DEVELOPMENT OF ALGINATE-GELATIN HYDROGEL BEADS: A BAITING TOOL FOR DENGUE VECTORS AS MOSQUITO CONTROL STRATEGY

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by

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LIST OF SYMBOLS

%	Percentage
θ	Theta
ºC	Degree celcius
cm	Centimeter
g	Gram
h	Hour
kV	Kilovolt
M	Molar mass
m^2	Square meter
mA	Miliampere
min	Minutes
min^{-1}	Per minute
mL	Milliliter
mm	Millimeter
pH	Potential of hydrogen
rpm	Revolutions per minute
sec	Second
sec^{-1}	Per second

LIST OF ABBREVIATIONS

AAA	Subgroup of the banana cultivar group
AChE	Acetylcholinesterase
Ae.	*Aedes*
AGHBs	Alginate-Gelatin Hydrogel Beads
AHBs	Alginate Hydrogel Beads
AI	Active ingredient
ALG	Alginate
An.	*Anopheles*
ANOVA	Analysis of Variance
ARASC	Animal Research and Service Centre
ATSB	Attractive Toxic Sugar Bait
B-O	Boron and oxygen bond
BOH_3	Boric acid
Bti	*Bacillus thuringiensis Israelensis*
C1	Chamber 1
C2	Chamber 2
Ca2+	Calcium Ion
CaCl2	Calcium chloride
C-H	Carbon and hydrogen bond
CHICKV	Chickungunya Virus
CL	Confidence Level
C-N	Carbon and Nitrogen bond
C=O	Carbonyl group
COC	Ester

COO^{-1}	Carboxylate ion
$CONH_2$	Amides
Cu.	Culex
Cu Ka	Copper K-alpha
CT	Crosslinking Time
Cr(VI)	Hexavalent Chromium
DDT	Dichlorodiphenyltrichloroethane
DENV	Dengue Virus
df	Degree of Freedom
DF	Dilaxonide furoate
D/N	Day/Night
DNA	Deoxyribonucleic acid
dsRNAs	Double-stranded ribonucleic acid
et al.	And others
FTIR	Fourier Transmission Infrared
GC-MS	Gas Chromatography Mass Spectrum
GLN	Gelatin
GLM	Generalized Linear Models
GSH	Glutathione transferase
HSD	Tukey's Honesty Significant test
HS-SPDE	Headspace solid-phase dynamic extraction
ICAR	Indian Agricultural Research Institute
i.e.	That is
IR	Insecticide Resistance
IRM	Insecticide Resistance Management
IGR	Insect Growth Regulator
IRS	Indoor residual spraying

JEV	Japanese encephalitis virus
Kdr	Knockdown resistance
L.	Linnaeus
N-H	Nitrogen and hydrogen bond
NPs	Nanoparticles
O-C-N	Cyanate
OH	Hydroxide
PI	Preference Index
PRINT	Particle Replication in Non-wetting Templates
SAPs	Superabsorbent Polymers
SD	Standard deviation
SE	Standard Error
Si	Swelling index
sig.	Significant
siRNA	Small interfering ribonucleic acid
SIT	Sterile Insect Technique
sp	Species
spp	Species plural
SPSS	Statistical Package for the Social Science
st.	Street
USA	United State of America
USM	Universiti Sains Malaysia
UV	Ultraviolet
VCRU	Vector Control and Research Unit
VIF	Variance Inflation Characteristics
WHO	World Health Organization
Wa	Weight attained

Wi	Attained weight
WNV	West Nile Virus
Wo	Initial weight
Wr	Weight reduction
w/v	Weight per volume
XRD	X-ray Diffraction
YFV	Yellow Fever Virus
ZIKV	Zika Virus

LIST OF APPENDICES

PEMBANGUNAN MANIK HIDROGEL ALGINAT-GELATIN: SATU ALAT PENGUMPAN UNTUK VEKTOR DENGGI SEBAGAI STRATEGI PENGAWALAN NYAMUK

ABSTRAK

Pengawalan nyamuk *Aedes* dengan menggunakan racun serangga kimia telah terganggu kerana wujudnya kerintangan dalam kalangan vektor nyamuk. Hal ini masih tidak dapat diselesaikan kerana perkembangan rintangan nyamuk terhadap aplikasi kimia. Satu alternatif untuk mengurus rintangan awalnya telah diperkenalkan melalui penggunaan umpan gula, namun ianya agak terbatas dalam melindungi cecair umpan untuk masa yang lama. Dengan memperkenalkan alginate-gelatin gel hydro (AGHBs), limitasi tersebut boleh dikurangkan melalui penghasilan alat pengumpanan yang berpotensi untuk menarik dan membunuh nyamuk *Aedes*. Polimer semulajadi, seperti natrium alginat (ALG) dan gelatin (GLN), telah dipilih utuk menghasilkan AGHBs disebabkan oleh sifat yang terbiodegradasi dan mesra alam. Parameter yang dioptimumkan dalam menyediakan AGHBs yang mempunyai indek serapan tinggi ialah gabungan 8% ALG bersama dengan 1% GLN dan 5 minit masa rangkai silang oleh kalsium klorida. Kemudian, pemerhatian morfologi terhadap AGHBs juga telah dilakukan dengan menggunakan mikroskop cahaya. Pemerhatian kriteria AGHBs, Fourier-transform infrared spectroscopy (FTIRs) kemudiannya dikaji untuk menganalisis kandungan kumpulan kimia, manakala X-ray diffraction (XRD) untuk menentukan kristaliniti sampel. Seterusnya, untuk memahami tahap ketahanan AGHBs, kadar kekeringan pada tiga kawasan kajian telah dinilai selama 14 hari sebelum ia menjadi kering dan keras. Tiga kawasan tersebut adalah di makmal, separuh-lapangan, dan lapangan. Kawasan makmal menunjukkan bacaan suhu dan

