehyde oxidation I
				ketogluconate metabolism
	Xenobiotic Biodegradation and Metabolism	3	21.4	methylgallate degradation
				gallate degradation II
				toluene degradation IV (aerobic) (via catechol)
	Metabolism of Cofactors and Vitamins	2	14.3	mono-trans, poly-cis decaprenyl phosphate biosynthesis
				superpathway of heme biosynthesis from glutamate
	Amino Acid Metabolism	1	7.1	creatinine degradation II
	Lipid Metabolism	1	7.1	methyl ketone biosynthesis
Total		**14**	**100.0**	

Table 6 Predicted pathways of larvae gut microbiome treated with NE_LC_{90} and EO_LC_{90}

Regulations Direction	Functional Cluster	Pathway Count	Percentage (%)	Pathway Affected
Supressed by NE_LC_{90} (22 pathways: 59%)	Carbohydrate and Energy Metabolism	8	21.6	superpathway of fucose and rhamnose degradation
				heterolactic fermentation
				Bifidobacterium shunt
				formaldehyde assimilation II (RuMP Cycle)
				TCA cycle VI (obligate autotrophs)
				formaldehyde oxidation I
				Calvin-Benson-Bassham cycle
				ketogluconate metabolism
	Genetic Information Processing	5	13.5	pyrimidine deoxyribonucleotides de novo biosynthesis I
				superpathway of pyrimidine deoxyribonucleotides de novo biosynthesis
				superpathway of pyrimidine deoxyribonucleotides de novo biosynthesis (*E. coli*)
				purine nucleotides degradation II (aerobic)
				adenosine nucleotides degradation II
	Amino Acid Metabolism	3	8.1	superpathway of polyamine biosynthesis II
				superpathway of L-threonine metabolism
				superpathway of L-serine and glycine biosynthesis I
	Metabolism of Cofactors and Vitamins	3	8.1	adenosylcobalamin biosynthesis I (early cobalt insertion)
				superpathway of tetrahydrofolate biosynthesis

				mono-trans, poly-cis decaprenyl phosphate biosynthesis
	Lipid Metabolism	2	8.1	fatty acid beta-oxidation I
				methyl ketone biosynthesis
Supressed by EO_LC$_{90}$ (15 pathways:41%)	Genetic Information Processing	6	16.2	adenine and adenosine salvage III
				purine ribonucleosides degradation
				pyrimidine deoxyribonucleosides salvage
				superpathway of pyrimidine deoxyribonucleosides degradation
				superpathway of pyrimidine ribonucleosides salvage
				superpathway of purine deoxyribonucleosides degradation
	Amino Acid Metabolism	5	13.5	superpathway of arginine and polyamine biosynthesis
				superpathway of polyamine biosynthesis I
				L-lysine biosynthesis II
				S-methyl-5-thio-alpha-D-ribose 1-phosphate degradation
				2-amino-3-carboxymuconate semialdehyde degradation to 2-oxopentenoate
	Xenobiotic Biodegradation and Metabolism	3	8.1	catechol degradation II (meta-cleavage pathway)
				2-nitrobenzoate degradation I
				catechol degradation to 2-oxopent-4-enoate II
	Energy Metabolism	1	2.7	superpathway of sulfur oxidation (*Acidianus ambivalens*)
	Glycan Biosynthesis and Metabolism	1	2.7	peptidoglycan maturation (meso-diaminopimelate containing)
	Total	**37**	**100**	

APPENDIX D

List of Publications

1. Nurul Syafiqahizzati, A.K., Tang, S.G.H., Othman, H., Ng, S.F., Chua, S.K., Kamari, A., Abdul Hamid, A.N.S., Janudin, M.R. & Rahman, H.S. Recent advancements in citral research: A comprehensive bibliometric overview and review of agricultural applications and future directions. *Phytochemistry Reviews*. WOS SCIE Q1, IF: 7.6. (accepted with publication)

2. Nurul Syafiqahizzati, A.K., Tang, S.G.H., Othman, H., Ng, S.F., Chua, S.K., Kamari, A., Abdul Hamid, A.N.S., Janudin, M.R. & Rahman, H.S. Phyto-nanoemulsion of lemon myrtle essential oil: Development and larvicidal efficacy against *Aedes aegypti* L. The 5th International Conference Advances in Medical Science. (2025). Medicine & Health, s20(2), 18. https://doi.org/10.17576/MH.2025.s2002. (WOS, ESCI, Q4)

