

THE EFFICACY OF BIOPESTICIDES, *Ipomoea cairica* (L.) LEAF EXTRACTS AND *Metarhizium anisopliae* (Metsch.) ON RESISTANT POPULATIONS OF *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) IN URBAN AND SUBURBAN AREAS OF PENANG ISLANDS, MALAYSIA

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THE EFFICACY OF BIOPESTICIDES, *Ipomoea cairica* (L.) LEAF EXTRACTS AND *Metarhizium anisopliae* (Metsch.) ON RESISTANT POPULATIONS OF *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) IN URBAN AND SUBURBAN AREAS OF PENANG ISLANDS, MALAYSIA

by

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LIST OF ABBREVIATIONS

<i>Ae.</i>	<i>Aedes</i>
<i>An.</i>	<i>Anopheles</i>
<i>Cx.</i>	<i>Culex</i>
<i>I.</i>	<i>Ipomoea</i>
<i>M.</i>	<i>Metarhizium</i>
PBO	Piperonyl butoxide
LT	Lethal Time
KT	Knockdown Time
WNV	West Nile virus
ppm	Parts per million
DENV	Dengue Virus
DF	Dengue Fever
DHF	Dengue Haemorrhagic Fever
DSS	Dengue Shock Syndrome
P450s	Cytochrome P450 monooxygenases
ESTs	Esterases

GSTs	Glutathione S–transferases
AChE	Acetylcholinesterase
MFO	Mixed Function Oxidase
α –EST	Alpha–esterase
β –EST	Beta–esterase
L.	Linnaeus
PNPA–EST	P–nitrophenyl acetate esterase
MFO	Mixed Function Oxidase
OD	Optical Density
Metsch.	Metschnikoff
USA	United States of America
WHO	World Health Organisation
<i>Bti</i>	<i>Bacillus thuringiensis israelensis</i>
μ g	microgram
<i>Bt</i>	<i>Bacillus thuringiensis</i>
IPM	Integrated Pest Management
sp.	species
OP	Organophosphate

N	North
E	East
DDT	dichloro-diphenyl-trichloroethane
MoH	Ministry of Health
<i>kdr</i>	Knockdown resistance
VGSC	Voltage-Gated Sodium Channel
SE	Standard Error
LLINs	Long-lasting insecticidal nets
<i>Pr1</i>	Subtilisin protease
CYPs	Cytochrome P450 enzymes
i.e.	in example
mL	milliliter
g	gram
h	hour
PDA	Potato Dextrose Agar
μl	microliter
mm	millimeter
cm	centimeter

°C	Degree Celcius
LC	Lethal Concentration
ANOVA	Analysis of variance
CRD	Completely Randomized Design
CI	Confidence Interval
df	Degree of freedom
MS	Mean-squared value
SE	Standard Error
SR	Synergistic Ratio
RR	Resistance Ratio
NA	Not Available
ND	Not Determined
VCRU	Vector Control Research Unit
USM	Universiti Sains Malaysia
F0	Filial 0
F1	Filial 1
IVM	Integrated Vector Management
TMBZ	Tetramethylbenzidine

nm	nanometer
M	Molar
mM	Milli Molar
mg	Milligram
DTNB	Ellman's Reagent
BSA	Bovine Serum Albumin
CDNB	1-chloro-2, 4-dinitrobenzene
nmol	nanomole
MMF	Mono Molecular Film

**KEBERKESANAN BIOPESTISID EKSTRAK DAUN *Ipomoea cairica* (L.)
DAN *Metarhizium anisopliae* (Metsch.) TERHADAP POPULASI RINTANG
NYAMUK *Aedes aegypti* (L.) dan *Aedes albopictus* (Skuse) DI KAWASAN
BANDAR DAN PINGGIR BANDAR, PULAU PINANG, MALAYSIA**

ABSTRAK

Biopestisid, yang diperoleh daripada sumber semula jadi seperti ekstrak tumbuhan dan kulat, adalah alternatif kepada racun serangga sintetik yang mesra alam, mengurangkan risiko terhadap kesihatan manusia dan alam sekitar sambil mengurangkan pembangunan rintangan. Ujian bioesei dewasa membuktikan bahawa *Ae. aegypti* dan *Ae. albopictus* bandar dan luar bandar rintang terhadap empat jenis racun serangga kimia (0.05% deltamethrin, 0.75% permethrin, 5% malathion, 0.25% pirimiphos–methyl), tetapi rintangan menurun apabila piperonil butosid (PBO) digunakan. Kajian ini bertujuan untuk menyiasat keberkesanan ekstrak daun *Ipomoea cairica* (pelarut: aseton) dan *Metarhizium anisopliae* (Meta–G4) dalam mengawal populasi *Aedes* yang rintang terhadap racun serangga sintetik di Pulau Pinang. Ekstrak daun *I. cairica* menunjukkan aktiviti larvisid yang tertinggi terhadap *Aedes aegypti* jenis bandar dan luar bandar, manakala *M. anisopliae* adalah paling berkesan terhadap *Aedes albopictus* luar bandar, dengan mortaliti optimum yang dicapai apabila 500 ppm daun ekstrak *I. cairica* atau 1×10^8 konidia/mL *M. anisopliae* yang dikultur selama 14 hari digunakan. Kesan sinergistik diperhatikan apabila 350 ppm *I. cairica* digabungkan dengan 1×10^6 konidia/mL *M. anisopliae* kerana dapat mencapai "dwi–serangan" yang luar biasa terhadap *Ae. aegypti*. Keserasian biopestisid dengan racun serangga juga meningkatkan keberkesanannya terhadap *Aedes* dewasa, terutamanya *Ae. albopictus*. Ujian biokimia esei mengesahkan mekanisme rintangan metabolik kerana *Ae. aegypti* menunjukkan kerentanan dan lebih bertoleransi terhadap kedua–

dua jenis biopestisid yang digunakan. Ini memaparkan potensinya dalam pengurusan rintangan bersepadu. Penemuan ini menyokong integrasi *I. cairica* dan *M. anisopliae* ke dalam program kawalan vektor, menawarkan penyelesaian yang mampan, berkesan dan saling melengkapi untuk membendung populasi *Aedes* yang rintang terhadap racun serangga sintetik serta mengurangkan wabak denggi.