kelembapan yang paling rendah diikuti dengan kawasan lapangan dan akhirnya separuh-lapangan. Selain itu, potensi cecair penarik dalam bentuk campuran yang mengandungi pisang dan manga juga dinilai bagi meningkatkan lagi daya penarik AGHBs. Apabila nyamuk diberi pilihan untuk memilih cecair penarik, keputusan menunjukkan pemilihan ketara terhadap penarik campuran berbanding dengan yang tidak bercampur. Walaubagaimanapun, tiada perbezaan ketara yang dapat diperhatikan terhadap tindak balas oleh nyamuk jantan dan betina terhadap cecair penarik yang disediakan. Penilaian potensi AGHBs sebagai alat pengumapanan yang berkesan adalah berdasarkan kadar mortaliti nyamuk dan kecenderungan nyamuk betina untuk menghisap darah. Hasil kajian mendapati bahawa peratusan mortaliti dapat dicapai selepas 72 jam, dan jumlah nyamuk betina yang memilih untuk menghisap darah berkurang selepas 6 jam terdedah kepada AGHBs. Ini dapat disimpulkan bahawa AGHBs berpotensi sebagai alat pengumpanan yang baik untuk membawa cecair umpan dalam usaha mencapai mortaliti nyamuk, dan pada masa yang sama mengurangkan kebarangkalian untuk nyamuk betina menghisap darah.

DEVELOPMENT OF ALGINATE-GELATIN HYDROGEL BEADS: A BAITING TOOL FOR DENGUE VECTORS AS MOSQUITO CONTROL STRATEGY

ABSTRACT

The control of the *Aedes* mosquito by using chemical insecticides is disturbed by the presence of resistance between the mosquito vectors. The issues are remain unsolved until now due to the development of insecticide tolerance of the mosquito to any chemical application. An alternative to handling the resistance is early recognized by using sugar bait, but they are somehow limited in protecting the liquid bait for long periods. By proposing alginate-hydrogel beads (AGHBs), the limitation can be narrowed by synthesizing a potential baiting tool that can attract and kill the population of the *Aedes* vector. Natural polymers, such as sodium alginate (ALG) and gelatine (GLN), were selected to develop AGHBs based on their biodegradability and environmental safety. The optimized parameters in preparing the highest swelling index of AGHBs is the combination of 8% of ALG with 1% of GLN and 5 min of crosslinking time (CT). Then, the morphological observation of AGHBs was observed by using a light microscope. Further characterization on AGHBs, Fourier-transform infrared spectroscopy (FTIRs) was done to analyze the chemical properties of AGHBs, while X-ray diffraction (XRD) were to determine their crystallinity. Then, to understand the durability of the AGHBs, the drying rate of the beads in three different studies site were observed for 14 days before it was dry and rigid. There selected studies sites are laboratory, semi-field, and field. There selected studies sites are laboratory, semi-field, and field. The laboratory displays the lowest humidity and temperature range compared to semi-field and field. Then, we also analyzed the potential of mixed fruit attractants, which are mango and banana, in enhancing the attractiveness of the AGHBs. As we exposed the mosquitoes to the attractant, it resulted in a significant preference towards the mixed attractant than the single attractant. However, there was no significant difference between males and female preference towards the attractants. The evaluation of AGHBs' effectiveness as the potential baiting tool based on the mortality rate of mosquitoes and females' biting preference. We found that the mosquitoes achieved total mortality after consumption, and the number of female attempts to blood-feed also reduced after 6 hour of exposure. We conclude that the AGHBs are applicable as a baiting tool to carry the liquid bait in achieving mosquito mortality and at the same time reduced in blood-feeding.

CHAPTER 1
INTRODUCTION

1.1 Background of Study

Almost every year, mosquitoes have caused more than 1.5 million mortality in the population of humans around the world (Ahmed *et al*., 2019; Sukumaran, 2016). *Aedes* borne diseases have become one of the critical issues that can cause disease to humans. These include chikungunya, dengue, zika, and yellow dengue fever (Roiz *et al*., 2018; Schubert, 2014). Disease caused by *Aedes* mosquitoes has increased incidence and expanded into new geographical areas. The increase in number correlated with increasing in population density, international travel, and the import and export of goods at the international level. Since the vaccines are unavailable, the only way to prevent epidemics is by controlling the mosquito vectors and thorough knowledge of its biology, behavior, and environmental factors that allow its transmission (García-Gutiérrez *et al*., 2012; Wilson *et al*., 2020).

Attractive toxic sugar baits are an effective method to attract mosquitoes based on their sugar foraging behavior. Since 1990, researchers have been following Lea's toxic sugar bait methodologies by introducing low toxic mosquito baits such as *Bacillus sphaericus* Meyer and Neide, boric acid, and spinosyns (Kline *et al*., 2018; Lea, 1965). The bait method is suitable to be combined with any type of gut active low toxin, which makes it a potentially valuable tool to fight rising resistance against conventional contact pesticides (Allan, 2010; Furnival-Adams *et al*., 2020; Gu *et al*., 2020). Hydrogel polymer can be a good candidate to be applied as bait since it is eco-friendly and biodegradable (Tay *et al*., 2017a). A researcher of Tay *et al*. (2017b) from the University of California, Riverside (UC Riverside) have developed a seaweed-based hydrogel bait to reduce the population of ant pests. The baits are made of sugar water (attractive to the ants) and contain 0.0001% of thiamethoxam. They found that the ant populations were reduced between 40 to 68% after four weeks of using the baits. Since the 1965s, attractive toxic sugar bait was introduced by entomologists against mosquito vectors in laboratory studies, and many methods have been explored to apply the techniques (Kline *et al.*, 2018; Lopes *et al.*, 2017; Shin *et al.*, 2021a; Tay *et al.*, 2017a). There were also studies integrating alginate hydrogel beads with mosquito biological control. Previously, the hydrogel beads were modified to carry temephos, spinosad, VectoBac, and essential oil by spreading in the potential oviposition site (Al-Solami *et al.*, 2019; Maia *et al.*, 2019; Silapanuntakul *et al.*, 2016).