3. Nurul Syafiqahizzati, A.K., Tang, S.G.H., Othman, H., Ng, S.F., Chua, S.K., Kamari, A., Abdul Hamid, A.N.S., Janudin, M.R. & Rahman, H.S. Lemon myrtle essential oil nanoemulsion: A next-generation nano-larvicide for sustainable dengue vector control. Research and Innovation Day 2025. https://www.ukm.my/fst/en/hapi2025-abstract

4. Nurul Syafiqahizzati, A.K., Tang, S.G.H., Othman, H., Ng, S.F., Chua, S.K., Kamari, A., Abdul Hamid, A.N.S., Janudin, M.R. & Rahman, H.S. Nanoemulsified Lemon Myrtle Essential Oil: An Effective Green Larvicide Against *Aedes aegypti.* International Conference on Bioengineering for Health & Environment (ICBHE 2025). https://manipal.edu.my/icbhe2025/

1.

Phytochemistry Reviews

Recent advancements in citral research: A comprehensive bibliometric overview and review of agricultural applications and future directions

--Manuscript Draft--

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Full Title:	Recent advancements in citral research: A comprehensive bibliometric overview and review of agricultural applications and future directions
Article Type:	Review Article
Keywords:	citral; scientific research; bibliometric; research hotspots; sustainable agrochemical; future directions
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Funding Information:	Malaysian Ministry of Higher Education (FRGS/1/2023/SKK06/UKM/02/15) — Dr Shirley Gee Hoon Tang
Abstract:	Citral (C6H16O), a key monoterpene aldehyde found in many plant essential oils, has contributed significantly to advancements in sustainable agriculture. However, its research evolution and future trends in agriculture remain underexplored. This work presents a comprehensive bibliometric overview of citral research from 2014 to September 2024 based on 363 citral-related papers from the Scopus database. It explores publication trends, research hotspots and future directions in agriculture through the analysis of publication outputs, journal rankings, influential research contributions (countries and authors), keyword co-occurrence and paper citations. Publication trends indicated a steady growth and rising interest in citral research, with untapped potential, while China emerged as the key contributor with the highest publication output. The top ten most cited articles and reviews were also analyzed for their field impact. Major research directions included antifungal and herbicidal activities, edible coatings, encapsulation technologies, therapeutic applications, and catalysis. An in-depth review of the agricultural applications of citral is presented, including its mechanisms of action and limitations. The review also highlights remaining research gaps and future opportunities. Finally, the challenges and future

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	directions of citral research were discussed to promote sustainable agricultural practices. This study offers valuable insights for researchers, industry stakeholders and policymakers in advancing citral-related research and its practical applications in agriculture.

2.

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OC12

Phyto-Nanoemulsion of Lemon Myrtle Essential Oil: Development and Larvicidal Efficacy against *Aedes aegypti* L.

AB KHALIM NURUL SYAFIQAHIZZATI[1]*, SHIRLEY GEE HOON TANG[1]*, HIDAYATULFATHI OTHMAN[1], SHIOW FERN NG[2], SIAW KIM CHUA[3], AZLAN KAMARI[4], SHARIFAH NUR SHAHIRAH BINTI SYED ABDUL HAMID[5], MOHD RIDZUAN BIN JANUDIN[5], HESHU SULAIMAN RAHMAN[6]

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[2]*Center for Drug Delivery Technology and Vaccine (CENTRIC), Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, Kuala Lumpur, 50300, Malaysia*
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[4]*Nanotechnology Research Centre, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900, Tanjung Malim, Perak, Malaysia*
[5]*Melaka Tengah District Health Office, Jalan Bukit Baru, Melaka 75150, Malaysia*
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**Presenting Author: P138286@siswa.ukm.edu.my*

Introduction: Mosquito-borne diseases remain a significant global health concern, with *Aedes aegypti* serving as the primary vector for multiple viruses, including the dengue virus. The rapid emergence of resistance to synthetic insecticides has compromised the effectiveness of vector control strategies. Therefore, the development of novel and efficient insecticides is crucial for sustainable mosquito management. Lemon myrtle (*Backhousia citriodora*) essential oil (LMEO) demonstrates potent antimicrobial, antifungal, antibiofilm, and insecticidal activities, but its hydrophobicity and susceptibility to environmental instability limit its efficacy.