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AND SUBURBAN AREAS OF PENANG ISLANDS, MALAYSIA**

ABSTRACT

Biopesticides, derived from natural sources like plant extracts and fungi, are eco-friendly alternatives to synthetic insecticides, offering minimal environmental and human health risks while reducing resistance development. Adult bioassays revealed that urban and suburban *Ae. aegypti* and *Ae. albopictus* were resistant to four chemical insecticides (0.05% deltamethrin, 0.75% permethrin, 5% malathion, 0.25% pirimiphos-methyl), but resistance decreased with piperonyl butoxide (PBO). This study evaluated the efficacy of *Ipomoea cairica* leaf extracts (solvent: acetone) and *Metarhizium anisopliae* (Meta-G4) in controlling insecticide-resistant *Aedes* populations on Penang Island. *Ipomoea cairica* leaf extracts demonstrated the highest larvicidal activity against *Aedes aegypti* urban and suburban strains, while *M. anisopliae* was most effective against *Aedes albopictus* suburban strains, with optimal mortality achieved using 500 ppm *I. cairica* extract or 1×10^8 conidia/mL of 14-day-old *M. anisopliae*. Synergistic effects were observed when 350 ppm *I. cairica* was combined with 1×10^6 conidia/mL *M. anisopliae*, achieving a robust “dual-attack” against *Ae. aegypti*. The compatibility of biopesticides with insecticides also enhanced efficacy against adult *Aedes*, particularly in *Ae. albopictus*. Biochemical assays confirmed metabolic resistance mechanisms, with *Ae. aegypti* showing susceptibility but some tolerance to biopesticides, highlighting their potential in integrated resistance management. These findings support the integration of *I. cairica* and *M. anisopliae* into existing vector control programmes, offering a sustainable, effective, and

complementary solution to curb insecticide-resistant *Aedes* populations and reduce mosquito-borne diseases like dengue.

CHAPTER 1

GENERAL INTRODUCTION

1.1 INTRODUCTION

Dengue, a leading viral infection transmitted by *Aedes* mosquitoes, presents a significant public health issue in tropical regions (WHO, 2012). Four closely related serotypes of the dengue virus (DENV) are responsible for dengue fever (DF), dengue haemorrhagic fever (DHF), and dengue shock syndrome (DSS) (WHO, 2022). Since the first major outbreak in 1902, dengue has become endemic in Malaysia, largely transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes (Abubakar and Shafee, 2002; Woon *et al.*, 2019). Urbanisation and infrastructure expansion have supported *Aedes* breeding, contributing to increased dengue incidence (Muhammad Azami *et al.*, 2011).

Despite ongoing efforts, controlling dengue-carrying mosquito populations remains challenging, especially in the absence of targeted antiviral treatments and a highly effective dengue vaccine (Rosilawati *et al.*, 2017). Without these medical tools, vector control remains essential, focusing on reducing larval habitats, minimising adult mosquito populations, and decreasing human-mosquito contact (Eisen *et al.*, 2009; Rohani *et al.*, 2011). Public health measures have used various synthetic insecticides, including carbamates, organochlorines, organophosphates, and pyrethroids, to manage mosquito populations (Rahim *et al.*, 2017a). However, issues such as high costs, environmental sustainability concerns, health risks, and increasing pesticide resistance have led to a decline in synthetic insecticide usage for mosquito control (Ghosh *et al.*, 2012). Resistance to commonly used pesticides, particularly in *Ae. albopictus*, has become a significant obstacle in vector control programmes worldwide (Nauen, 2007; Vontas *et al.*, 2012).

For the past 43 years, the organophosphate larvicide temephos has been the primary chemical approved in Malaysia to control container–breeding *Aedes* larvae (Chen *et al.*, 2013). However, tolerance to temephos has been reported in Malaysia (Ishak *et al.*, 2015; Rahim *et al.*, 2016). Insects use behavioural, physiological, and genetic adaptations to mitigate the effects of insecticides (Kliot and Ghanim, 2012). Resistant insects often face fitness costs, such as reduced fecundity, shorter survival, and delayed development, compared to susceptible individuals (Fang *et al.*, 2011; Chan and Zairi, 2013). Understanding these fitness costs is crucial for designing effective vector control strategies, as resistance can influence disease transmission and pest management dynamics (Kliot and Ghanim, 2012; Rahim *et al.*, 2017b). While some studies report fitness disadvantages in resistant insects, others indicate no biotic penalties (Lyons *et al.*, 2016), underscoring the importance of monitoring resistance within vector populations.

Plant–derived pesticides offer an effective alternative to synthetic insecticides for integrated pest management programmes (Waliwitiya *et al.*, 2009; Ghosh *et al.*, 2012). Plants produce a wide range of bioactive compounds that can target insect vectors while degrading more quickly in the environment and posing fewer risks to public health (Demirak and Canpolat, 2022; Mann and Kaufman, 2012). Historically, natural plant–based treatments have been used to control insect pests, with around 2,000 plant species reported to have insecticidal properties (Das *et al.*, 2007; Ghosh *et al.*, 2012). Notable examples include azadirachtin from neem, known for its mosquitocidal effects (Isman, 2006), and other plants such as *Tagetes minuta*, *Piper nigrum*, *Piper longum*, and *Phyllanthus niruri*, all of which have demonstrated larvicidal activity against mosquitoes (Perich *et al.*, 1994; Park *et al.*, 2002a; Lee, 2000; Moniharapon *et al.*, 2020).

The "railway creeper," *Ipomoea cairica* (Linnaeus) from the Convolvulaceae family, is a tropical, climbing perennial plant with medicinal properties (Liu *et al.*, 2011). Often used in fencing, it also has traditional medicinal uses, such as treating body rashes with a beverage made from its leaves (Ferreira *et al.*, 2006; Thomas *et al.*, 2004). *Ipomoea cairica* contains bioactive compounds with insecticidal properties, making it an environmentally friendly alternative to synthetic chemicals. These natural compounds can help reduce reliance on conventional pesticides, which often harm non-target species and pollute ecosystems. Additionally, *I. cairica* offers novel modes of action, which can aid in managing insecticide resistance in mosquito populations (Zuharah *et al.*, 2017). Essential oil from steam-distilled *I. cairica* leaves has shown effectiveness in repelling *Anopheles stephensi* and killing mosquito species like *Culex tritaeniorhynchus*, *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus* (Rajkumar and Jebanesan, 2007; Thomas *et al.*, 2004). Due to its natural abundance, identifying its active larvicidal compounds could provide an alternative to chemical larvicides (AhbiRami *et al.*, 2014a).