Our study aims to integrate the function of hydrogel beads in adult mosquito control strategies. The outcomes may propose an adult mosquito baiting system that could significantly change the mosquito surveillance and control strategies. The introduction of alginate-gelatin hydrogel beads (AGHBs) can be a potential candidate to carry the liquid bait for the adult control strategies. Two issues will be highlighted are: 1) The applicability and durability of AGHBs to be applied in three selected study sites; 2) The efficiency of AGHBs with the combination of attractant and oral-toxicant to alter the behavioural responses of adult *Aedes* mosquitoes in laboratory work.

1.1.1 Alginate-gelatin hydrogel beads (AGHBs) as *Aedes* control baiting tools

The application of attractive hydrogel beads as a sugar bait was an effective method for mosquito control since their diet for energy sources is sugar. However, the design of early bait stations was not durable for application in extended periods, especially from dust and rain (Müller & Galili, 2016). The ingredients used in early crude "home-made" baits were not suitable for a stable commercial product. In this study, sodium alginate (ALG) and gelatin (GLN) were selected as the potential biopolymers in the preparation of the hydrogel beads. ALG is a polysaccharide derived from brown algae composed of β (1→4) linked -D-mannuronic acid, and α-(1→4) linked L-guluronic acid units. This unit can form a hydrogel with the presence of divalent cation (Ca^{2+}) (Ghanbari *et al*., 2021; Shin *et al*., 2021b). In contrast to ALG, GLN is a protein-based derived from animal body parts such as bone, skin, tissue, etc. (Nazmi *et al*., 2017). GLN is also a type of natural polymers that can degrade naturally in the environment *(*Pathak & Navneet, 2017; Yao *et al*., 2019).

This study aims to optimize the synthesis conditions of alginate-gelatin hydrogel beads (AGHBs) as a liquid carrier for mosquito bait. The durability of the AGHBs in adapting different environmental conditions (relative humidity and temperature), and the carrying capacity of the beads in storing the liquid bait for mosquito control strategies will also be explored.

1.1.2 Potential of mixed mango and banana as the bait attractant

An attractive toxic sugar bait (ATSB) is a baiting tool proposed to reduce the population of insect pests by exploiting their sugar-feeding behavior (Meza *et al*., 2020a). Previous ATSBs studies were applied to malaria vectors and have successfully reduced population management (Tizifa *et al*., 2018; Traore *et al*., 2020a). Therefore, there is a need to further improved and expanded the methods, especially for different types of mosquitoes species (Barbosa *et al*., 2019a, 2019b). This current study is keen to explore the behavior of *Ae. aegypti* and *Ae. albopictus* on our proposed attractants to induce the stimulant of these species towards the bait station. The attractant components, such as sugar, sucrose, or nectar, with enormous floral and fruity scents, will emit the volatile compounds to attract the mosquito. Adult male and female mosquitoes will land on a flower in their habitat as carbohydrate sources. Studies on fruit-based attractants also had proven the attractancy of mosquitoes to certain subtropical fruit such as guava, mango, and banana (Peach & Gries, 2020a). Waterlily mango (*Mangifera indica* L) comes from the family Anacardiaceae originated from Thailand, which contains a delicate tropical flavor with extremely juicy and sweet flesh. Mango fruit has been selected as an attractant due to the high sugar concentration and detectable chemical compounds. The volatile compounds in mango fruit, such as aldehydes, monoterpenes, sesquiterpenes, were previously explored as mosquitoes attractants (Meza *et al*., 2020b; Olale *et al*., 2019).

However, bananas can also be a good candidate for fruit-based attractants since they contain octanol, which is also attractive to mosquitoes. Cavendish banana (*Musa acuminata) is* a subgroup of the AAA Group, rich in volatile compounds from a complex mixture including acetates, butanoates, and 3-methyl butyl ester (Maduwanthi & Marapana, 2019; Zhu *et al.*, 2018). Few studies have been done on the response of mosquitoes towards mango and banana attractants, and they have proven a positive attractancy towards the bait. To enhance the findings, it is really important to understand the effect of the mixture between mango and banana as mosquito attractants. The combination of the two fruit attractants could induce synergism of compounds interaction, making it more attractive as a baiting tool. The presence of volatile compounds from both fruits shall improve the attractancy of the bait since they contain more attractive functional groups. The volatile compounds from the fruits will be identified by using headspace gas chromatography-mass spectrometry (GC-MS). Some researchers have used GC-MS to detect the compounds from the targeted attractant in developing a synthetic volatile as an attractant (Cai *et al.*, 2020; Jagodič *et al.*, 2017; Nyasembe *et al*., 2012; Sarkar *et al*., 2016). The introduction of bananas in the mango solution with a biocide may introduce a new functional groups that can increase the attractiveness of the bait. The findings of this study may provide crucial information in the production of killing baiting tools in dengue control programs and long-term towards a potent and inexpensive invention.

1.1.3 The response of *Aedes* vectors after Alginate-Gelatin Hydrogel Beads consumption

For decades, the application of ATSBs has been improvised to imply the successive vector control methods. As we identified the optimized formulation and the preferred attractant for the AGHBs, we are keen to determine the response of *Ae. aegypti* and *Ae. albopictus* towards the application, especially their mortality rate and female biting attempts. It is an obligatory part to ensure the efficacy of AGHBs as a baiting trap in the mosquito control strategies. The mortality rate of mosquitoes is a good indicator in determining the efficacy of the AGHBs baiting tool. Previous studies explored the application of low-oral toxicants in terminating insect pests by targeting their gut functions (Fiorenzano *et al*., 2017; Sippy *et al*., 2020). Some oral toxicants that have been used before is boric acid, dinotefuran, permethrin, and eugenol (Lucia *et al.*, 2020; Tenywa *et al*., 2017a; Traore *et al.*, 2020b). Boric acid was selected as a biocide in this study because it is safe for mammals, and its uses can be recyclable. For the bait dispensing system, boric acid is safe for the environment since it can turn into natural fertilizer, namely boron that act as a nutritional source for soil.