Objectives: This study aims to develop a phyto-nanoemulsion-LMEO system (LMEON) and evaluate its larvicidal efficacy against *Ae. aegypti*.

Methods: The main constituents of LMEO were identified using gas chromatography-mass spectrometry (GC-MS). LMEON was prepared using the high-energy ultrasonication method, incorporating LMEO, Tween 80, corn oil, and deionized water at optimized concentrations. The fabricated LMEON were characterized using a dynamic light scattering particle size analyzer. The larvicidal study was then performed following the standardized protocol established by the World Health Organization (WHO).

Results: GC-MS analysis identified neral and geranial as two major phytochemicals in LMEO. Characterization of the selected formulations revealed that Formulation A (containing 5% Tween 80) and Formulation B (containing 10% Tween 80) exhibited nanoscale droplet sizes. Stability testing over 28 days at three different temperatures (4°C, 25°C, and 37°C) indicated that Formulation A

Med & Health Jul 2025; 20(2) (Suppl): 01-72 https://doi.org/10.17576/MH.2025.s2002

was the most stable, as no phase separation was observed, and droplet size remained consistent throughout the observation period. Larvicidal assays demonstrated that Formulation A achieved a higher lethal effect at a lower concentration compared to LMEO, suggesting its potential as an effective and sustainable approach for integrated mosquito control programs.
Conclusion: These findings highlight the potential of nanoemulsified LMEO as an eco-friendly and effective alternative to synthetic insecticides for *Ae. aegypti* control.

3.

Lemon Myrtle Essential Oil Nanoemulsion: A Next-Generation Nano-Larvicide for Sustainable Dengue Vector Control

NURUL SYAFIQAHIZZATI BINTI AB KHALIM[1], SHIRLEY GEE HOON TANG*[,1], HIDAYATULFATHI OTHMAN[1], SHIOW FERN NG[2], AZLAN KAMARI[3], SHARIFAH NUR SHAHIRAH BINTI SYED ABDUL HAMID[4] and MOHD RIDZUAN BIN JANUDIN[4]

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[4]*Melaka Tengah District Health Office, Jalan Bukit Baru, 75150 Melaka, Malaysia*
**Corresponding author email: shirleytgh@ukm.edu.my*

ABSTRACT

Aedes aegypti is a primary vector of dengue, and increasing resistance to synthetic insecticides necessitates innovative and sustainable vector control alternatives. This study presents a nanotechnology-enhanced approach utilizing lemon myrtle essential oil (LMEO), recognised for its antimicrobial, antibiofilm and insecticidal properties. A stable LMEO nanoemulsion (LMEON) system was developed through high-energy emulsification and displayed good colloidal stability over 28 days with stable values of size, polydispersity index, and zeta potential. Comparative larvicidal assays against *Ae. aegypti* revealed that LMEON exhibited significantly higher efficacy ($P<0.05$) at lower concentrations compared to bulk LMEO. LMEON treatment caused severe morphological deformations in *Ae. aegypti* larvae, including cephalic, thoracic, abdominal, and siphon abnormalities. The superior performance of LMEON is attributed to enhanced bioavailability and targeted delivery, ensuring effective larval control while minimizing environmental impact. This innovation highlighted the potential of LMEON as a dual-action nano-larvicide addressing both resistance challenges and ecological concerns.