Entomopathogenic fungi, such as *Metarhizium anisopliae*, are effective biological agents against various insect pests, including Lepidoptera, Hemiptera, Homoptera, and Coleoptera, offering benefits like reduced pesticide use, lower environmental contamination, and decreased pest resistance (Golshan *et al.*, 2013; Herdatiarni *et al.*, 2014; Ambethgar, 2009). Combining fungi with plant extracts has shown promising results in pest control. For example, combining fungal isolates with essential oils effectively controlled *Acanthoscelides obtectus*, with outcomes influenced by the interaction between the two agents (Lak *et al.*, 2022). Essential oils like *Foeniculum vulgare*, *Artemisia dracunculus*, and *Lavandula angustifolia* have demonstrated synergistic effects with *M. anisopliae* (Lak *et al.*, 2022). Similarly, a

formulation of *I. cairica* extract (450 ppm) and *M. anisopliae* (1×10^6 conidia/mL) was highly effective against *Ae. aegypti* and *Ae. albopictus* (Zuharah *et al.*, 2018).

Dengue vector control globally relies on four main chemical insecticides: carbamates, pyrethroids, organochlorines, and organophosphates (WHO, 2006). However, widespread use has led to significant insecticide resistance in mosquito populations, undermining chemical control efforts (Hemingway and Ranson, 2000; Ranson *et al.*, 2010). Resistance monitoring is crucial to set effective thresholds and sustain insecticide-based control against *Ae. aegypti* and *Ae. albopictus* (Donnelly *et al.*, 2009). This study evaluates the susceptibility of these mosquito species to various adulticides in Penang Island and assesses the impact of biopesticides on insecticide-exposed *Ae. aegypti* and *Ae. albopictus* adults, as resistance can hinder both chemical and biopesticide effectiveness.

Resistance that disrupts *Aedes*-borne disease control efforts can increase disease transmission (Amelia-Yap *et al.*, 2018). In mosquitoes, resistance often arises from enhanced metabolic detoxification, where enzymes break down insecticides before they can act. This metabolic resistance frequently involves the upregulation of specific detoxifying enzymes, such as cytochrome P450 monooxygenases (P450s), esterases (ESTs), and glutathione S-transferases (GSTs), which degrade or sequester insecticides (Liu, 2015; David *et al.*, 2013; Kamita *et al.*, 2016). For instance, P450s are linked to pyrethroid metabolism, while ESTs hydrolyse pyrethroids, and GSTs aid in the detoxification process, though not directly metabolising pyrethroids in mosquitoes (Amelia-Yap *et al.*, 2018; Tchigossou *et al.*, 2018). This study examines the effect of biopesticides on insecticide-resistant *Aedes* mosquitoes to understand the role of metabolic enzymes in potentially reducing dengue cases in Penang Islands, Malaysia.

1.2 PROBLEM STATEMENT

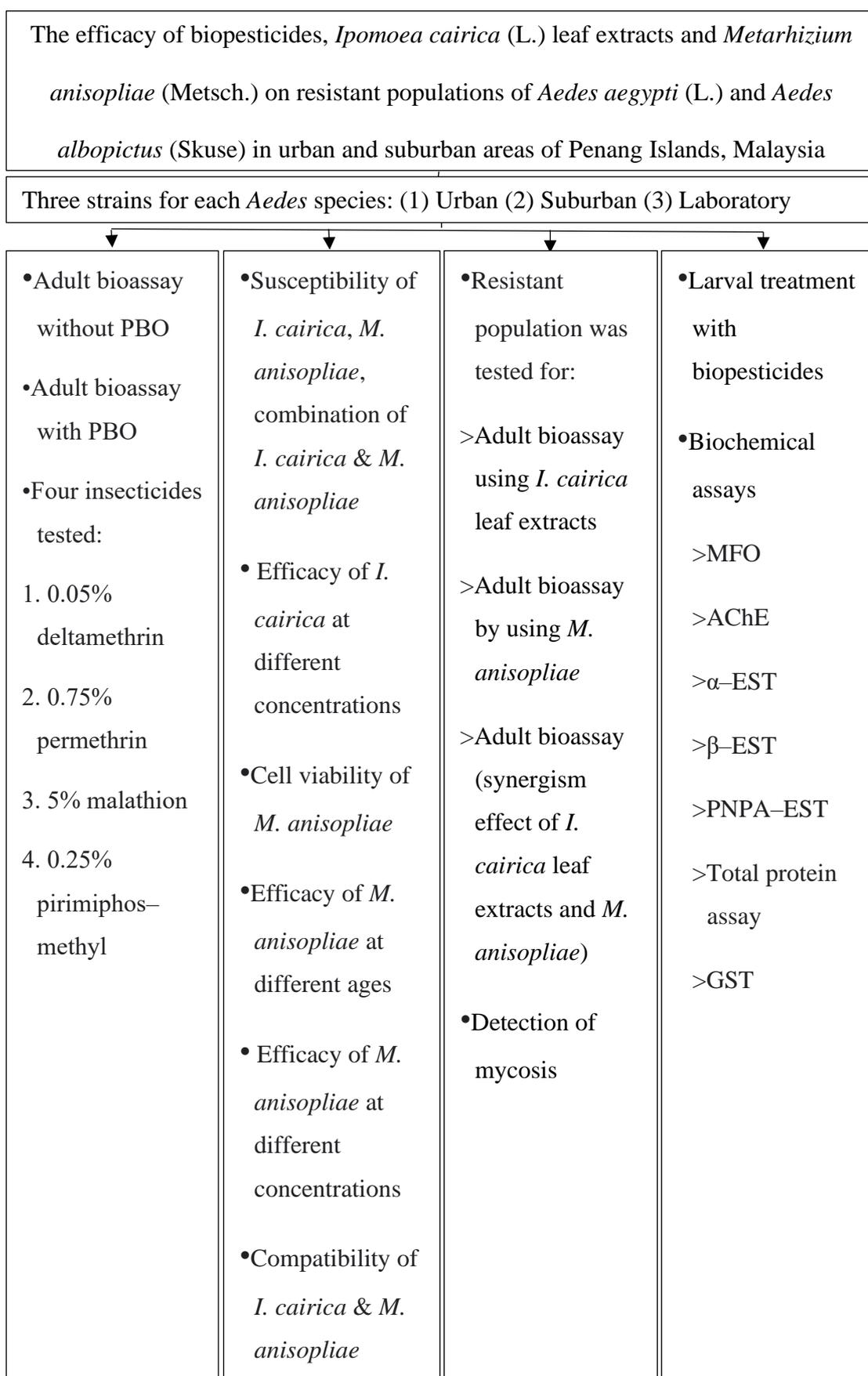
Dengue, a severe viral disease, is primarily transmitted by *Ae. aegypti* and *Ae. albopictus*, posing a major public health issue in tropical regions like Malaysia. Despite ongoing vector control efforts, resistance to synthetic insecticides is rising, with high environmental and health costs. This resistance complicates mosquito management and necessitates alternative, eco-friendly strategies to reduce disease transmission. Plant-based biopesticides, such as *I. cairica* extracts, in combination with entomopathogenic fungi like *M. anisopliae*, may offer effective solutions for mosquito control while mitigating resistance and environmental risks. This study aims to assess these biopesticides' efficacy and potential to enhance vector control outcomes against resistant *Aedes*.