Furthermore, the second indicator for determining the efficacy of AGHBs is by observing the female biting attempts. AGHBs that carry liquid bait will function in initiating bait consumption and altering the blood-feeding behavior of the mosquitoes. The trial of female mosquito attempts biting is measured to determine the effect of AGHBs consumption on the fertility of mosquitoes. It is very crucial in reducing the generation survival of the vector. The female that displays a slower blood-feeding attempt will indicate the successiveness of the AGHBs application. The finding will conclude the potential application of AGHBs as attractive baiting tools in *Aedes*-borne diseases surveillance studies.

1.2 Problem Statement

Factual measurement of dengue vector populations is important in epidemiological surveillance, disease prevention, and control. The ATSB's used the combination of attractant and toxicant to attract and kill the targeted pest. However, the earlier designs lack in protecting the liquid for long periods, especially from rain and dust. Then, the application of synthetic polymer in previous hydrogel applications might cause long-term effects such as environmental pollution and genetic mutation (Deman & Van Larebeke, 2001). For example, they may not degrade naturally in the environment or pollute the water (Andrady, 2011; Moore, 2008). Plus, the degradation of polyacrylamide, a synthetic polymer, will produce acrylamide, which a potential carcinogen listed by International Agency for Research on Cancer 1994 (WHO 1985) (Tay *et al*., 2020).

Since we were using alginate and gelatin to produce the hydrogel beads, the size and swelling index were influenced by various processing parameters such as crosslinking time and concentration of the ingredients. Then, the placement of the AGHBs during application may affect the evaporation rate, which affects its durability. Furthermore, the attractiveness of the AGHBs may be restricted by the presence of natural sugar sources. Thus, modifying the liquid baits component may attract and enhance the mosquito imbibing the bait. In Mali, West Africa, the application of ATSBs has used fermented fruits solutions with 1% (w/v) boric acid to control the population of *An. gambiae* within 30 days (Gu *et al*., 2011).

Understanding the role of the biology of key vector mosquitoes in outbreaks of mosquito-borne disease can assist the development of surveillance and control technologies. While mosquitoes can transmit pathogens they acquire during blood feeding from an infected host, the frequency of blood feeding may also be allayed by sugar feeding. Hence, the choices mosquitoes make in obtaining food resources greatly impact pathogen transmission dynamics. These anticipated outcomes of the proposed study will assist the development of affordable and sustainable attract-and-kill strategies with the potential to reduce insecticide use by utilizing different modes of action (i.e. gut toxin delivered to the midgut) of mosquito control to overcome resistance to current toxins (i.e. pyrethroids) in mosquitoes.

1.3 Hypothesis

This study aims to integrate the function of hydrogel beads in adult mosquito control strategies. The outcomes may propose an adult mosquito kill baiting tools that could significantly change the way mosquito surveillance and control strategies. In addition, the effective control can be improved with the collection of both sexes, allowing for new alternatives that interfere with the normal foraging-nectar behavior of dengue vectors. Therefore, our study attempts to determine the synergism effect of the mixed of fruit extracts: bananas in mango solution. The introduction of bananas in the mango solution with a biocide may introduce a new chemical compound that can increase the bait's attractiveness. Despite evidence that fruits are full of aromas and other chemicals that occur naturally and during ripening, surprisingly their roles as modulators of mosquito foraging activity have attracted remarkably little research interest. The findings may provide a potential in the production of killing baiting tools in control programs and long-term towards a potent and inexpensive invention that could be utilized to control the spread of dengue vectors.

1.4 Objectives

To propose a potential baiting tool with the participation of the mixed fruit attractants in targeting the survivability of the *Aedes* mosquito: Implication in mosquito-borne disease risk.

This present study aims to:

1. To optimize the synthesis conditions of Alginate-Gelatin Hydrogel Beads as a baiting tool for mosquito bait.
2. To determine the effect of mixed fruit consisting of mango (*Mangifera incida*) and banana (*Musa accuminata*) in improving the attractancy of mosquitoes towards the Alginate-Gelatin Hydrogel Beads bait.
3. To evaluate the efficacy of the interaction between Alginate-Gelatin Hydrogel Beads bait and fruit attractants in controlling *Aedes* mosquitoes under laboratory conditions.

CHAPTER 2
LITERATURE REVIEW

2.1 *Aedes* Mosquitoes

2.1.1 *Aedes* morphology and biology

Aedes mosquito is a disease vector of dengue virus (DENV), chikungunya virus (CHICKV), zika virus (ZIKV) and yellow fever virus (YFV) (Huang *et al*., 2020; World Health Organization, 2020). The two *Aedes* species, famously known in *Aedes*-borne diseases, are *Aedes aegypti* and *Aedes albopictus*. Both are Dipterans morphologically with two pairs of wings, but the hind wings of the mosquitoes had reduced as halters to support their balance during flying while the fore wings are dark scaled (Hung *et al*., 2019). The things that distinguish the mosquitoes from other types of flies are their proboscis which have long tubular mouthparts for sucking up fluids, and their hair-like scales on the body (Helvacı, 2021; Seale *et al*., 2018).

