EFFECT OF SUGAR VARIANTS ON THE *Aedes* MOSQUITO MIDGUT MICROBIOME AND SUGAR-FEEDING BEHAVIOR

RANJITHA A/P SAMBANTHAN

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by

RANJITHA A/P SAMBANTHAN

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LIST OF SYMBOLS

%	Percentage
°C	Degree Celcius
°	Degree
nm	Nanometer
cm	Centimeter
μL	Microliter
a.u	Absorbance unit
w/v	Weight per volume
M	Molar
μm	Micrometer

LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
16S rRNA	16S ribosomal ribonucleic acid
AGL	*Aedes aegypti* lab strain
AGW	*Aedes aegypti* wild strain
ALL	*Aedes albopictus* lab strain
ALW	*Aedes albopictus* wild strain
ATSBs	Attractive Targeted Sugar Baits
C	Carbon
Ca	Calcium
$CaCl_2$	Calcium Chloride
CDC	Centers for Disease Control and Prevention
Cl	Chlorine
DDT	Dichlorodiphenyltrichloroethane
DENV	Dengue Virus
EDX	Energy Dispersive X-ray
FESEM	Field Emission Scanning Electron Microscope
FTIR	Fourier Transform Infrared
FWHM	Full Width at Half Maximum
IVM	Integrated Vector Management
K	Potassium
Mg	Magnesium
Na	Sodium
O	Oxygen
OCRO	Obligate Co-Receptors
OR	Odorant Receptors
P	Phosphorus
PI	Preference Index
RIDL	Release of Insects with Dominant Lethality
SBS	School of Biological Sciences
SE	Standard Error
Si	Silicone

SIT	Sterile Insect Technique
USM	Universiti Sains Malaysia
UV	Ultraviolet
VCRU	Vector Control Research Unit
VOC	Volatile Organic Compounds
WHO	World Health Organization
XRD	X-Ray Diffraction

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KESAN VARIASI GULA TERHADAP MIKROBIOM USUS TENGAH DAN PERLAKUAN MAKAN GULA NYAMUK *AEDES*

ABSTRAK

Nyamuk *Aedes* terkenal untuk penyebaran penyakit disebabkan virus denggi dan Chikungunya. Usaha kawalan telah diperkukuhkan dengan menggunakan teknik pengurusan vektor bersepadu yang inovatif seperti penggunaan manik hidrogel alginat bersama umpan gula beracun. Namun, terdapat kekurangan sumber yang meneroka hubungan antara umpan gula sasaran dengan mekanisme tarik dan bunuh nyamuk. Oleh itu, kajian ini mengenal pasti ciri-ciri asas variasi gula yang mempengaruhi tingkah laku nyamuk terhadap sumber gula. Tambahan pula, respon olfaktori nyamuk *Aedes* liar dan makmal terhadap pelbagai umpan gula sasaran (ATSBs) diuji menggunakan olfaktometer yang diubah suai. Komuniti mikrobiom dalam saluran usus tengah nyamuk dianalisis menggunakan penjujukan gen 16S rRNA. Akhir sekali, perubahan struktur usus tengah nyamuk yang diberi makan ATSBs dikenal pasti menggunakan teknik histologi. Penyediaan sampel ATSBs berdasarkan tiga variasi gula yang berbeza seperti *Mangifera indica*, *Chrysanthemum morifolium* dan campuran *M. indica* dengan *C. morifolium.* Semua sampel ATSBs dikeringkan di bawah kawalan suhu sebanyak 40°C selama 3 jam dan diteruskan dengan analisis Field Emission Scanning Electron Microscopy (FESEM), UV-Vis-NIR Spectroscopy (UV-Vis), X-ray Diffraction (XRD) dan Fourier Transform Infrared (FTIR). Eksperimen respon olfaktori nyamuk dijalankan menggunakan olfaktometer yang diubahsuai melalui ujian tanpa pilihan dan ujian pilihan menggunakan 20 ekor nyamuk *Aedes* (Afify et al., 2014). Selain itu, nyamuk diberi makanan ATSBs yang berlainan jenis selama 3 hari dan seterusnya usus tengah dibedah untuk mengkaji komuniti mikrobiom. Tambahan pula, usus tengah dibenamkan dalam acuan lilin untuk mengkaji perubahan morfologi usus nyamuk. Data XRD menunjukkan variasi kehabluran sampel kawalan yang menunjukkan nilai tertinggi berbanding ATSBs *Mangifera indica* yang mempunyai nilai terendah. Kedua-dua spesies nyamuk *Aedes* liar dan makmal lebih cenderung memilih *M. indica* berbanding campuran tetapi menunjukkan minat kedua terhadap campuran berbanding *M. indica*. ATSB *C. morifolium* menunjukkan kesan penolakan yang kuat terhadap *Ae. aegypti* (-0.65 ± 0.07) dan *Ae. albopictus* (-0.72 ± 0.18). Usus tengah nyamuk yang diberi makan ATSBs *M. indica*, *C. morifolium* dan campuran menunjukkan gangguan morfologi menurut kajian histologi. Proteobacteria dan Firmicutes adalah dua filum bakteria paling dominan dalam usus tengah nyamuk jantan dan betina. Secara konklusinya, kajian ini menjadi asas untuk mencipta strategi kawalan vektor nyamuk yang lebih berkesan dan sebagai salah satu inisiatif pencegahan penyakit bawaan nyamuk pada masa depan.