1.3 RESEARCH OBJECTIVES

The specific objectives of this study are as follows:

1. To determine the current resistance status of *Ae. aegypti* and *Ae. albopictus* populations in Penang Island.
2. To determine the effectiveness and the synergistic effects of *Ipomoea cairica* leaves plant extract and *Metarhizium anisopliae* fungi Meta-G4 against larvae of *Ae. aegypti* and *Ae. albopictus*.
3. To study the impact of biopesticides *Ipomoea cairica* leaf extracts and *Metarhizium anisopliae* fungi (Meta-G4) against resistant adult females of *Ae. aegypti* and *Ae. albopictus*.
4. To investigate the impact of biopesticides on the metabolic enzymes of resistant *Ae. aegypti* and *Ae. albopictus* populations from Penang Island.

1.4 STUDY FRAMEWORK



The study aims to evaluate the efficacy of biopesticides, specifically *I. cairica* leaf extracts and *M. anisopliae*, against resistant populations of *Ae. aegypti* and *Ae. albopictus* mosquitoes collected from urban, suburban, and laboratory sources in Penang Islands, Malaysia. The research framework is organized into four key objectives to systematically investigate the potential of these biopesticides:

Objective 1: To determine the current resistance status of *Ae. aegypti* and *Ae. albopictus* populations in Penang Island

Adult bioassay without PBO: An initial bioassay is conducted on adult mosquitoes using four synthetic insecticides—0.05% deltamethrin, 0.75% permethrin, 5% malathion, and 0.25% pirimiphos–methyl – to establish baseline resistance levels across strains without the synergistic action of PBO. This allows the application of biopesticides on *Ae. aegypti* and *Ae. albopictus* larvae in integrated vector management in the next chapter. Adult bioassay with PBO, an enzyme inhibitor, was also assessed in this study. This allows the detection of metabolic resistance mechanisms by revealing any shifts in susceptibility due to inhibited detoxification processes.

Objective 2: To determine the effectiveness and the synergistic effects of *Ipomoea cairica* leaves plant extract and *Metarhizium anisopliae* fungi (Meta–G4) against larvae of *Ae. aegypti* and *Ae. albopictus*

Susceptibility tests: Initial tests assess the susceptibility of each mosquito strain to *I. cairica*, *M. anisopliae*, and a combined treatment of both. This establishes baseline efficacy for each agent across the urban, suburban, and laboratory populations. In this chapter, we investigated the effects of multiple concentration of *I. cairica* leaf extracts to determine the optimal dose for each mosquito strain. The viability of *M. anisopliae*

spores was also examined to confirm their effectiveness prior to application, ensuring that the fungal spores are active and potent.

Whereas age and concentration effects of *M. anisopliae* spores were examined to identify the most effective spore conditions for mosquito control to be used in the subsequent chapter. We also analysed the compatibility of *I. cairica* and *M. anisopliae*: This part of the study investigates potential interactions—synergistic or antagonistic—between *I. cairica* and *M. anisopliae*, assessing the feasibility and effectiveness of a combined treatment approach. This allows the application of biopesticides on *Ae. aegypti* and *Ae. albopictus* female adults in integrated vector management in the next chapter.

Objective 3: To study the impact of biopesticides *I. cairica* leaf extracts and *Metarhizium anisopliae* fungi (Meta-G4) against resistant adult females of *Ae. aegypti* and *Ae. albopictus*

Using the information from the objective 2, adult mosquitoes were tested with *I. cairica* leaf extracts to evaluate their standalone effectiveness as a biopesticide. Adult bioassay were also performed with *M. anisopliae*. The application of *M. anisopliae* alone provides insights into the fungal infection's impact on resistant mosquito populations.

Following that, the synergistic effect of combined treatment: The combined formulation of *I. cairica* extracts and *M. anisopliae* were tested to identify any potential enhancements in efficacy against adult mosquitoes. This effect is crucial for conducting enzymatic assays to investigate the metabolic resistance mechanisms in treated mosquito populations in the next chapter. Following treatment with *M.*

anisopliae, the presence of mycosis is checked in adult mosquitoes to confirm fungal infection and to measure mortality due to fungal pathogenicity.