BB27	Siti Noor Syafarehan Mohd Isa, Nurul Azira Mohd Zamziba, Shamsul Khamis, Hamdan Omar dan Norhayati Ahmad *Taburan Flora Pada Mikrohabitat Berbeza di Gunung Lang, Ipoh, Perak*
BB28	Nurul Syafiqahizzati Ab Khalim, Shirley Gee Hoon Tang, Hidayatulfathi Othman, Shiow Fern Ng, Azlan Kamari, Sharifah Nur Shahirah Syed Abdul Hamid and Mohd Ridzuan Janudin *Lemon Myrtle Essential Oil Nanoemulsion: A Next-Generation Nano-Larvicide for Sustainable Dengue Vector Control*
BB29	Johari Jalinas, Meor Muhamad Syahmi Meor Adam, Farah Hanisya Sidek, Nur Zahirah Khusaini, Siti Norhafizah Ahmad Tarmidzi dan Mohamad Ruzi Abdul Rahman *Kembara Kasturi: Mengenal Sahabat & Musuh Limau Kasturi*
BB30	Muhammad Hariz Asraf, Nik Ahmad Nizam Nik Malek and Imran Qashfi Ismail *Colloidal* Persicaria odorata*-Silver Nanoparticles as Antibacterial Spray and Cream*

4.

5th International Conference on Bioengineering for Health and Environment

Nanoemulsified Lemon Myrtle Essential Oil: An Effective Green Larvicide Against *Aedes aegypti*

Ab Khalim Nurul Syafiqahizzati[1], Shirley Gee Hoon Tang[1*], Hidayatulfathi Othman[1], Shiow Fern Ng[2], Siaw Kim Chua[3], Azlan Kamari[4], Sharifah Nur Shahirah binti Syed Abdul Hamid[5], Mohd Ridzuan bin Janudin[5] and Heshu Sulaiman Rahman[6]

[1]Center for Toxicology and Health Risk Studies (CORE), Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, Kuala Lumpur 50300, Malaysia
[2]Center for Drug Delivery Technology and Vaccine (CENTRIC), Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, Kuala Lumpur, 50300, Malaysia
[3]Malaysian Palm Oil Board, 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia
[4]Nanotechnology Research Centre, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900, Tanjung Malim, Perak, Malaysia
[5]Melaka Tengah District Health Office, Jalan Bukit Baru, Melaka 75150, Malaysia
[6]Department of Basic Medical Sciences, College of Medicine, University of Sulaimani, Sulaimaniyah, Iraq

Mosquito-borne diseases remain a pressing global health issue, with *Aedes aegypti* recognized as the primary vector of viruses such as dengue. The growing resistance of mosquito populations to synthetic insecticides has reduced the effectiveness of conventional control measures, highlighting the urgent need for safer and more sustainable alternatives. Lemon myrtle (*Backhousia citriodora*) essential oil (LMEO) has demonstrated strong antimicrobial, antifungal, antibiofilm, and insecticidal properties; however, its hydrophobic nature and susceptibility to environmental degradation limit its direct application. This study aimed to develop a phyto-nanoemulsion system incorporating LMEO (LMEON) and evaluate its larvicidal efficacy against *Ae. aegypti*. The major constituents of LMEO were identified by gas chromatography–mass spectrometry (GC-MS), revealing neral and geranial as the predominant compounds. LMEON formulations were prepared using a high-energy ultrasonication method with LMEO, Tween 80, corn oil, and deionized water at optimized ratios. Characterization via dynamic light scattering confirmed nanoscale droplet sizes for two selected formulations: Formulation A (5% Tween 80) and Formulation B (10% Tween 80). Stability studies conducted over 28 days at 4°C, 25°C, and 37°C showed that Formulation A was the most stable, maintaining consistent droplet size with no phase separation. Larvicidal bioassays following World Health Organization (WHO) guidelines demonstrated that Formulation A exerted higher mortality against *Ae. aegypti* larvae at lower concentrations than LMEO alone. Overall, these findings demonstrates that nanoemulsified LMEO offers enhanced stability and larvicidal potency, supporting its potential as an eco-friendly and effective alternative to synthetic insecticides in integrated mosquito management programs.