EFFECT OF SUGAR VARIANTS ON THE *AEDES* MOSQUITO MIDGUT MICROBIOME AND SUGAR-FEEDING BEHAVIOR

ABSTRACT

The *Aedes* mosquito species are known for spreading illnesses caused by dengue and Chikungunya virus. Control efforts have been strengthened by the employment of novel integrated vector management techniques such as alginate hydrogel beads in combination with toxic sugar bait. However, limited sources are available on the relation of toxic sugar bait with the attract and kill mechanism. Therefore, this study identified the fundamental properties of sugar variants that affect the behaviour of mosquitoes towards the sugar sources. In addition, olfactory response of wild and laboratory strain *Aedes* mosquitoes towards the various Attracted Targeted Sugar Baits (ATSBs) also investigated using modified olfactometer. Finally, post-effect of ATSBs ingestion that influence microbiome community colonize the midgut were detected by using 16S rRNA gene sequencing and the changes in midgut structure of mosquitoes were analysed using histology technique. The ATSBs were prepared based on three different sugar variants such as *Mangifera indica*, *Chrysanthemum morifolium* and mixture of *M. indica* and *C. morifolium*. All of the ATSBs samples were dried for 3 hours in 40°C prior to analysis such as Field Emission Scanning Electron Microscopy (FESEM), UV-Vis-NIR Spectroscopy (UV-Vis), X-ray Diffraction (XRD) and Fourier Transform Infrared (FTIR). Mosquito olfactory response experiments were conducted using a modified olfactometer through no-choice and choice tests by using 20 *Aedes* mosquitoes (Afify et al., 2014). Additionally, mosquitoes were fed with different types of ATSB for 3 days, after which their midguts were dissected to study the microbiome community. Moreover, the midguts were embedded in wax mould to study morphological changes in mosquito guts. The X-ray Diffraction (XRD) analysis indicates that the samples crystallinity varies with the control having the highest while the mango ATSBs having the lowest crystallinity. Both laboratory and wild strain *Aedes sp.* are more likely to choose *M. indica* attractant compared to mix. However, the mosquitoes show a second interest to mix attractant compared to *M. indica*. Overall, *C. morifolium* ATSBs strongly repels *Ae. aegypti* (-0.65 ± 0.07) and *Ae. albopictus* (-0.72 ± 0.18) from reaching the ATSBs. The midgut of mosquitoes fed with *M. indica*, *C. morifolium* and the mix ATSBs displayed disturbed morphology according to histology studies. Proteobacteria and Firmicutes were the two most prevalent phylum that found in both male and female mosquito midgut. In conclusion, this study provides the groundwork for creating effective mosquito vector control and disease prevention initiatives for future.

CHAPTER 1

INTRODUCTION

1.1 Research Background

According to the taxonomic classification, *Aedes* mosquito are categorized under Kingdom Animalia, which is further divided into the Order Diptera and Family Culicidae (Talaga et al., 2015). There are approximately 3500 types of mosquito species which can be found worldwide and it is a medically importance flying insects (Cilek, 2022). Essentially, there are three genus of mosquitoes that took part in human transmission such as *Culex*, *Anopheles* and *Aedes* (Tolle MA, 2009). Majorly, *Aedes* mosquitoes can cause mosquito-borne diseases such as dengue, chikungunya, yellow fever and Zika that are quickly expanding and turning into a serious health risk.

Overall in 2023, more than 6.5 million cases and over 6,800 deaths attributed to dengue virus were recorded (Haider et al., 2024). The disease is now endemic in more than 100 countries in the WHO Regions of Africa, Americas, Eastern Mediterranean, South-East Asia and Western Pacific. The most severely impacted regions are Americas, South-East Asia, and Western Pacific, with Asia accounting for around 70% of the world's disease load (World Health Organization, 2024). The reported outbreaks of dengue in Malaysia have considerably climbed up to 185% from 13,650 cases between January-May in 2022 to 25,283 cases during the same period in 2023, resulting to the rise in the number of fatalities from 7 to 24 cases (Wan-Fei et al., 2023). The number of cases rise up is directly influenced by the growth in number of international travellers, population density, and international trade in goods. However, there is lack of vaccines, the only method to stop this epidemic is by managing the mosquito vectors and having a deep understanding of their biology, behavior, as well as environmental elements that facilitate its spread (Wilson et al., 2020).

Common ways for reducing *Aedes* population includes residual spraying for resting areas, interior space spraying, routine of larval control insecticides usage and the use of personal protective measures. In the control programme, insecticides such as pyrethroids and organophosphates are frequently utilized (Centers for Disease Control and Prevention (CDC), 2020). These insecticides work well to minimize the spread of disease and lower the mosquito populations. However, the excessive and indiscriminate use of these chemicals can lead to environmental issues and the development of insecticide resistance. Therefore, an alternative method, bait application with sugar and low toxic mosquito baits such as *Bacillus sphaericus*, boric acid, and spinosyns has been adopted. Thus, this type of bait is known as attractive toxic sugar bait which mainly focusing on mosquito foraging behavior (Kline *et al*., 2018).

The bait approach is a potentially useful tool in fighting against growing resistance to conventional contact pesticides because this bait is paired with any kind of gut active low toxin (Allan, 2010; Gu et al., 2020). Since hydrogel polymer is biodegradable and eco-friendly, it can be a viable option to be use as bait (Tay *et al*., 2017a).

The study on the molecular works in the organ of mosquitoes is a multidisciplinary approach to study mosquito behaviour and microbiome dispersal in mosquito body. The integration of mosquito behavioural and molecular studies has offered ideas on the means of achievement for the controlling of mosquitoes around the world to be readily used, applied and modified by these researchers has arisen.

1.2 Problem Statement

In recent years, the emergence of insecticide resistance in populations of key mosquito vectors has made managing outbreaks of mosquito-borne diseases, such as dengue, increasingly difficult. Affordable, effective, and sustainable alternatives to traditional insecticides are required to assist authorities to reduce the threat of mosquito-borne disease. Attractive toxic sugar baits are being developed to control mosquitoes by combating the issues of insecticidal resistance and at the same time, they have a low impact on non-targeted organism (Fiorenzano et al., 2017). This implementation used the combination of an attractant sugar bait and toxicant mixture to attract and kill mosquitoes. However, effective mosquito management depends on a thorough understanding of the biology and feeding patterns of the key vector mosquito species. All mosquitoes rely on sugar feeding to gain energy for their body development. Moreover, there are gaps in our understanding of how mosquito species behave in response to sources of sugar and how sugar feeding influences the midgut microbiome, a potentially critical determinant of mosquito life traits and vector competence. Understanding sugar-feeding behaviour is necessary before moving to develop and operationally deploy novel mosquito surveillance and control strategies.