Objective 4: To investigate the impact of biopesticides on the metabolic enzymes of resistant *Ae. aegypti* and *Ae. albopictus* populations from Penang Island

Larval bioassay with biopesticides: The larvicidal potential of *I. cairica* and *M. anisopliae* is assessed across strains to evaluate the biopesticides' impact on larvae with various resistance profiles. The resistance profile was analysed using the biochemical assays to investigate the metabolic resistance mechanisms in treated mosquito populations. These include:

- i. AChE (Acetylcholinesterase): Assays to detect resistance to organophosphate insecticides.
- ii. MFO (Mixed Function Oxidases): Analysis of P450 enzyme activity related to metabolic resistance.
- iii. α -EST, β -EST, and PNPA-EST (Esterase Assays): Different esterase assays to examine esterases' role in resistance.
- iv. GST (Glutathione S-Transferase): Assessed for its role in detoxification processes linked to pyrethroid resistance.
- v. Total Protein Assay: This assay measures total protein levels to provide insight into changes associated with resistance mechanisms.

This structured approach is designed to provide a thorough understanding of the impact of *I. cairica* and *M. anisopliae*, both individually and in combination, on resistant

Aedes populations and to elucidate the role of metabolic enzymes in mosquito resistance.

CHAPTER 2

LITERATURE REVIEW

2.1 Geographical distribution of *Aedes aegypti* and *Aedes albopictus*

The *Aedes* (Stegomyia) *aegypti* (Linnaeus 1762) and *Aedes albopictus* (Skuse 1895) mosquitoes inhabit tropical and subtropical regions worldwide, with overlapping geographic ranges but distinct historical introductions to the Americas. *Aedes aegypti*, transported from Africa to the Americas aboard slave trade ships between the 15th and 18th centuries, is primarily a domestic mosquito preferring human hosts despite other available hosts and abundant artificial containers (Brown *et al.*, 2014; Harrington *et al.*, 2001). In contrast, *Ae. albopictus*, or the Asian tiger mosquito, is native to Southeast Asia and often thrives in rural or suburban areas with more natural breeding sites. Nonetheless, their habitats can overlap, with both species occasionally occupying similar environments and aquatic breeding sites. Due to global travel and trade, *Ae. albopictus* has spread extensively beyond its native range in recent decades (Lwande *et al.*, 2020; Scholte and Schaffner, 2007).

2.2 Biology, ecology, and behaviour of *Aedes aegypti* and *Aedes albopictus*

Aedes aegypti and *Ae. albopictus* are closely related but differ in biology, ecology, and behaviour, which may influence the efficacy of control measures and their role in disease transmission. *Aedes aegypti* is primarily anthropophilic, favouring humans as hosts. In contrast, *Ae. albopictus* is an opportunistic, generalist feeder, consuming a range of vertebrates, including amphibians, birds, reptiles, and mammals (Delatte *et al.*, 2010). However, studies indicate that *Ae. albopictus* displays notable anthropophilic in suburban and urban areas. In regions with limited human presence,

it feeds on various other vertebrates, as observed in its native Southeast Asia and as an invasive species in the USA and Europe (Kim *et al.*, 2017).

Aedes aegypti is more closely associated with urban, densely populated areas and thrives in artificial habitats like flowerpots, tyres, and water tanks, particularly in tropical and subtropical climates. Although it also exists in some temperate regions, *Ae. albopictus* inhabits a broader range of environments—rural, suburban, and urban. In regions with co-occurrence, *Ae. aegypti* favours containers in densely built areas, while *Ae. albopictus* prefers those near vegetation (Kamgang *et al.*, 2018). Both species develop in stagnant water, often in peri-domestic containers, and undergo distinct life stages: egg, larva, pupa, and adult. Under ideal conditions, these holometabolous insects can produce five to seventeen generations per year, facilitating the spread of diseases like dengue, chikungunya, and Zika (Gatt *et al.*, 2009). Adult females lay eggs in various water-holding containers, with *Ae. aegypti* generally preferring clean water indoors and *Ae. albopictus* using a wider range of sources, including those with organic material. Eggs are fertilised upon laying but require specific temperature and humidity conditions to develop, typically hatching when the oxygen content in the water decreases.

Larvae progress through four instars before becoming pupae, from which adults emerge, with males often emerging slightly earlier (Estrada-Franco and Craig, 1995; Foster and Walker, 2002). Newly emerged adults obtain energy from sugar sources like rotting fruit or nectar, while females blood-feed within a few days to acquire protein for egg development, potentially living for several weeks in the wild and taking multiple blood meals (Hawley, 1988; Estrada-Franco and Craig, 1995). Unlike *Ae. aegypti*, *Ae. albopictus* can enter a diapause, a form of hibernation triggered

by temperature and photoperiod, enabling its eggs to survive colder winters (Estrada–Franco and Craig, 1995; Diniz *et al.*, 2017).

Aedes albopictus, known as the "forest day mosquito," is an exophilic species and diurnal biter, primarily active in wooded areas. Its biting times can vary based on location, season, habitat, and host availability, including dawn and dusk. *Aedes aegypti*, also a diurnal biter, exhibits a more aggressive and persistent biting behaviour, with a strong preference for humans. This species is highly anthropophilic, endophilic, and endophagic, meaning it bites indoors, remains indoors post-feeding, and rests inside while digesting its blood meal and maturing its eggs (Reinhold *et al.*, 2018; Scott and Morrison, 2010). As a result, *Ae. aegypti* is a major vector of several arthropod-borne diseases affecting humans and animals, including flaviviruses like West Nile virus (WNV), dengue virus (DENV), Zika virus, and yellow fever virus (YFV) (Bhatt *et al.*, 2013; Camila and Claudia, 2016).

2.3 Epidemiology of dengue fever and dengue haemorrhagic fever in Malaysia

Dengue fever (DF) and dengue haemorrhagic fever (DHF) remain significant public health concerns in Malaysia. Dengue is caused by four closely related mosquito-borne serotypes of the dengue virus (DENV) (WHO, 2022). Since the first major outbreak in 1902, dengue has been endemic in Malaysia, posing a persistent health risk (Abubakar and Shafee, 2002). Infection can range from mild to severe, with the risk of severe illness heightened in cases of secondary infection from a different DENV serotype (St John and Rathore, 2019; WHO, 2022). Early dengue symptoms resemble other febrile illnesses, leading to frequent misdiagnosis and underreporting (Capeding *et al.*, 2013; Fernández *et al.*, 2016; Tsheten *et al.*, 2021). The World Health Organization (WHO) classifies dengue into three categories: dengue with or without

warning signs, and severe dengue (WHO, 2019). Nonetheless, Malaysia continues to report cases under the 1997 WHO criteria: dengue fever (DF), dengue haemorrhagic fever (DHF), and dengue shock syndrome (DSS) (Nealon *et al.*, 2016; Woon *et al.*, 2019).