Aedes aegypti is a dark mosquito with two conspicuous white markings and banded legs with all proboscis are in black. The palps are white-tipped, while the scutum has a dorsal pattern of white scales in the form of a ‘lyre’ with curved lateral and two central stripes contrasting with the general covering of narrow dark scales. The clypeus part shows the presence of white scale patches (Figure 2.1a) (Singh *et al*., 2021). *Aedes aegypti* locally distributed in an urban area, close to households, and can bite in indoor and outdoor conditions (Ryan *et al*., 2019). Compared to *Ae. albopictus*, the thorax, a medium longitudinal line displays the presence of a white scale lyre but lacks in the clypeus part (Figure 2.1b). They were more prevalent in cemetery sites and abundant near the coastal area (Westby *et al*., 2021). Besides, they are predominantly distributed in rural areas, far from human households, and prefer biting outdoor (Yin *et al*., 2019).

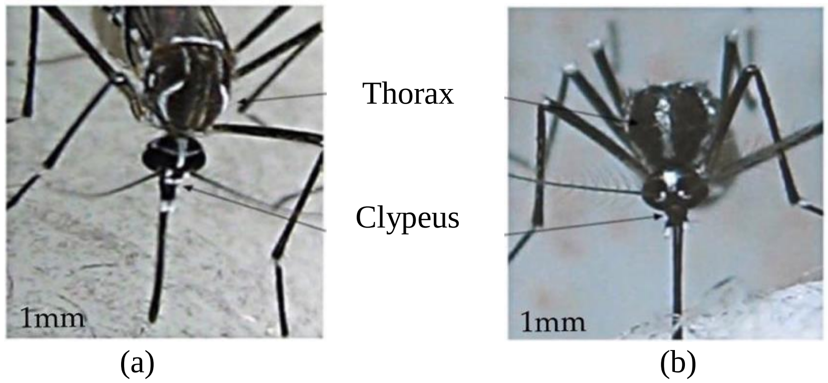

Figure 2.1 Different thorax morphology between *Aedes* species (a) *Aedes* aegypti, and (b) *Aedes albopictus* (adapted from Marlina, 2014)

The peak biting time of *Aedes* mosquito is during dusk and dawn. Both species are container-breeding mosquitoes, where their habitat will usually associate with the human area (Champion & Vitek, 2014; Westby *et al*., 2021). The female mosquito lays eggs in the water-holding containers around or further away from homes, tree holes, and bamboo internodes after 4 or 5 days of feeding in blood. The eggs will hatch by water supply, and the life cycle from egg to adult can occur in 7-9 days to complete, while the life span for adult mosquitoes is around three weeks (Naziri, 2015). For both species, the morphological features of males and females can be differentiated by their body sizes, where the males are smaller than females, and the presence of feathery antennae that helps them sense their potential wingbeats of the mate. Compared to the females, the antennae are plain, while their proboscis is designed to allow penetration into human skin (Bar & Andrew, 2013).

2.1.2 *Aedes*-borne diseases

The *Aedes*-borne disease has been a serious global issue for the past 50 years, causing almost 390 million infections per year to humans (WHO 2021, 2021). The vector is categorized under order Diptera, which plays a role as a biological vector where the disease agents can develop or multiply inside them. The pathogens may be in the form of viruses or parasites. Viruses that *Aedes* mosquitoes can be transmit include zika virus (ZIKV), west nile virus (WNV), chikungunya virus (CHIKV), dengue virus (DENV1, DENV2, DENV3, & DENV4), japanese encephalitis virus (JEV), and yellow fever virus (YFV) (Bibbs *et al*., 2018; Gaye *et al*., 2019; Pearson *et al*., 2020; Teo *et al*., 2017). Parasitic protozoa such as *Plasmodium* spp. and nematode such as *Brugia malayi* can also be transmitted by mosquitoes from the species of *Culex* spp. and *Anopheles* spp. (Diarra *et al*., 2021; Tananchai *et al*., 2019; Vaughan & Turell, 2017).

The transmission spreads when the infected mosquito bites a healthy human or animal (domestic and wild). The transmission may occur in the way of mosquitoes to humans, and the infection can be between humans or from animals to humans (World Health Organization, 2020). Mosquito bites on the infected person may pick the pathogen, then multiplies or develop inside the body. Once it reaches the complete cycle, the pathogen will be transmitted to a healthy host through the proboscis. The effect may take time to show the symptoms starting from mild and short-term illness to severe and long-term illness. Up to 14 days (about 2 weeks), the patients may display signs of fever, headache, retro-orbital pain, myalgia, exanthema, prostration, arthralgia, nausea, vomiting, diarrhea, sleepiness, weakness, coryza, and cough (Hung *et al*., 2019; Teixeira *et al*., 2017). Some serious signs such as bleeding, warning signs (intense abdominal pain, persistent vomiting, painful hepatomegaly, pre-syncope, mucosal bleeding, sleepiness/irritability, and respiratory distress), date of onset and duration, and presence of signs of shock (hypotension, cold extremities, cyanosis, and rapid pulse) may also occur due to the infections (Teixeira *et al*., 2017). Severe cases of mosquito-borne diseases can lead to death (WHO 2021, 2021).