1.3 Significance of Study

This research contributes to the knowledge in the evaluation of sugar variant in developing the bait. The sugar variants representing three different types of readily available, sustainable, and affordable sugars with a variety of concentrations including flower nectar, fruit juice, and a mixture of both. Direct influences on mosquito behaviour and physiology was investigated such as the attractiveness of the sugar variant compared to naturally occurring sugar sources on key vector mosquitoes.

In addition, the synergistic effect of sugar variant feeding and the gut microbiome colonization that influence mosquito life history or susceptibility to toxic substances. While mosquitoes can transmit pathogen 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 this study assist the development of 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.

The sustained release of the sugar variants would furnish a novel and sustainable mosquito bait in integrated vector management (IVM) of mosquito-borne diseases control formulation technology, which is currently limited in the market. Moreover, this research study can be a novel fundamental outcome in promising a new tool for the *Aedes* vector control in the Attractive Targeted Sugar Baits (ATSBs) methods in future.

1.4 Hypothesis

The purpose of this project is to incorporate the function of hydrogel beads into adult mosquito control plans. Thus, this finding might have the potential to drastically alter mosquito surveillance and control tactics. Furthermore, the accumulation of both sexes can enhance effective control which disrupt the typical nectar-foraging activity of vectors. Consequently, our research aims to discover the overall impact of different sugar variant which extracted from fruit and flower. Firstly, the physical properties of different Attractive Targeted Sugar Baits (ATSBs) were analysed to understand the fundamental characteristics of it. Then, the olfactory response of mosquitoes was determined to identify the attractiveness of ATSBs towards the mosquitoes. Finally, the impact of ATSBs in terms of altering the mosquito midgut structure and midgut microbiome diversities were figured out.

1.5 Objectives

This study aimed to address the effect of ATSBs on the targeted mosquito population of *Aedes* sp in both wild and laboratory strains. In order to accomplish the goal, this study was designed with certain precise objectives as below:

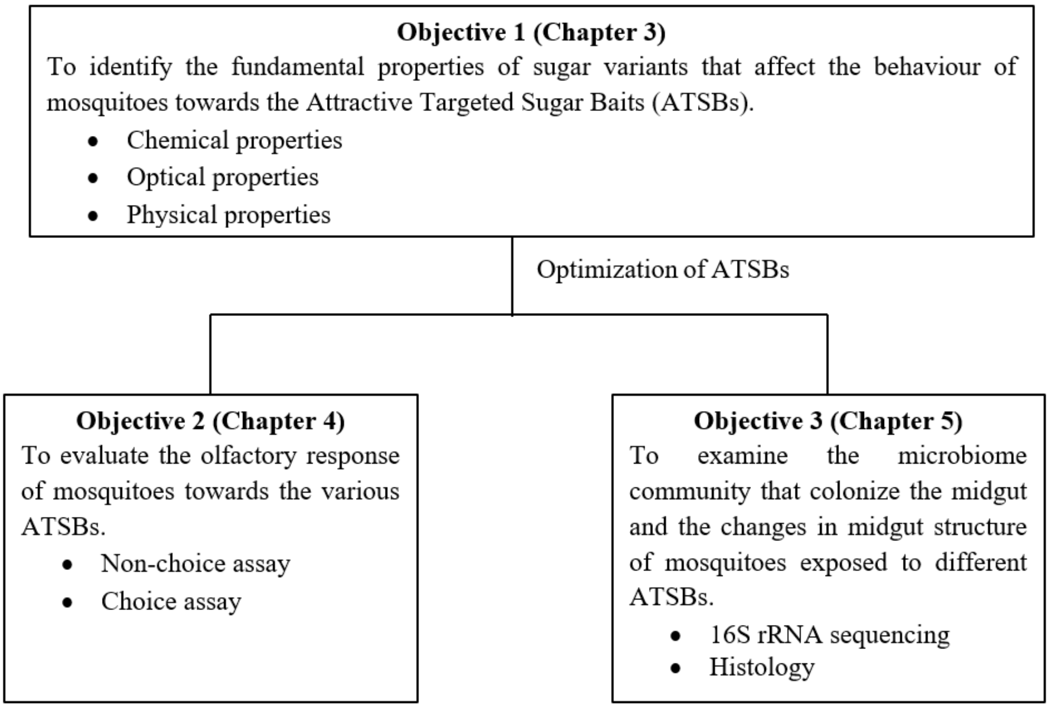


Figure 1.1 Conceptual framework of the research objectives

CHAPTER 2

LITERATURE REVIEW

2.1 Biology of *Aedes sp*

2.1.1 General biology of *Aedes sp*

Biotic and abiotic factors are the main factor that cause the changes in life history traits and population level of the *Aedes* mosquitoes. Adult body size, lifespan, fecundity and gonotrophic cycles are the life history traits while survival and population growth are the population level changes that determine Aedes number. Environmental factors such as rainfall, photoperiod, temperature of the surrounding and humidity are the most commonly studied for mosquito ecology and disease transmission by vector mosquito (Waldock et al., 2013, Roiz et al., 2010).

Generally, male adult is smaller in size compared to female adult. However, body size is not a main parameter in sexual identification because environmental factors such as food resources availability and larval number in breeding sites influence body size of the mosquitoes (Carvalho & Moreira, 2017). Antenna with bushy and feathery appearances indicate the identification for male while less dense antennal hairs indicates female adult. The proboscis of these mosquitoes is long, thin and straight (Andrew & Bar, 2013). Adult female *Aedes sp* have a pointed abdomen and the size of the maxillary palp is shorter compared to the male.