2.4 Current vector control in Malaysia

Vector-borne viral diseases are a pressing public health issue, especially in tropical regions. In Kuala Lumpur, Malaysia, field releases of mosquitoes carrying bacterial symbionts to reduce vector competence are underway. Preliminary findings indicate that wAlbB *Wolbachia* can establish in urban mosquito populations and lower dengue cases in humans (Chrostek *et al.*, 2020).

Wolbachia, a maternally inherited bacterial symbiont widely present in insects, has emerged as a promising tool for managing vector-borne diseases. Infected insects often exhibit reduced viral loads compared to uninfected ones, a phenomenon referred to as "virus blocking" (Teixeira *et al.*, 2008; Hedges *et al.*, 2008). While *Ae. aegypti* mosquitoes do not naturally carry *Wolbachia*, laboratory studies have successfully introduced several strains into this species, resulting in stable, heritable infections. These *Wolbachia* infections notably reduce the mosquitoes' ability to acquire and transmit arboviruses (Moreira *et al.*, 2009).

These field interventions typically involve rearing *Wolbachia*-infected mosquitoes in controlled facilities and releasing them into dengue-affected areas. *Wolbachia* is maternally transmitted, so it spreads across mosquito populations through a process known as cytoplasmic incompatibility, which promotes its establishment over generations. Once established, *Wolbachia* effectively hinders the

replication of arboviruses in mosquitoes, thereby preventing transmission of disease-causing viruses to humans (O'Neill, 2018).

The wAlbB strain of *Wolbachia* has proven effective for dengue control in densely populated, dengue-endemic urban regions of Malaysia (Nazni *et al.*, 2019). To test this approach, *Wolbachia*-infected mosquitoes were released in several strategically chosen areas with varying structures and population densities, while comparable control sites without interventions were monitored simultaneously. Findings indicate that *Wolbachia* can achieve high prevalence in most local mosquito populations across these complex urban landscapes. Significantly, this intervention led to a reduction in human dengue cases by 5–65%, with the exact impact varying by location (Chrostek *et al.*, 2020).

2.5 Biopesticides in vector control

Biopesticides are pest control agents derived from natural materials, such as animals, plants, bacteria, and minerals. Their environmentally friendly action aims to mitigate the adverse impacts of synthetic pesticides. Biopesticides employ various mechanisms, including disrupting pest lifecycles, deterring feeding, and infecting pests with natural pathogens (Fenibo *et al.*, 2020).

There are three types of biopesticides: (I) Microbial biopesticides: These include microorganisms like bacteria, fungi, and viruses. For instance, *Bacillus thuringiensis* (*Bt*) produces toxins lethal to specific insect larvae and is widely used in crop protection (Mishra *et al.*, 2018). (II) Plant-incorporated protectants (PIPs): These are pesticidal substances produced by plants after the introduction of specific genetic material, such as *Bt* toxins in genetically engineered corn (Nelson and Alves, 2014). (III) Biochemical biopesticides: These consist of naturally derived chemicals, such as

pheromones and plant extracts. Examples include neem oil and *I. cairica* leaf extracts, which exhibit larvicidal effects against mosquitoes (Stankovic *et al.*, 2020).

Biopesticides tend to target specific pests, sparing beneficial species, and are generally less toxic to humans and the environment. They also lower the risk of pest resistance due to their varied modes of action, making them increasingly valuable in integrated pest management (IPM) programmes (Fenibo *et al.*, 2020).

The heavy use of synthetic pesticides has driven resistance in mosquito populations, leading to environmental and health concerns (Ohia and Ana, 2015). This has heightened the demand for safer alternatives, with biopesticides, derived from plants and fungi, being preferred for their biodegradability and low toxicity (Murray, 2006).

Studies have shown certain plant extracts as effective larvicides. *Cinnamomum* extracts demonstrate high efficacy (Cheng *et al.*, 2004), while *Piper nigrum* extracts have lethal effects on mosquito species, causing mortality and behavioural disruptions (Park *et al.*, 2002b). Neem oil is toxic to *An. gambiae* larvae (Okumu *et al.*, 2007), and extracts of *Lantana camara* and *Catharanthus roseus* show high toxicity to *Ae. aegypti* even at low doses (Remia and Logaswamy, 2010).

Additionally, fungi such as *Coelomomyces*, *Beauveria*, *Metarhizium*, and *Entomophthora* play significant roles in vector control by penetrating mosquito cuticles, degrading internal tissues, and sometimes producing toxic metabolites (Kamareddine, 2012; Abdul-Ghani *et al.*, 2012). Due to the lower resistance rates associated with biopesticides, they are increasingly a focus in mosquito-borne disease research (Ohia and Ana, 2015).

2.5.1 *Ipomoea cairica* plant extracts



Plate 2.1: *Ipomoea cairica* presented with a trumpet-shaped flower and five-lobes of leaf blades.

Ipomoea cairica, commonly known as the "railway creeper" or "mile-a-minute vine," is a perennial herb from the Convolvulaceae family, identifiable by its striking, trumpet-shaped flowers and typically five-lobed, star-like leaves (Gong *et al.*, 2014; Srivastava and Rauniyar, 2020). Originally cultivated as an ornamental plant from South or Central America, *I. cairica* has become invasive in many areas, including southern China, where it was first naturalised in Hong Kong in 1921 (Li *et al.*, 2012a). It thrives in sunny, well-drained areas like roadsides and open shrublands, where its aggressive growth enables it to overtake other plants by blocking sunlight, ultimately reducing biodiversity and disrupting ecosystems (Li *et al.*, 2012b; Wang *et al.*, 2011).

With a high growth rate and significant photosynthetic efficiency, *I. cairica* is widely considered a harmful invasive species. It is highly adaptable, tolerating a variety of soils, drought, and nutrient-poor conditions (Srivastava and Rauniyar, 2020). In Malaysia, the vine is commonly planted as a decorative fencing option in residential settings (AhbiRami *et al.*, 2014a).