Table 2.1 Summary of mosquito-borne diseases

Mosquito-Borne Disease	Vector Species	Type of Pathogen	Parasite and Pathogen	Symptoms	References
Yellow Fever	*Aedes spp.*	Virus	Flavivirus, YFV	Fever; headache; muscle aches, particularly in your back and knees; sensitivity to light; nausea; vomiting; loss of appetite; dizziness; red eyes, face or tongue.	(Akram *et al.*, 2021; Barnett, 2007)
Dengue Hemorrhagic Fever	*Aedes aegypti* *Aedes albopictus*	Virus	Flaviviridae, (DENV-1, DENV-2, DENV-3 and DENV-4)	Fever; rash; muscle and joint pain; nausea and vomiting	(Akram *et al.*, 2021; Ooi & Gubler, 2011)
Chikungunya	*Aedes spp.*	Virus	Togaviridae, CHIKV	Muscle pain; joint swelling; headache; nausea; fatigue, and rash.	(World Health Organisation, 2017)
Zika	*Aedes aegypti* *Aedes albopictus*	Virus	Flaviviridae, ZIKV	Fever; rash; headache; joint pain; red eyes; muscle pain; no to mild symptom, diagnosis through urination test.	(Rubio-Solis *et al.*, 2019)

Table 2.1 continued

Lymphatic Filariasis	*Culex spp.* *Anopheles* spp. *Aedes* spp. *Mansonia spp.*	Nematodes	*Wuchereria bancrofti*, *Brugia malayi* *Brugia timori*	Limb or genital swelling - Repeated episodes of inflammation and lymphedema lead to lymphatic damage, chronic swelling, and elephantiasis of the legs, arms, scrotum, vulva, and breasts	(World Health Organization, 2018) (Van den Berg *et al*., 2013)

2.1.3 Resistance management

Insecticide resistance (IR) has become a serious issue in reducing the successfulness of vector control since it will decrease the susceptibility of the targeted pest to the insecticide. Chemical insecticides from the classes of organophosphate, neonicotinoids, phenylpyrazole, and carbamates share a common chemical structure and mode of action (Martín Reina *et al*., 2017). It functioned in killing the insect or inhibiting their growth. One of the mechanical effects is by interfering with the enzyme activities within its metabolic pathways (Esterace, Monooxygenases, & GSH S-Transferases) and alteration of target-sites (kdr & AChE) (O'Neal *et al*., 2019). Besides, genetic mutation also can disturb the vector susceptibility towards the insecticide. The resistance to the insecticide that has not been prevented will cause genetic changes within the parents and pass to the offspring, then expand within the population (Karunaratne *et al*., 2018). Thus, many initiatives have been introduced globally in overcoming these issues to protect the public from mosquito-borne diseases.

The avhievement in resistance management can strongly depend on the biological specific of the vector system (Sternberg & Thomas, 2018). Insecticide resistance management (IRM) is currently being explored in reducing the fitness of individual resistance among the population. First, rotational strategies employ the alternative of insecticide rotation from different classes of insecticide (Dusfour *et al*., 2019). Second, by mixing two or more insecticides with a single or more formulation, then applied in the same spraying tank or dwelling areas. Current researchers are formulating an insecticide with more than one active ingredient (AI) to reduce the issue of cross-resistance between the mosquitoes (Richards *et al*., 2020). The third is implementing a mosaic strategy in various places but one locality, which involves a spatial alternation of two or more insecticides with a different mode of action.

Despite all the implemented techniques, the control of insecticide resistance between the vectors is still limited. Studies by Dusfour *et al*. (2019) mentioned that IRM is currently lacking in several aspects and absent a strategic plan in terms of a systematic association with non-insecticidal tools to reduce the evolution of resistance. In May 2012, Global Plan Insecticide Resistance Management for malaria was announced as a collective strategy to tackle malaria outbreaks in the community (Chanda *et al*., 2016). The challenges that can be highlighted include the limited availability of vector control tools with a new mechanism of action, critical finance potential, and resource deficiencies (Mnzava *et al*., 2015). In addition, the threat of insecticide resistance may occur due to the lack of action by the global community to address the issues. Furthermore, the rotation of indoor residual spraying (IRS) techniques to avoid resistance can be disturbed due to the cost increases, difficulties in preparing formulations with multiple active ingredients, positive synergistic interaction, and safety for the environment (Mnzava *et al*., 2015).

2.2 Mosquito control strategies

Vector control for disease-borne issues requires a proper strategical method to avoid cost and time waste. The methods are categorized as chemical, biological, mechanical, and physical control. The control of *Aedes* mosquitoes by using chemical insecticide has been applied for a decade. The insecticides such as permethrin, deltamethrin, and cyfluthrin are used against *Aedes* spp., either in the form of space treatment, indoor residual spraying, insecticide-treated bed nets, or as larvicides (Auteri *et al*., 2018; Hamid *et al*., 2018; Smith *et al*., 2016). For example, a study by Kring (2009) on metofluthrin mesh sheets resulted in enormous potential mosquito control in Queensland, Australia since the application displays positive feedback and the method is widely accepted (Darbro *et al*., 2017).

The national-level collaboration fight for mosquito-borne diseases focused on the unity of the countries in Asia against the spread of those diseases. The acquaint of 'World Mosquito Day' opens the opportunity for the governments, interest groups, businesses, and local communities to play a role in spreading awareness throughout the regions (O'Neill *et al*., 2018). The collaboration of these respective countries can overcome the challenges by reinforcing preparedness planning at a collective regional level and helping establish public-private partnerships to exploit the reach and resources of the private sectors to reduce the impact of a disease outbreak (Rolfe, 2017). Through the investment of community and faith-based organizations in spreading awareness, higher level of engagement with the private sectors such as the tourism, food and beverage sector to share the best method would ensure a consistent culture of prevention and control (Mely *et al*., 2015; O'Neill *et al*., 2018).

In Malaysia, the development of urbanization caused an increase in the intrusion of mosquitoes in their natural habitats and could lead to the evolution of co-habitation between mosquito vectors and humans (Anoopkumar & Aneesh, 2021; Lee *et al.*, 2020). The government has implemented prevention and control measures at the local level and policy measures at the state level to control the population of mosquitoes. The Deputy Prime Minister, Tan Sri Muhyiddin Yassin, headed the creation of a Dengue Task Force in July 2014 at a commendable national level (Mely *et al*., 2015). However, improvement in the infrastructure and human resources investment is needed in rural areas to ensure the programs are well-designed.