2.1.2 Biology of *Aedes albopictus*

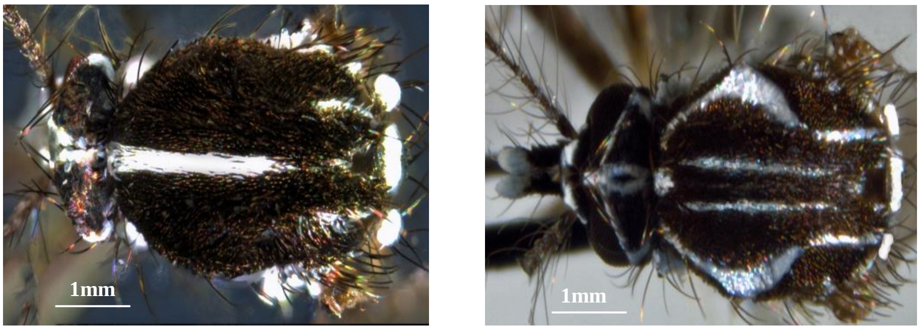
Aedes albopictus (Skuse) is referred as *Stegomia albopicta* and commonly known as 'Asian Tiger Mosquito'. Human activities made the distribution of this species to spread worldwide. According to previous studies, *Ae. albopictus* was originated from Asian forests (Satho et al., 2015). The expanding of this species all around the world due to oviposition behaviour since they can differentiate high quality place to deposit their eggs. Around 25 countries from Europe were reported the availability *of Ae. albopictus*, for instance, Greece, Italy and France. *Aedes albopictus* also classified by Invasive Species Specialist Group as one of the most invasive species. This is due to the ability of this species in withstanding drastic climate change (Medlock et al., 2015).

Adult *Ae. albopictus* has a silver strip on the middle of its scutum. Hence, this characteristic distinguishes them with other *Aedes* species. Additionally, this species is in black and white pattern since there are bold black and silver white scales on some parts of the body (Medlock et al., 2015). This species is commonly found outdoor because it is an exophagic and exophilic species (Aiman et al., 2016).

2.1.3 Biology of *Aedes aegypti*

Aedes (*Stegomia*) *aegypti* (Linnaeus) is the popular species that is also known as yellow fever mosquito. This is due to the potential of this species in becoming the vector for yellow viral fever. According to previous study, Africa is the native habitat of *Ae. aegypti* and now it is expanded worldwide especially in tropical and subtropical areas (Mousson et al., 2005). There are two groups of *Aedes aegypti* form such as domestic and sylvan form. Sylvan form is the ancestor of the domestic form because it is more rural while domestic form prefers the urban areas (Paupy et al., 2008).

The *Ae. aegypti* sometimes show colour tone variation such as light brown (Carvalho & Moreira, 2017). Dorsal surface of the thorax has a pair of longitudinal stripes with a pair of white scales in lyre or violin pattern (Plate 2.1). Commonly, black with white alternating patterns cover the whole body of this species (Zettel Nalen & Kaufman., 2008).



(a) (b)

Plate 2.1: Different thorax morphology between *Aedes sp*. (a) *Ae. albopictus*, and (b) *Ae. aegypti*

2.1.4 Life cycle of *Aedes sp*

Mosquitoes undergoes four life stages in life which are egg, larva, pupa and adult. Hence, it is known as holometabolous insect which go through a complete metamorphosis (Denysiuk et al., 2015). This most successful adaptation is great in inducing less competition for resources within each of the life stages (Wang et al., 2019). The life cycle of mosquito may take about seven to ten days for an adult emerge from egg. The breeding site of *Aedes sp* are either natural breeding sites such as as bamboo trunks or in artificial container breeding sites like tires, pots, vases and cans. Prior to oviposition adult female must take a complete blood meal to ensure sufficient amount of iron and amino acids are delivered to the egg development. Estimated number of eggs produced in one batch of complete blood meal of female mosquito is around 100 to 200 eggs (Miesfeld, 2009). These mosquitoes can produce up to five batches of eggs during their life span (Muktar et al., 2016).

The eggs of *Aedes sp* are laid singly, however, the female will spread their eggs randomly in the breeding sites with water sources. Eggs surface are smooth and in ovoid shape, approximately one millimetre long. The eggs are said to be last for a year because the outer layer of the egg is covered with an outer shell known as chorion. It acts as a place of gas exchange site and also protect the eggs from loss of water (Mundim-Pombo et al., 2021). Initially the deposited eggs are white in colour. However, in one minute of time it will quickly change its colour into shiny black. The hatching rate is totally depending on the environment. If the environment is favour to the eggs then it will hatch to become larval in two days. Meanwhile, the eggs will enter dormant stages if the condition is unfavourable. Optimal temperature limit for embryogenesis ranged from 16°C to 31°C with a relative humidity of 80%. The eggs will hatch once it is submerged in water but it can withstand the desiccation up to months. Hence, it is very hard to control the *Aedes sp* population (Muktar et al., 2016).

After the egg hatching process, larva will develop into four stages of instars where it will last for five to seven days. Larva undergoes moulting process that enable them to develop into next instar. The size of the larva increases continuously (Zettel Nalen & Kaufman., 2008). Larval stages will only survive in presence of aquatic environment. Tiny organisms and algae in the breeding sites are the main food sources for larvae. Their fan-like mouth brush is essential in filtering the food sources. Adequate amount of food and optimum temperature in each of the larval stage will ensure the survivability of the larva (Posidonio et al., 2021). The wiggle movement of larva takes place when they feel disturbed during resting on the water surface. Larval posterior end posse siphon for respiration process is hanging upside down and vertically during larval resting period (Lee et al., 2017).

Following larval stage, the next stage in *Aedes* life cycle is pupal stage. This is an active inactive stage because pupal stage is a non-feeding stage. Anyhow, the pupa still highly response to the external stimuli such as light and vibrations by swimming actively in the water. This pupal stage will last for approximately two days. Whenever the pupa detects the external disturbance, it will swim quickly to the bottom of water surface to protect themselves. A pair of trumpets in pupa used to facilitate their respiration. Commonly, pupa prefers to stay at the water surface so that it can quickly escape from water surface once metamorphosis into adults (Zettel Nalen & Kaufman., 2008).

Final stage in the life cycle is adult stage. The adult will quickly escape to a dry place to feed on sugar source. Teneral period is a physiological state where sexual maturation and exoskeleton hardening process took place with 24 hours of adult emergence. The female *Aedes sp* have a long-life span compared to the male (Zettel Nalen & Kaufman., 2008).

2.2 Feeding behavior of *Aedes sp*

2.2.1 Larval feeding

The larvae of *Aedes sp* fed on microorganism and suspended organic matter in the aquatic environment through filter-feeding. Sometimes, they may demonstrate scavenging behaviour because it feed on the carcasses. Hence, the larvae are categorized under omnivorous feeding behaviour (Ponlawat & Harrington, 2005).