Ipomoea cairica contains diverse bioactive compounds with pesticidal and medicinal potential. Key chemicals include phenolic compounds such as flavonoids (e.g., quercetin, kaempferol) known for antioxidant, anti-inflammatory, and

insecticidal effects; tropane and indole alkaloids, which can be toxic to pests; terpenoids (monoterpenes, sesquiterpenes, diterpenes) with insecticidal properties in repelling or killing pests.; saponins that disrupt insect cell membranes of insects, leading to mortality; fatty acids like palmitic and linoleic acids with antimicrobial and insecticidal effects; and phytosterols such as stigmasterol and β -sitosterol. Together, these compounds hinder mosquito development, interfere with enzyme activity, and ultimately increase mortality (Fenibo *et al.*, 2020).

This species has also shown promise in pest control, as its leaf extracts exhibit notable larvicidal properties. Acetone extracts at 450 ppm induce rapid paralysis and death in mosquito larvae, achieving 100% mortality in species like *Cx. tritaeniorhynchus*, *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus*, making it a candidate for biopesticide development (AhbiRami *et al.*, 2014a; Thomas *et al.*, 2004; Rajkumar and Jebanesan, 2007).

2.5.2 *Metarhizium anisopliae* entomopathogenic fungus

Entomopathogenic fungi have shown effectiveness in controlling both adult mosquitoes and larvae, with several species specifically targeting mosquito larvae. These fungi, such as *Metarhizium anisopliae*, are used as biological agents against various insect pests, including mosquitoes. *Metarhizium anisopliae* produces hyphae–branching filaments that spread throughout the host insect's body. Its main infectious structures, conidia (cylindrical, single–celled spores), are formed on specialised conidiophores and appear as a white, powdery layer on the host's surface after colonisation (Tiago *et al.*, 2014).

Metarhizium anisopliae infects mosquitoes by adhering to, penetrating, and spreading through the cuticle. It can also form appressoria to pierce the cuticle,

ultimately leading to host death as it proliferates within the insect (Ayele *et al.*, 2020). The fungal metabolites gradually degrade the internal organs of mosquito larvae and adults, producing secondary compounds that enhance the lethality (Wang and Wang, 2017). Tests of *M. anisopliae* crude metabolites significantly reduced mortality in *Ae. aegypti* larvae, pupae, and adults across concentrations from 10 to 75 µg/mL (Vivekanandhan *et al.*, 2022).

2.6 Synergistic effect of the combination of entomopathogenic fungus *Metarhizium anisopliae* with *Ipomoea cairica* plant extracts

The diverse mechanisms of bioactive “green” metabolites highlight the value of combining chemical insecticides with plant and fungal bioactive compounds for mosquito control (Srivastava *et al.*, 2011). Research has shown that bioactive metabolites from fungi and plants possess notable insecticidal properties (Dhanasekaran *et al.*, 2010; Benelli, 2015; Benelli, 2016; Amerasan *et al.*, 2016; Vivekanandhan *et al.*, 2017). Among promising biological control agents are entomopathogenic fungi, valued for their selective toxicity, non-persistence, and efficacy without ingestion (Keller, 1991; Maurya *et al.*, 2011).

Studies indicate that *M. anisopliae* exhibits strong larvicidal and adulticidal effects on *Ae. aegypti* and *An. stephensi* through its secondary metabolites (Silva *et al.*, 2008; Vyas *et al.*, 2007). Enhanced efficacy through synergistic actions of plant extracts may weaken the mosquito's defences (e.g., disrupting the cuticle), facilitating fungal invasion. This will increase the mortality rates due to simultaneous targeting of multiple physiological pathways. Recent investigations into binary biopesticide mixtures reveal synergism, where one component amplifies the effect of another, leading to a greater combined impact than either alone (Benelli *et al.*, 2017a, Benelli

et al., 2017b). Additionally, certain pesticides can increase insect stress and alter behaviour, potentially boosting entomopathogen efficacy (Santos *et al.*, 2007). However, the effect of *I. cairica* leaf extracts alone, *M. anisopliae* alone and the combination of both agents on urban (Desa Permai Indah) and suburban (Taman Indah) field strain of *Ae. albopictus* and *Ae. aegypti* larvae and adult female have not been reported.

2.7 Insecticide resistance in worldwide

Insecticide resistance typically arises through selection of individuals capable of surviving lethal doses. Repeated insecticide applications favour resistant genotypes, reducing susceptible populations and driving resistance (Brown, 1986).

Current studies are examining global, country-specific resistance rates to insecticides like DDT, deltamethrin, permethrin, and malathion. In Indonesia, *Ae. aegypti* shows high resistance to deltamethrin (69%), permethrin (70%), and malathion (54%). In Thailand, *Ae. aegypti* has significant resistance to DDT (94%), permethrin (43%), and deltamethrin (21%), while *Ae. albopictus* is notably resistant to DDT (30%). In Cambodia, *Ae. aegypti* exhibits high resistance to permethrin (99%) and deltamethrin (58%), while in Singapore, it shows strong resistance to permethrin (89%) and deltamethrin (51%) (Zulfa *et al.*, 2022).

Strengthening resistance management programmes is essential to control pesticide resistance in *Ae. aegypti* and *Ae. albopictus*, key vectors in arbovirus transmission (Asgarian *et al.*, 2023).

2.8 Insecticide resistance in Malaysia

In Malaysia, dengue control primarily involves larviciding with temephos and *Bacillus thuringiensis israelensis* (*Bti*), alongside adulticiding with permethrin, deltamethrin, and malathion (MOH, 2011). Various groups, including private businesses, communities, and the Ministry of Health (MoH), use insecticides to manage household pests, including mosquitoes (Rohani *et al.*, 2011). However, the effectiveness of these measures is increasingly compromised by rising insecticide resistance in *Aedes* populations, both in Malaysia and globally, largely due to the overuse of pesticides (Ranson *et al.*, 2010). *Aedes* species in Kuala Lumpur and Penang have shown resistance to temephos and permethrin (Nazni *et al.*, 2009; Chan *et al.*, 2011), though resistance to other insecticides remains unclear. Furthermore, the full extent of pesticide resistance in *Ae. aegypti* and *Ae. albopictus* across Malaysia is still unknown, highlighting the need for further research to inform effective control strategies (Ishak *et al.*, 2015). Moreover, there is no recent data on the susceptibility status of adult mosquitoes and a lack of information comparing adult *Ae. aegypti* and *Ae. albopictus* in urban and suburban areas of Penang Island, Malaysia using pyrethroids and organophosphates.