Regarding WHO (2016) statistics in Malaysia, the cumulative number of dengue cases in 2015 is 58% higher than those reported during the same periods in 2014 (Suppiah *et al.*, 2018). The vector control for dengue that has been practiced in Malaysia focused on human resources dependent, which required a large human workforce to perform the variety of dengue of control vector, surveillance, and prevention activities at the district level (Lim *et al*., 2020). The health professional is well-trained to conduct premise inspections, fogging, and larviciding activities. The involvement of specialist doctors and entomologists in providing technical support contributes to the success of this control program (Gachelin *et al*., 2018; Packierisamy *et al*., 2015).

2.2.1 Chemical control

Chemical control of pests like mosquitoes refers to the use of any chemical pesticide to kill the pest and vector populations. Pesticide is categorized into two groups, natural and synthetic pesticides. Thus, previous chemical control focused on synthetic organic compounds. It includes organochlorines, organophosphates, carbamates, and pyrethroids. However, natural pesticides can be obtained from mineral oils or plant-based such as pyrethrum and azadirachtin, as illustrated in Figure 2.2 (Al Naggar *et al*., 2019; ICAR, 2016; Wojciechowska *et al*., 2016). Decades ago, various kinds of chemical insecticides were introduced and improved from diverse groups regularly in response to the recent resistance issues. Dichlorodiphenyltrichloroethane (DDT) insecticides were discovered by Muller in 1939 to control the prevalence of malaria, marine typhus, and other mosquito-borne diseases (Talapko *et al*., 2019). Since their application covers broad-spectrum, they were functionally useful in targeting insect pests. Also, they are persistent in the environment and thus reducing the repetition appliance and saving cost (Jyp *et al*., 2013; Umulisa *et al*., 2020). In the 1972s, however, DDT was banned in the United States since it caused bioaccumulation of DDT in food chains and human fat (Merhaby *et al*., 2019). Some countries still use DDT as a pesticide due to its effectiveness and inexpensive manufacturing costs.

Organophosphate was introduced in the year 1944s to fulfil the insecticide requirements to control pests (Faiz *et al*., 2020; Woodrow *et al*., 2019). For mammals, the organophosphate can affect the oral and dermal of insects. The application may hurt mammals primarily through endocrine disruption, Acetylcholinesterase (AChE) inhibitors. And to the worth, it may introduce bioaccumulation in the food chains where the toxins built up and the organism at the top sequence is severely affected (Ding *et al*., 2020). On the other hand, carbamates are widely used in homes, gardens, and agriculture as an insecticide to control insect pests. The mode of action for carbamates resembles the organophosphate, where they will inhibit AChE and induce similar symptomatology after exposure (Routt *et al.*, 1999). However, its effect on the human system expresses a rapid recovery pattern compared to organophosphate. It is due to the ability of the acetylcholinesterase enzyme to break apart (Routt Reigart & Roberts, 1999).

Synthetic pyrethroid was introduced as it resembles natural insecticides, pyrethrins. Their photostability under sunlight exposure can prolong the appliance since the poison can remain functional even after being given prolonged exposure to sunlight (Ding *et al*., 2020; Elliott, 1980). Their toxicity can cause harm to the population of fishes and the colonies of bees (Straub *et al*., 2019). Lastly, the miscellaneous compound neonicotinoids, a neurotoxicant, was used to initiate broad-spectrum insecticide into the field but it may affect the non-target organisms and not only targeting's pests (Ding *et al*., 2020). These include the heteropteran, dipteran, and lepidopteran. Acetamiprid is an example of the active ingredient that can act as neuropeptide acetylcholine. It targets the central nervous system of the insect (Wojciechowska *et al.*, 2016).

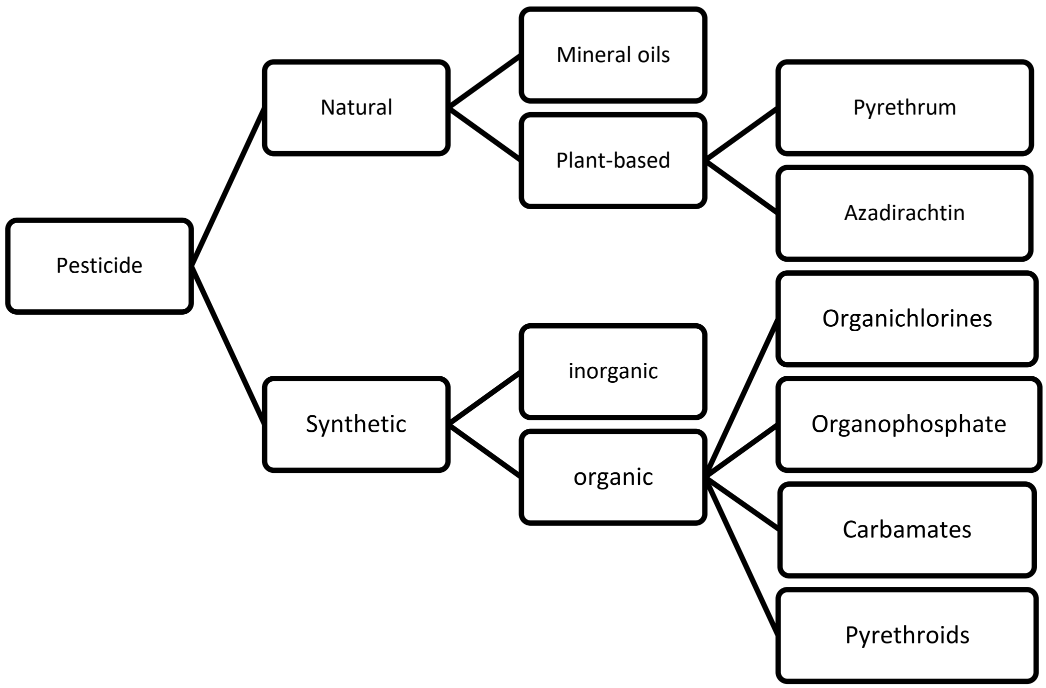

Figure 2.2 Classification of pesticides based on their chemical nature (adapted from ICAR, 2016)