2.2.2 Adult sugar feeding

All adult mosquitoes depend on sugar feeding for survival and reproduction because it gives them the energy to fly, body development and mating process. Approximately 22 to 24 hours following adult emergence, sugar-feeding takes place right away prior to females reacting to host cues (Foster and Walker, 2019).

In order to acquire the carbohydrates sources, both male and female mosquitoes ingest sugar meals. Flying insects, especially mosquitoes, mostly obtain their energy from variety of sources, such as fruits, plant nectar, and other liquids that contain sugar. The simple sugars fructose and glucose as well as the disaccharide sucrose are produced when mosquitoes consume plant nectar. These simple carbohydrates used right away or they can be transformed into storage molecules like trehalose, glycogen, or triacylglycerols. The muscles and liver of the mosquito can store glucose polymers called glycogen. Trehalose, a disaccharide of two glucose molecules, plays a significant role in insects' storage of carbohydrates. Lipid molecules called triacylglycerols can be produced from an oversupply of carbohydrates and stored in adipose tissues. When energy demands are high or fasting periods are approaching, these store molecules can be broken down and used as energy sources (Jessica, et.al. 2019).

2.2.2 Adult blood feeding

Anautogenous female mosquitoes need a blood meal to finish the development process of their matured eggs. Female mosquitoes need complete blood meal to finish the gonotrophic cycle and it is greatly influenced by the females' age, circadian rhythm and nutrient content in its body. The eggs in ovary started to mature after intake of blood. During this period, the female mosquitoes stop its feeding process and remain inactive until the eggs oviposited (Hill and Ignell, 2021).

2.3 Sensory system of mosquitoes in nectar-foraging behavior

According to previous studies, there are three sensory systems in mosquitoes that facilitate their nectar-foraging behaviour. The systems involved are olfaction, gustation and vision. Olfaction is crucial in locating the nectar from natural resources (Barredo & DeGennaro, 2020). Various number and type of sensilla found on their olfactory appendages such as labials, maxillary palps and antennae. It depends on the mosquito species and their current stage of development. In the beginning, the odorant will diffuse through countless pores on the sensilla surface. Then, the absorbed odorant enters aqueous lymph to contact with the spectrum of molecular receptors on dendrites of olfactory receptor neurons (Montell and Zwiebel, 2016). Odorant receptors (OR) and obligate co-receptors (OCRO) are responsible in identifying plant-related volatiles (Barredo & DeGennaro, 2020).

In mosquito gustation system, the tarsi and proboscis of insects include gustatory hairs are very important. The unique bristles formed by insect taste receptor cells and their support cells. Moreover, male and female reproductive organs along with several other organs covered with insect gustatory receptors which function as sensors of nutritional and metabolic state (Montell and Zwiebel, 2016).

Apart from that, the adult mosquito visual organs consist of compound eyes with each ommatidium having eight photoreceptor cells and 200–300 ommatidia in overall (Montell & Zwiebel, 2016). Opsins are universal photoreceptor molecules present in visual systems. Opsins may switch from resting to signalling when exposed to light, activating the G protein and starting a signal transduction cascade that causes physiological reactions (Shichida and Matsuyama, 2009). The genomes of the mosquitoes include an exceptionally high number of opsin genes. For example, the genome of *Ae. aegypti* encodes 10 opsins, while the genome of *Anopheles gambiae* is anticipated to contain 11 opsins (Montell and Zwiebel, 2016).

All of these three cues are integrated with one another (Figure 2.1). For example, mosquitoes can identify flowers from a distance by utilizing their visual and olfactory organs. The antenna and maxillary palp express olfactory receptors, which mediate attraction reactions to specific plant volatiles. When sugars and other substances are near a plant, it encourages nectar eating response (Roth et al., 2021).

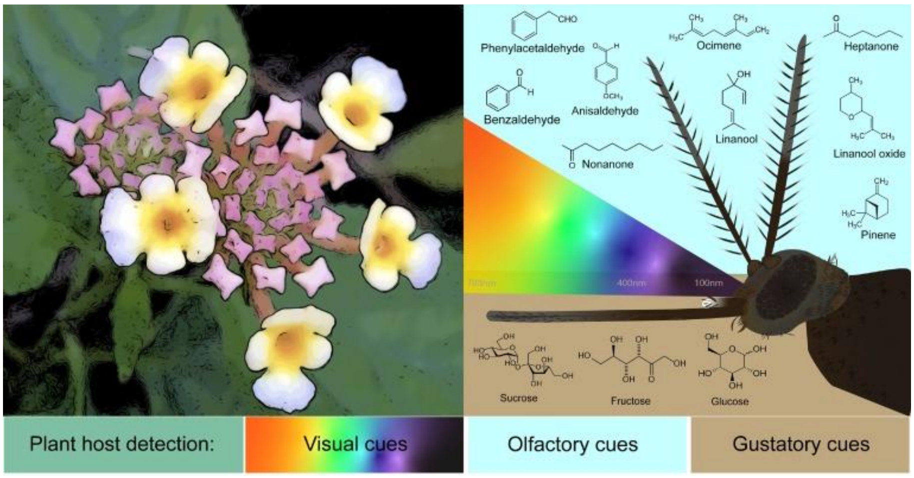


Figure 2.1 Multimodal Attraction of Mosquitoes to Nectar Sources (Roth et al., 2021).

2.4 Role of *Aedes sp* as a vector

Human illnesses caused by parasites, viruses, and bacteria that are spread by vectors are known as vector-borne diseases. Diseases include malaria, dengue, schistosomiasis, human African trypanosomiasis, leishmaniasis, Chagas disease, yellow fever, Japanese encephalitis, and onchocerciasis cause about 700,000 deaths annually. Thus, mosquitoes are one of the main biological vectors which enhance parasites grow or proliferate within them (WHO., 2020).