2.9 Type of resistance

Four main types of resistance exist in insects that allow them to withstand the effects of pesticides: metabolic resistance, target–site resistance, penetration resistance, and behavioural resistance (Corbel and N’Guessan, 2013).

2.9.1 Metabolic resistance

Metabolic resistance, a common defense mechanism in insects, relies on enzymatic processes that detoxify or sequester pesticide compounds. Insects have evolved enzymes to protect against plant toxins such as phenols, terpenes, and alkaloids, which also helps them resist synthetic insecticides derived from botanical sources (Despres *et al.*, 2007; War *et al.*, 2012; Heidel–Fischer and Vogel, 2015; Rane *et al.*, 2016). This adaptation enables rapid development of resistance to insecticides, particularly those with botanical origins (Isman, 2006).

Enzymes play a key role in converting harmful xenobiotics into less toxic forms, which are then excreted from the body. Resistant insects often exhibit higher enzyme levels or variants with enhanced catalytic activity, allowing more efficient insecticide metabolism. Detoxification occurs in two phases: Phase I involves hydrolysis or oxidation, while Phase II involves conjugation with compounds like glutathione for excretion (Li *et al.*, 2007; Yu, 2008; Berenbaum and Johnson, 2015).

In addition to detoxification, sequestration is another defence mechanism, where insects absorb, transport, and store specific plant metabolites to avoid physiological disruption (Erb and Robert, 2016; Petschenka and Agrawal, 2016). For mosquitoes, this may be an adaptive strategy to ensure efficient egg formation (Moore, 2015).

Key enzyme families, such as esterases, oxidases, and GSTs, are crucial for detoxifying xenobiotics (Panini *et al.*, 2016). Organophosphate insecticides inhibit acetylcholinesterase, but mosquitoes can develop resistance by increasing detoxifying enzymes that break down these chemicals. Pyrethroids, which affect mosquito nerve systems, are resisted by upregulating enzymes like cytochrome P450s, which degrade pyrethroids (Leong *et al.*, 2019). Field strains of *Ae. aegypti* in Malaysia, for example,

show increased glutathione S-transferase and mixed-function-oxidase levels after treatment with deltamethrin and permethrin, indicating metabolic resistance (Leong *et al.*, 2019). The rising use of pyrethroids in vector control may be contributing to this resistance. However, the impact of biopesticides on resistant *Ae. aegypti* and *Ae. albopictus* populations from Penang Island based on metabolic enzyme analysis have not been reported.

2.9.2 Target-site resistance

Pesticides, including DDT, pyrethroids, carbamates, and organophosphates, mainly target the nervous system of insects. In resistant strains, alterations at the pesticide target sites reduce insecticide binding efficiency. Mutations in the protein-encoding genes decrease receptor sensitivity, such as in acetylcholinesterase (AChE), the target of carbamate and organophosphate pesticides. Multiple mutations in the AChE gene have been found to lower susceptibility to these pesticides (Fournier, 2005; Alout *et al.*, 2009). Similarly, changes in nerve cell membrane sodium channels reduce sensitivity to pyrethroids and DDT (Davies *et al.*, 2007).

"Knockdown resistance" (*kdr*) describes insects with these mutations, allowing them to withstand prolonged pesticide exposure (Donnelly *et al.*, 2009). Hemingway and Ranson (2000) identified the *kdr* mutation as key to pyrethroid and DDT resistance. Specific mutations in the Voltage-Gated Sodium Channel (VGSC) gene at codons I1011M/V, V1016G/I, and F1534C are associated with resistance in *Ae. aegypti* (Bregues *et al.*, 2003; Harris *et al.*, 2010). Additionally, S989P is linked to pyrethroid resistance (Kawada *et al.*, 2014). The F1534C mutation has also been found in *Aedes* populations in Vietnam, Thailand, and Singapore (Kawada *et al.*, 2009; Yanola *et al.*, 2011; Kasai *et al.*, 2011).

2.9.3 Penetration resistance

Some resistant insects have modifications in their cuticle or digestive lining that reduce or block the absorption of pesticides. This mechanism, known as cuticular resistance, can apply to various insecticides and is not specific to one type (Zulfa *et al.*, 2022). This is particularly relevant for pests that ingest insecticides, but for malaria control, where insecticides are often applied to bed nets or wall surfaces, resistance may involve reduced permeability in the tarsal cuticle to lipophilic pesticides, affecting insecticide bioavailability. Cutaneous resistance has been observed in vectors like *Cx. quinquefasciatus* and *Musca domestica* (Plapp, 1984; Georgiou *et al.*, 1975). Scanning electron microscopy on *An. funestus* show that pyrethroid-tolerant mosquitoes have a significantly thicker cuticle compared to susceptible strains (Wood *et al.*, 2010).

2.9.4 Behavioural resistance

Mosquitoes displaying behavioural resistance modify their actions to minimise exposure to pesticides or control methods, which can reduce the effectiveness of these interventions and complicate disease management (Chandre *et al.*, 2000).

The first study on irritancy from residual DDT deposits involved *An. quadrimaculatus*, showing that females quickly left treated areas before feeding on blood (Gahan and Lindquist, 1945). This reaction can be classified as either irritancy (direct contact excitation) or spatial repellency, where mosquitoes avoid treated areas before contact (Chareonviriyaphap *et al.*, 1997). In Tanzania, increased use of pyrethroid-treated nets led to *An. funestus* shifting its biting activity from indoors to outdoors (Russell *et al.*, 2011). Similarly, in Benin, the use of long-lasting insecticidal nets (LLINs) caused *An. funestus* to bite earlier in the morning rather than at night, indicating significant behavioural changes (Moiroux *et al.*, 2012).