Keywords: Lemon myrtle, nanoemulsion, *Aedes aegypti*, larvicidal

[*]**Correspondence:** Shirley Gee Hoon Tang, shirleytgh@ukm.edu.my

APPENDIX E

List of Attended Conferences and Innovation Competitions

1. 5th International Conference on Advances in Medical Science


UNIVERSITI KEBANGSAAN MALAYSIA The National University of Malaysia

NARATIF BAHARU UKM UNIVERSITI WATAN KITA

FACULTY OF MEDICINE THE NATIONAL UNIVERSITY OF MALAYSIA

ICAMS

CERTIFICATE

OF ORAL PRESENTATION

THIS CERTIFICATE IS PROUDLY AWARDED TO

NURUL SYAFIQAH IZZATI BINTI AB KHALIM

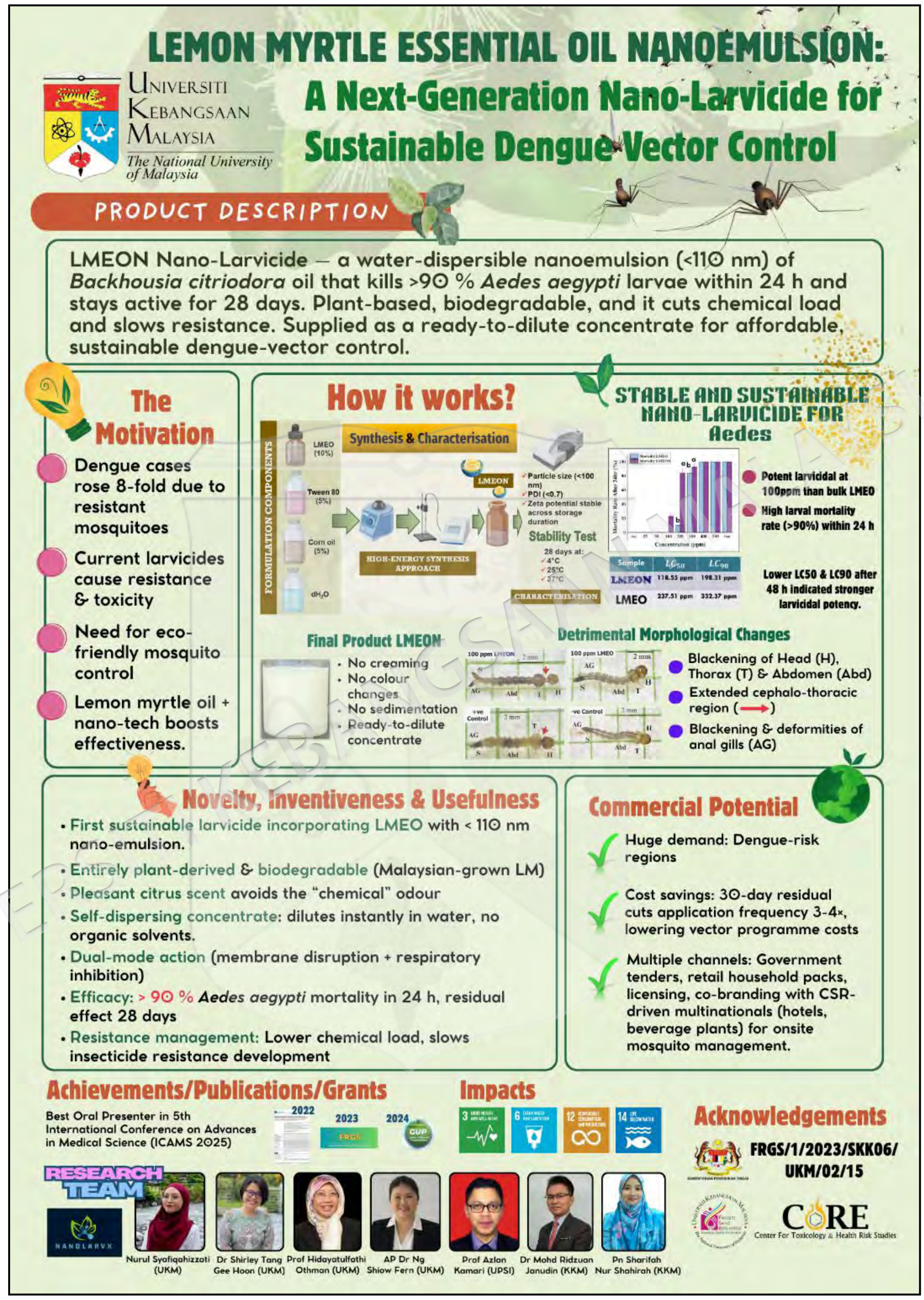
WITH THE PRESENTATION TITLE

Phyto-Nanoemulsion of Lemon Myrtle Essential Oil: Development and Larvicidal Efficacy Against Aedes Aegypti L.