2.4.1 *Aedes* borne diseases

For the past 50 years, the *Aedes*-borne disease has been a major global concern, infecting approximately 390 million people annually (Table 2.1). Severe cases caused by *Aedes* mosquitoes cause death (WHO., 2021). When an infected mosquito bites a healthy person or animal (both domestic and wild), the transmission spreads. Humans and mosquitoes may propagate the infection, and humans and animals can also susceptible the infection from one another (World Health Organization, 2020)

Table 2.1 Summary of *Aedes*-borne diseases

***Aedes*-borne diseases**	**Dengue Fever**	**Yellow Fever**	**Zika**	**Chikungunya**
Symptoms	Fever, frontal headache, body aches, nausea and vomiting, joint pains, weakness, and rash	Fever, chills, intense headache, back pain, aches all over the body, nausea, vomiting, lethargy, and exhaustion	Rashes, fever, conjunctivitis, muscle and joint discomfort, malaise, and headache that last from 2-7 days	Muscle pain; joint swelling; headache; nausea; fatigue, and rash.
Pathogen and Parasite	DENV-1, DENV-2, DENV-3, DENV-4, Flaviviridae	Yellow fever virus, Flavivirus	Flavivirus, Zika Virus	Togaviridae, Chikungunya virus
References	(Akram *et al*., 2021; Ooi & Gubler, 2011)	(Centers for Disease Control and Prevention., 2024)	(World Health Organisation, 2022)	(World Health Organisation, 2017)

2.5 Microbial communities in mosquitoes

Microbiome are bundle of microorganisms that colonize internal or external organ of a living organism. All of the organisms have a complex and rich microbial diversity (Dharne et al., 2006). It has been discovered that the microbiome of mosquitoes differs in a variety of tissues, such as the cuticle surfaces, reproductive tracts, salivary glands, and the midgut (Cansado-Utrilla et al., 2021; Segata et al., 2016). These microbial populations in many tissues influence mosquito-borne illnesses directly or indirectly. Changes in vectorial competence or capacity of mosquitoes to sustain a pathogen-induced infection are referred to as direct effects. While the majority of indirect effects are caused by vectorial capacity, which is the average number of pathogens a population of vectors inoculates into a host within a given amount of time. The mosquito microbiome had an impact on number of characteristics that indicate vectorial capability, including pathogen extrinsic incubation duration, vector survival rate, vector density, and vector bite rate (Ma et al., 2016).

According to Scolari et al., (2019), Gammaproteobacteria was observed as predominant taxon in the salivary glands of female *Ae. albopictus* mosquitoes. But, the salivary gland of *Ae. aegypti* is dominated by species from *Burkholderia, Pantoea, Acetobacter, Sphingomonas, Cupriavidus,* and *Escherichia–Shigella* genera. These results suggested that *Ae. albopictus* and *Ae. aegypti* have different microbial compositions in their salivary glands with *Ae. aegypti* showing more diverse and richer microbiome in this specific organ (Scolari et al., 2019).

According to previous studies, the microbiomes have a tissue-specific tropism where they express their preference for particular organs. One significant finding is that the microbiota presents in the female and male mosquito reproductive organs are more diverse than the bacteria discovered in the gastrointestinal tract and salivary glands. For example, almost 30% of bacteria in the reproductive organs of *Ae. aegypti* are Alphaproteobacteria (Mancini et al., 2018).

2.6 Significance of microbiome in vector competency

Mosquitoes vector competence or capacity to spread pathogens can be influenced by the makeup and variety of its body microbiota. Numerous studies and reviews have clarified the importance role of gut microbiota in altering the ability to cause infections. For instance, a research had demonstrated that the presence of *Enterobacter* bacteria in the midgut of *Anopheles* mosquitoes prevent the growth of *Plasmodium* parasites in it. Thus, we can control malaria disease (Dennison et al., 2016). In addition, *Aedes* mosquitoes carrying Wolbachia can considerably lower their ability to transmit arboviruses such as the Zika virus (Caragata et al., 2016). Contamination with Wolbachia triggers number of actions that limit the virus capacity to multiply and propagate inside the mosquito body (Dutra et al., 2016). The bacteria restrict the parasite growth before they reach the midgut epithelium. Overall, there is an opportunity of modifying the vector competency and inhibit the spread of pathogens by altering the function and structure of microbiota (Cirimotich et al., 2011).

2.7 Natural sugar variants

Many mosquito species have been shown to exhibit genotypic and metabolic resistance to common insecticides such as pyrethroid, organophosphate, and organochlorine as a result of abuse (Hardy Abdullah et al., 2021). The creation of pesticides based on plant volatiles has increased as a result of this serious scenario. Plant extract can be added to the bait as an attractant or repellent in addition to its insecticidal properties. Several plant extracts from diverse plant components were employed in earlier research, and their effectiveness as an attractant for adult *Ae. aegypti* was evaluated. The parts that have been selected can originate from the plant's fruits (Von Oppen et al., 2015), flowers (Sissoko et al., 2019), peel (Nur Athen et al., 2020), fruit seed (Briones et al., 2012), and infusion (Benelli & Govindarajan, 2017; Velan et al., 2021).

2.7.1 Fruit based sugar

The mango, or *Mangifera indica*, is a well-known native fruit tree in South-Eastern Asia. It is a member of the Anacardiaceae family and is classified in the genus Mangifera (Lizada, 1993; Morton, 1987). The mango tree, which typically reaches a height of 15 to 18 meters with a circular and broad canopy, is thought to be an evergreen fruit tree that grows quickly (Morton, 1987). Nonetheless, in tropical climates, mango trees can reach heights of 30 to 40 meters however in subtropical climates their height is limited. Mango trees have evergreen leaves that are lanceolate in shape. Young leaves can be in pink, deep rose, wine-red, or yellow-green in colour, while mature leaves are deep green in colour (Lauricella et al., 2017). The topmost portion of the mature leaves have a shiny top surface (Morton, 1987). Based on the mango fruit morphology, the fruit skin has a leathery texture and can be reddish pink or light green in color. The fruit emit a nice fragrance as it ripe. Ripe *M. indica* fruits are abundant in provitamin A, vitamin B1, and vitamin C. In addition, there is a high concentration of micronutrients like vitamins and minerals as well as macronutrients like lipid, amino acid, carbs, protein, fatty acids, and other organic acids (Battistelli et al., 2019). Furthermore, *M. indica* fruits are abundant in dietary fibre and fermentable sugars. It is interesting to note that they even include non-nutrient substances such volatile chemicals, flavonoids, phenolic compounds, and other polyphenols (Ajila et al., 2007). Additionally, according to Maldonado-Celis et al. (2019), the ripened *M. indica* contain roughly 15% of the overall sugar content. Thus, it is a prime candidate for attractant bait since mosquitoes use sugar as their main food source (Sissoko et al., 2019). Next, according to Fowomola (2010), the amino acids found in *M. indica* may serve as an additional attraction for *Aedes spp*. The concepts are based on the knowledge that *M. indica* contains protein, certain other nutrients, and amino acids (isoleucine, lysine, phenylalanine, and tyrosine) that are necessary for *Aedes* species to perform their physiological functions (Maldonado-Celis et al., 2019).

