Effect of *Pithecellobium dulce* extract on vector competence of *Aedes aegypti* to chikungunya virus

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Reg. No: I56/8631/2017

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF THE DEGREE OF MASTERS OF SCIENCE IN MEDICAL AND VETERINARY ENTOMOLOGY

FACULTY OF SCIENCE AND TECHNOLOGY

DEPARTMENT OF BIOLOGY

UNIVERSITY OF NAIROBI

NOVEMBER 2021.

DECLARATION

I testify that this thesis is my original work and has not been presented for examination or award of a degree in any other university. Where other people's work has been used, it has been properly acknowledged and referenced in accordance with the University of Nairobi requirements.

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To my loving mother, Ms. Lilian Muhambe Chahilu, my husband Mr. Lawrence Shivairo Misango and our adorable daughter Aviv Aryel Khasoa

ACKNOWLEDGEMENT

My sincere appreciation goes to CAP Africa-Norad project for funding this study and the International Centre of Insect Physiology and Ecology (ICIPE) through the Dissertation Research Internship Programme (DRIP) for hosting and according to me the chance to pursue this study. Special mention of the University of Nairobi (UoN) for the registration and the award of the UoN Scholarship Award that sponsored my tuition fees. My perpetual gratitude goes to my supervisors; Dr. Eunice Owino and Dr. George Ongamo (UoN) and Dr. David P. Tchouassi, Prof. Rosemary Sang and Prof. Baldwyn Torto (ICIPE), for the tremendous intellectual, technical and moral support that I received during the course of study. I am deeply indebted to all of you. I owe special gratitude to several colleagues who went out of their way to assist me achieve my objectives. Special thanks go to Dr. Xavier Cheseto, Dr. Trizah Milugo, Mr. Benson Limo, Ms. Joan Lichoti (ICIPE), Dr. Edith Chepkorir, Ms. Hellen Koka and Ms.Yvonne Kimata (KEMRI) who provided tremendous technical support. More specifically, I am thankful to members of Behavioral and Chemical Ecology Unit (BCEU) and the Martin Luscher Emerging Infectious Disease (MLEID) laboratory, ICIPE. My heartfelt gratitude goes to all my friends and family members who have contributed morally and materially for the successful completion of this thesis. More specifically is to my husband, Mr. Lawrence Misango and our adorable daughter Aviv Aryel for being a great pillar in completion of this study.

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ABBREVIATIONS AND ACRONYMS

ATSBs	Attractive targeted sugar baits
CHIKV	Chikungunya virus
CHIK	Chikungunya disease
CDC	Center for Disease Control
CI	Confidence interval
CPE	Cytopathic effects
DALYs	Disability adjusted life years
DENV	Dengue virus
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic acid
DMSO	Dimethyl sulfoxide
ECSA	East Central South Africa lineage
ECSA-IOL	East, Central and South Africa-Indian Ocean Lineage
EIP	Extrinsic incubation period
GIS	Geographic Information System
GC-MS	Gas chromatography-mass spectrometry
GC-EAD	Gas chromatographic-electroantennographic detection
HEPA	High efficient particulate air
LC-QqQ-MS	Liquid chromatography triple quadrupole tandem mass spectrometry
ICIPE	International Centre of Insect Physiology and Ecology
IOL	Indian Ocean Lineage
KEMRI	Kenya Medical Research Institute
JAK-STAT	Janus kinase signal transducer and activator of transcription
LD	Light and Day
LC-MS	Liquid chromatography-mass spectrometry
MEB	Midgut escape barrier
MIB	Midgut infection barrier
NACOSTI	National commission for science, technology and innovation
NIST	National Institute of Standards and Technology
NHPs	Non-human primates
NSAIDs	Non-steroid anti-inflammatory drugs
PAHO	Pan-American Health organization
QTI	Quantitative trait loci
SGIB	Salivary gland infection barrier
SIT	Sterile insect technique
VC	Vectorial capacity
WHO	World Health Organization