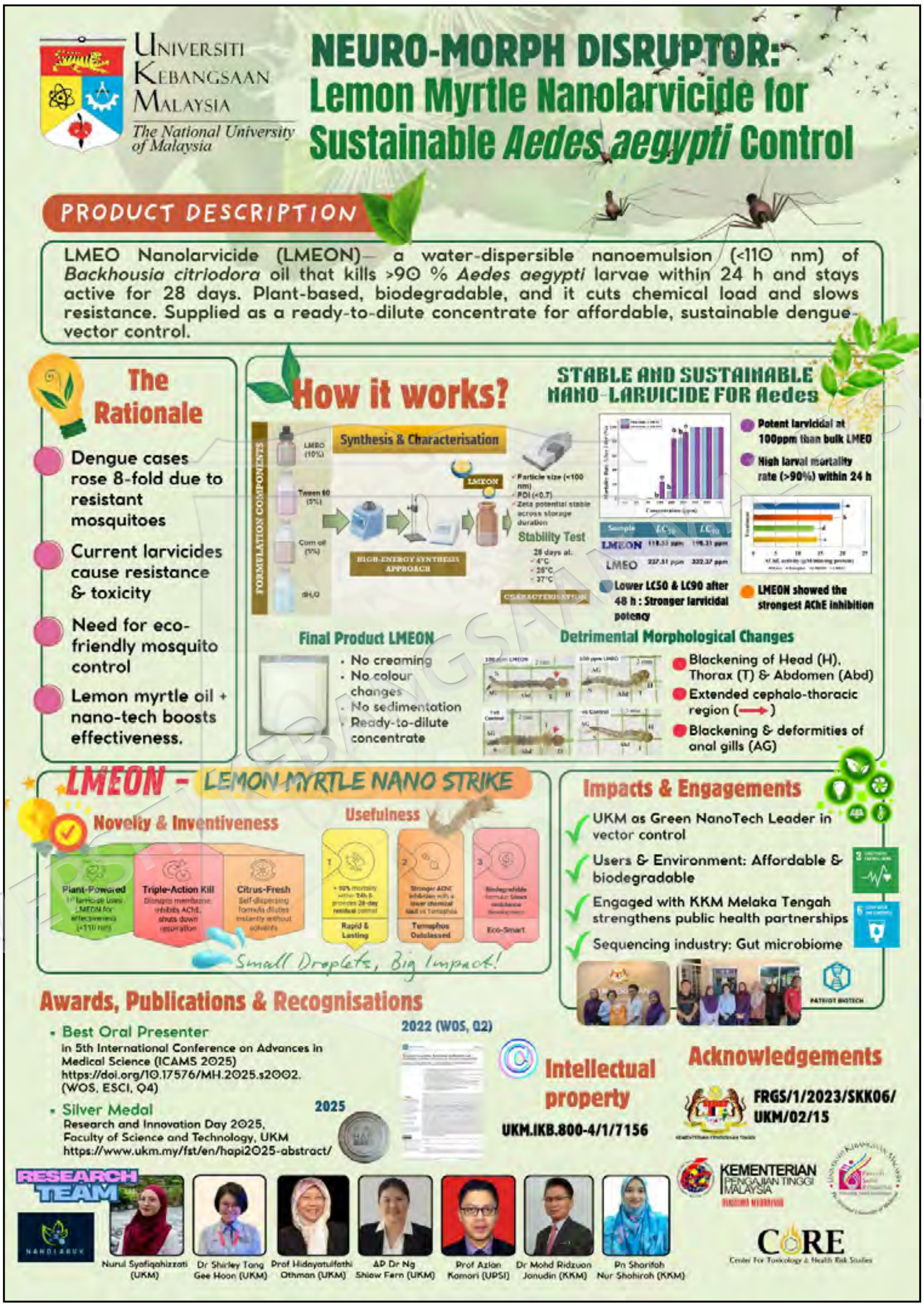
during the **5th International Conference on Advances in Medical Science** on 29th & 30th April 2025 at Concorde Hotel, Kuala Lumpur, Malaysia