Apart from mango, flesh of *Carica papaya* (Papaya) and *Cucumis melo* (Honeydew melon) showed to be attractive to *Ae. aegypti* (Sissoko *et al.*, 2019). It was remarkable that the papaya fruit peel from *C. papaya* could draw adult *Ae. aegypti* in both choice and no-choice tests (Nur Athen *et al.* 2020). Müller et al., (2008) conducted experiment using decaying nectarine (*Prunus persica var. nectarina*), guava and honeydew melon juice in one of their studies. They attempt to minimize the *Culex pipiens* population by applying the solution to the non-flowering vegetation near a sewage treatment pond. Additionally, they attempted to attract *Anopheles claviger, Anopheles sergentii,* and *Aedes caspius* in an oasis.

2.7.2 Flower based sugar

One of the major energy sources that mosquitoes can readily obtain in the outdoors is the nectar of flowers (Barredo & DeGennaro, 2020). Following this, the blooming segment of plant enable mosquitoes to either attract or repel by the nectar source. Based on Sissoko et al., (2019), blooming branches of various flowering plant species attract both male and female *Ae. aegypti*. For examples, *Acacia macrostachya, Acacia salicina, Galphimia gracilis,* and *Prosopis juliflora*. However, *Lantana camara* and *Bougainyillia glabra* did not draw adult mosquitoes. Conversely, it has been demonstrated that the scent and nectar of *Leucanthemum vulgar* commonly known as

Ox-eye daisy had been demonstrated to be alluring to *Ae. aegypti*. The flowering plant *Chrysanthemum indicum*, occasionally referred to as Indian chrysanthemum, is a member of Asteraceae family. This species originating from East Asia has been cultivated and valued for both its eye-catching and therapeutic qualities (Britannica, 2023). The pyrethrin extracted from this flower has a fatally poisonous impact on insects by acting on their neurological systems (Bowman et al., 2018).

2.8 Current application of mosquito control

Since mosquitoes can spread disease, controlling mosquito populations is a major public health initiative in many different countries. Effective strategies of vector control are necessary for mosquito-borne disease, in order to minimize expenses and time waste. Chemical, biological, genetic and mechanical and physical controls are the current mosquito control methods in application.

2.8.1 Chemical control

The application of any chemical pesticide to eradicate the insect and its vector populations is known as chemical control. There are two types of insecticides such as natural and synthetic (manmade) sources.

Mineral oils and plant-based chemical are examples of natural insecticides that have been used to control mosquitoes. Bioinsecticides originate from natural sources including pheromones and the bioactive substances found in plants, as well as microorganisms like bacteria, fungi, viruses, and protozoa. Based on the methods by which they were made, phytochemicals, microbial pesticides, plant-incorporated protectants (PIPs), and pheromones are the four main categories of bioinsecticides (Chengala & Singh, 2017). As good substitutes for synthetic substances, they are less hazardous, target-specific, extremely effective in small quantities, and biodegradable.

Also referred to as "phytochemicals," these physiologically active substances operate as toxic substances, growth regulators, feeding deterrents, and repellents on insects (Tyagi., 2016).

An efficient plant-based insecticide can be made from a variety of plant parts, including the entire body of small herbs, leaves, roots, stems, seeds, barks, fruits, fruit peels, and resin (Shaalan et al., 2005). A phytochemical activity differs depending on the type, age and element of plant and the species of mosquito. Phytochemicals influence insect physiology and target essential cell components to demonstrate their effects. The effect of phytochemicals is blocking calcium channels, activating nicotinic acetylcholine receptors, triggering octopamine receptors, disrupting sodium-potassium ion exchange and nerve cell membrane action (Souto et al., 2021). Moreover, it also inhibits Acetylcholinesterase inhibitors and Gamma-aminobutyric acid -gated chloride channel function (Sharma et al., 2006). Additionally, phytochemicals have the ability to disrupt mosquito midgut epithelial cells and have an impact on metamorphosis (Al-Mekhlafi., 2018).

Natural plant oil known as citronella (3, 7-dimethyloct-6-en-1-al) is derived from various kinds of Cymbopogon lemongrasses. Citronella can be added to candles and flame pots in concentrations ranging from 0.5% to 20% as a lotion, oil, or solid wax. Citronella has a brief period of effect due to its high volatility, yet it can prevent bothersome mosquito bites for up to two hours (Carroll & Loye, 2006).

Mosquito control operations primarily employ four groups of insecticides which are pyrethroids, organochlorines, organophosphates, and carbamates. The majority of synthetic pesticides mostly target insect central nervous systems and have cause effect on mosquito physiology or behaviour (WHO., 2018).

Firstly, pyrethroid group insecticides such as permethrin, cypermethrin, deltamethrin and lambda-cyhalothrin are commonly used in chemical control strategies. They are usually used to make insecticide-treated mosquito nets and to perform indoor residual spraying (WHO., 2020). Pyrethroids are less toxic to mammals but it causes a harming and repellent effects to the mosquitoes (Soderlund, 1989). Working mechanism of pyrethroid is by blocking voltage-gated sodium channel in neuronal membranes. Pyrethroids bind to open channels and inhibit them from closing, which might cause an action potential to last longer or interfere with the nervous system ability to send electrical signals (Narahashi.,1996). This results in the insect's constant nerve stimulation, paralysis and cause death (Bowman et al., 2018). Organochlorines also block the voltage-gated sodium channels of insects. Muller is the scientist that found dichlorodiphenyltrichloroethane (DDT) in 1939 to reduce the occurrence of malaria, marine typhus, and other diseases spread by mosquitoes (Talapko *et al*., 2019). The DDT is the most widely employed in residual spraying. It is a good chemical for indoor wall spraying because of its low cost and great efficacy. However, the harmful effects of DDT on people and non-target organisms, along with the resistance that some mosquito species have acquired to it, have prohibited its use (Eskenazi et al, 2009). However, DDT bioaccumulates in food chains and human fat, it was outlawed in United States in the 1972s. But, since DDT is cheap and effective as a pesticide, some nations continue to use it to minimize their production expenses (Merhaby et al., 2019).