2.2.2 Biological control

Besides chemical approaches, biological control is one of the approachable methods since it can suppress the population of specific pest organisms by using other living organisms. It works by importing natural enemies to destroy pests or conserving the natural enemies that are already available (Plouvier & Wajnberg, 2018). The control agents include predators, parasitoids, parasites, and pathogens that are naturally available in the habitat, which will destroy the targeted pest by invading their habitat (Rossbacher

& Vorburger, 2020). Even though this method does not eliminate the population of the pests, it will keep them at low levels (Lenteren *et al*., 2018). The control agents can be classified into three different types; 1) conservative, 2) classical, and 3) augmentative biological control, where each term describes the strategies specifically.

2.2.2(a) Conservative biological control

Conservation biological control is more specified to the targeted species, and this method takes advantage of the presence of natural enemies and develops control strategies. The natural enemy can be a predator for several pests such as thrips and aphids, or else, parasitoids for *Helicoverpa armigera* (tomato's pest) or *Plutella xylostella* (cabbage's pest) (Giles *et al*., 2017; Vandervoet *et al*., 2018). Since the use of insecticide harms the population of natural enemies, these control methods can maintain the natural enemies' survivability in their habitat. The natural enemies need a suitable habitat and sufficient natural resources for their development (Shields *et al*., 2019). They may prefer the source of pollen, nectar, or honeydew, harvested products, provision of clean air and water, regulation of climate, and maintenance of biodiversity for their sustainability (Costanza *et al*., 1997; El-Wakeil *et al*., 2017). Some approaches have been made recently, such as increasing the attractiveness of natural enemies, prolific production of pollen or nectar, improving accessibility of floral sources, flowering phenology, and the availability of seeds (Irvin & Hoddle, 2021).

Fiedler *et al*. (2008b) evaluated the plants that can be used in habitat management by providing multiple benefits in the biological control strategies (Shields *et al*., 2019). Plants like phacelia (*Phacelia tanacetifolia*), alyssum (*Lobularia maritima*), and native buckwheat (*Fagopyrum esculentum*) planted in New Zealand vineyard region provided the biological control for the leafroller caterpillar, which is the larvae of the light-brown apple moth (*Epiphyas postvittana*) (Bui, 2018; González-Chang *et al.*, 2019; Tiwari *et al.*, 2020). These plants nurture the needs of parasitic wasp, *Dolichogenidea tasmanic,* by providing a nectar source for their natural fitness. Ecosystem services have been recognized as a promising approach in sustainability research and implementation to maintain primary producers within a particular system (Daily, 1997; Terêncio *et al*., 2021). The focus on small scales such as establishing home landscapes, urban or golf course settings, flowering plants in orchards, vineyards, and annual crops fields can be a good strategy to implement the actions. Ellis *et al.* (2005) studied the potential of conservation biological control for the bagworm (pest), *Thyridopteryx ephemeraeformis* (Haworth). They reported that the biological control potentially can preserve a suitable habitat for their natural enemies, hymenopterous parasitoids (Tillman, 2017). The researchers hypothesized that the presence of flowering forbs in proximity to host shrubs can regulate the abundance of parasitoid populations.

2.2.2(b) Classical biological control

Classical biological control is the method that will introduce the control agent for the permanent establishment and long-term pest control strategies. Most of the agents include parasites, pathogens, predators, and herbivores for weed control (Jongen, 2005; Stenberg *et al*., 2021). It is useful in controlling lands and reducing the population of pests that cause potential ecological and economic damage. The success of this method was proven in the 1800s when the vedalia beetle (*Rodolia (Vedalia) cardinals* Mulsant (Coleoptera: Coccinellidae) was released against the cottony cushion scale *Icerya purchasi* Mask. (Homoptera: Margarodidae) in California (Cass *et al*., 2020; Eilenberg *et* *al.*, 2001). In June 1898, a ladybird predator, *Cryptolaemus montrouzieri*, was introduced to control the crop pest for the Indian subcontinent (Maqbool *et al.*, 2020; Subramanian *et al.*, 2021). The first intention was to target the specific species, soft green scale (*Coccus viridis*). Unfortunately, the predators were more interested in mealybugs, the insects that infest fruits, coffee, and ornamental plants (Kundoo & Khan, 2017). Recently, the ladybird predator was actively suppressing the infestation of mealybugs pest on other commercial fruits such as guava, grapes, mulberry, coffee, mango, pomegranate, custard apple, etc., in South India (Singh *et al*., 2021). In India, a woolly aphid, *Erisoma lanigerum*, was accidentally introduced from the United States and caused damages to the apple crops. To overcome this issue, an exotic aphelinidae parasitic wasp (*Aphelinus mali)* native from North America was introduced to Saharanpur (Uttar Pradesh, India), but the problem was unsolved. The parasitoid failed to establish itself in that area due to the presence of ladybird predators that fed on the parasitized and unparasitized woolly aphids (Bisht & Giri, 2019; Singh *et al*., 2021). As a result, the population of the parasitoid diminished.

2.2.2(c) Augmentative biological control

Augmentative biological control is a method that releases a large number of insectary-reared natural enemies in the pest population with the existed natural enemies to establish them in that area (Collier & Van Steenwyk, 2004; Tefera *et al.*, 2019). Van Lenteren *et al*. (2003) mentioned that augmentative biological control is a more environmentally and economically successful potential pest reduction strategy (Begg *et al*., 2017; Romeis *et al.*, 2019; Rossbacher & Vorburger, 2020). It can be idealized as a long-term solution for controlling the pest population without using chemical pesticides. The augmentative control strategies are grouped under two conditions based on the