Ts. Dr. Wong Sok Kuan
Chairperson

2. Hari Penyelidikan dan Inovasi (HAPI) 2025, Fakulti Sains dan Teknologi, UKM, Bangi



3. Hari Penyelidikan dan Inovasi Fakulti Sains Kesihatan 2025, UKM, Kuala Lumpur



APPENDIX F

List of Awards

1. Best Oral Presentation at International Conference on Advances in Medical Sciences (ICAMS) 2025.

2. Silver Award at Hari Penyelidikan dan Inovasi (HAPI) 2025, Fakulti Sains dan Teknologi, UKM, Bangi.

3. Gold Award at Hari Penyelidikan dan Inovasi Fakulti Sains Kesihatan 2025, UKM, Kuala Lumpur.

1.


UNIVERSITI KEBANGSAAN MALAYSIA
The National University of Malaysia
NARATIF BAHARU UKM
UNIVERSITI WATAN KITA
FACULTY OF MEDICINE
THE NATIONAL UNIVERSITY OF MALAYSIA
3 GOOD HEALTH AND WELL-BEING
ICAMS
BEST ORAL PRESENTATION AWARD
THIS CERTIFICATE OF AWARD IS PRESENTED TO
NURUL SYAFIQAHIZZATI BINTI AB KHALIM
WITH THE PRESENTATION TITLE
PHYTO-NANOEMULSION OF LEMON MYRTLE ESSENTIAL OIL: DEVELOPMENT AND LARVICIDAL EFFICACY AGAINST Aedes Aegypti L.
during the 5th International Conference on Advances in Medical Science on 29th & 30th April 2025 at Concorde Hotel, Kuala Lumpur, Malaysia
Ts. Dr. Wong Sok Kuan
Chairperson

2.

UNIVERSITI KEBANGSAAN MALAYSIA The National University of Malaysia

NARATIF BAHARU UKM UNIVERSITI WATAN KITA

FAKULTI SAINS & TEKNOLOGI

25 FST SAMBUTAN JUBLI PERAK 1999-2024

PINGAT PERAK

Pencapaian ini dianugerahkan kepada

Nurul Syafiqahizzati Ab Khalim, Shirley Gee Hoon Tang, Hidayatulfathi Othman, Shiow Fern Ng, Azlan Kamari, Sharifah Nur Shahirah Syed Abdul Hamid, Mohd Ridzuan Janudin and Heshu Sulaiman Rahman

atas penyelidikan dan inovasi bertajuk

Lemon Myrtle Essential Oil Nanoemulsion: A Next-Generation Nano-Larvicide for Sustainable Dengue Vector Control

sempena

Hari Penyelidikan & Inovasi
HAPI 2025

FST Menyerlah: 25 Tahun Kecemerlangan Penyelidikan

21 Mei 2025

Dewan Tun Abdullah Mohd. Salleh (DTAMS), UKM

Prof. ChM. Dr. Ishak Ahmad
DEKAN
FAKULTI SAINS DAN TEKNOLOGI

3.

UNIVERSITI KEBANGSAAN MALAYSIA
The National University of Malaysia

Universiti Kebangsaan Malaysia
FAKULTI SAINS KESIHATAN
Peneraju Sains Kesihatan
The National University of Malaysia

SIJIL ANUGERAH

Sekalung Tahniah kepada

NURUL SYAFIQAHIZZATI BINTI AB KHALIM,
SHIRLEY GEE HOON TANG,
HIDAYATULFATHI BINTI OTHMAN,
NG SHIOW FERN,
AZLAN BIN KAMARI,
SHARIFAH NUR SHAHIRAH BINTI
SYED ABDUL HAMID,
MOHD RIDZUAN BIN JANUDIN,
HESHU SULAIMAN RAHMAN

Telah memenangi

PINGAT EMAS
Kategori : INOVASI PENYELIDIKAN

Sempena

HARI PENYELIDIKAN & INOVASI FSK 2025
Kajian Membara Inovasi Menyala

30 September 2025

....
PROF. DR. SUZANA SHAHAR
Dekan
Fakulti Sains Kesihatan
Universiti Kebangsaan Malaysia