Organophosphate such as malathion and fenitrothion came into use in the 1944s (Faiz et al., 2020; Woodrow et al., 2019). The organophosphate has an impact on mosquito oral and cutaneous tissues of mammals. Thus, the usage of this harmful chemical may disrupt the endocrine system which is mediated by Acetylcholinesterase (AChE) suppressors. Furthermore, it might cause bioaccumulation in the food chains, which would be detrimental to the organism at the top of the food cycle impacted by the accumulation of toxins (Ding *et al*., 2020). Apart from that, carbamates are a common insecticide used in homes, gardens, and agriculture to manage pest insects. Examples of carbamates are propoxur and bendiocarb. Carbamates work similarly to the organophosphate in that they inhibit AChE and cause comparable symptoms following to the exposure. In contrast to organophosphate, its impact on the human system exhibits a quick recovery pattern. It results from the acetylcholinesterase enzymes capacity to disintegrate eventually.

2.8.2 Biological control

Biological control is a viable way that can suppress the population of specific pest organisms by utilizing other living things, in addition to chemical measures. It functions by bringing in natural enemies to eradicate pests or preserving the present natural enemies (Plouvier & Wajnberg, 2018). The command Natural pathogens, parasitoids, parasites, and predators are examples of biological agents in the environment, which will eradicate the intended pest by invading their habitat (Rossbacher & Vorburger, 2020).

The introduction of predatory organisms has been crucial to the biological control of mosquito populations. For instance, the utilization of the mosquito fish, *Gambusia affinis*, dates back more than a century, and it has demonstrated a high degree of compatibility with the concurrent use of other chemical or biological control agents (Ec, 1967). They are appropriate to use with chemical control methods since they are adaptable to insecticides, especially larvicides. Furthermore, mosquito control can be further maintained through the combination of *G. affinis* with additional biological control agents. They are comparatively having high survival rate, low reproductive rate and able to flourish in permanent water bodies (Ns et al., 1991). The *G. affinis* has been shown to be useful in the temporary extinction or suppression of specific mosquito species. A study conducted in Taiwan demonstrated the use of *G. affinis* to manage arboviral infections including dengue. They were released in artificial water containers in household. This method produced a lower container index for *Aedes* species mosquito larvae, particularly *Ae. aegypti* and *Ae. albopictus* (Wang et al., 1990).

In the past, it has been documented that larvae of several species of mosquitoes in the genus *Toxorhynchites* prey on larvae of other mosquito species. Since *Toxorhynchites* lack the need for blood feeding, they are an excellent mosquito species to release into the environment for biological control without raising the danger of disease transmission. Examples of *Toxorhynchites* larvae that have been used were *Toxorhynchites splendens*, *Tx. brevipalpis*, *Tx. moctezuma*, and *Tx. rutilus* (Sherratt & Tikasingh, 1989). Nevertheless, due to predation occurs at the larval stage, the population of prey and *Toxorhynchites* species must cohabit in the same water reservoir. For instance, it has been discovered that *Tx. splendens* prefers to oviposit in man-made containers with larger apertures than in small breeding habitats. Thus, the eggs emerge into larval stage and starts to fed on the *Aedes* larva (Mohamad & Zuharah, 2014).

Bacterial endotoxins were discovered to be an efficient mosquito control measure for more than thirty years ago. The toxins generated by *Bacillus thuringiensis israelensis* and *Lysinibacillus sphaericus* have the added benefit of being extremely powerful larvicides for variety of arbovirus vector species. The *Lysinibacillus sphaericus* toxicity is mostly restricted to *Culex* and *Anopheles* species mosquitoes. Meanwhile, *Bacillus thuringiensis israelensis* toxicity is generally effective against medically significant mosquitoes in the genera of *Aedes, Culex,* and *Anopheles* (Bellows & Fisher,1999). Furthermore, it has been established that certain mosquito species, including *Aedes aegypti*, *Culex quinquefasciatus* and *Culex tarsalis Coquillett* are at risk from these bacteria. They are able to significantly reduce the mosquito population (Alves *et al.*, 2020).

The principle of entomopathogenic fungus usage for mosquito control was introduced initially on *Anopheles gambiae* by using fungus from genus of *Coelomomyces* (Muspratt,1963). Fungal agents can also be successfully used as a biological control method against mosquitoes by altering the mosquito physiology, which reduces the possibility of blood feeding and eventually blocking disease transmission. For instance, contamination with *Beauveria bassiana* lowers the reproduction rate, survivability and blood feeding frequency of *Ae. aegypti* (Darbro et al., 2012).

2.8.3 Genetic control

Idea of replacing the natural population of competent mosquitoes with genetically modified mosquitoes is the foundation of the genetic control theory (Higgs, 2013). The sterile insect method (SIT), which obstructs the mating process in insemination phase, is one excellent example for genetic control. This SIT method causes lethality at earlier of embryo stage. Release of radioactively sterile male carry sperm with chromosomal damage. This male able to mate with females but are unable to effectively produce viable offspring. Thus, the population of mosquitoes turn to be decreasing in number. However, systematic release spots and mass sterilization of male mosquitoes are essential to achieve the reduction aim of SIT (Lacroix et al., 2012).

In order to combat mosquitoes carrying viruses, a new version of SIT has been successfully designed to target *Ae. aegypti* (Phuc et al., 2007). In order to inhibit the emergence of viable progenies, "Release of Insects with Dominant Lethality" (RIDL) was introduced. In this method, male transgenic mosquitoes harboring the dominant lethal gene are released into vector populations to stimulate the expression of the