ABSTRACT

Chikungunya virus (CHIKV) is among re-emerging arboviruses that affect human health globally. The spread has been associated with lack of sustainable vector control and viral preventive measures. Studies of the biology and ecology of the key vector, *Aedes aegypti* can open avenues for control of this virus. Despite the increasing evidence linking plant feeding to the survival and pathogen transmission dynamics as observed in the *Anopheles*-malaria parasite system, little is known as to whether plant feeding can influence pathogen-*Ae. aegypti* interaction. This study aimed to determine the effect of *Pithecellobium dulce*, a host plant for this vector, on the competence of *Ae. aegypti* to CHIKV. Adult *Ae. aegypti* females fed orally on dimethyl sulfoxide (DMSO) extracts of *P. dulce,* reduced female survival in a dose-dependent manner ($P<0.0001$). Chemical analysis of pools of midgut content after ingestion of the plant extract detected by coupled liquid chromatography triple quadrupole tandem mass spectrometry (LC-QqQ-MS) and coupled gas chromatography-mass spectrometry (GC-MS) identified several plant metabolites namely the amino acid proline, the flavonoid glycoside kaempferol 3-O-rhamnoside, the sterol β sitosterol and the fatty acid linoleic acid. Further, the females were orally exposed to a CHIKV infectious blood using a membrane-feeding assay before and after feeding on an optimal survival dose of the plant extract. Virus infection in the mosquito vector was determined by plaque assays. Highly significant infection and dissemination rates and respective mean titers were observed in the control and post-exposed (mosquitoes fed on glucose solution then the plant extract) treatment ($P<0.001$). No significant effect was observed in mean titers of the control and the pre-exposed (mosquitoes fed on plant extract then glucose solution) cohort ($P<0.001$). Although there was no observed significant difference while using either frozen or freshly cultured virus, transmission, which is a measure of vector competence, was only observed in the freshly cultured virus type. The pre-exposed, control and pre + post-exposed treatments recorded transmission although with significantly reduced titer in the latter. The post-exposed treatment recorded no transmission further suggesting possibility of *P. dulce* activity. These results demonstrate that *Ae. aegypti* feeding on this plant i) influences its survival, ii) leads to ingestion of secondary metabolites and iii) modulates infection success to chikungunya virus. The known anti-pathogenic effect of the identified metabolites suggests the potential impact on virus transmission occurs through reduced virus titers, thus these findings open a novel avenue towards the development of antiviral strategies targeting vector plant feeding behavior.

CHAPTER 1.0: INTRODUCTION

1.1 Background

Chikungunya (CHIK) is a mosquito-borne viral disease first identified during the 1952-53 outbreak in Tanzania (Robinson, 1955). The name chikungunya which means "that which bends up" describes the stooped posture of infected patients suffering from severe arthralgia besides the abrupt onset of fever and rash (Thiberville *et al*., 2013). Even though infection in humans is self-limiting and acute symptoms resolve within 5-7 days, chikungunya virus (CHIKV) is recurrent in 30-40% of infected patients and may persist for years, impacting productivity (Owino, 2018; Schwartz and Albert, 2010a). Since its discovery, sporadic outbreaks of CHIK have been reported in Africa and Asia (Powers and Logue, 2007).

In Kenya, chikungunya virus emerged in Lamu Island in 2004 before spreading to Comoros and La Reunion Islands, India and South East Asia infecting millions of people and causing severe cases of the disease and deaths (Sergon *et al*., 2008, 2007; Renault *et al*., 2007). These outbreaks resulted in importation of the virus in Europe and America in 2007 and 2013 respectively (Watson, 2007;Yactayo *et al.,* 2016). Currently, cases of CHIKV have been reported in over 60 countries globally with Asia and America being the most affected (WHO, 2020). The virus re-emerged in 2016 in Mandera County with reports of over 1792 cases (Konongoi *et al*., 2018), followed by an outbreak in Mombasa County in 2017-2018 involving a novel CHIKV strain (Eyase *et al*., 2020). The most recent outbreak was reported in Hagadera, Garissa County where 109 cases were recorded (WHO, 2020). In addition to the aforementioned outbreaks, sero-prevalence studies have

shown evidence of CHIKV transmission in western Kenya among asymptomatic children (Nyamwaya *et al*., 2021; Grossi-Soyster *et al*., 2017; Mease *et al*., 2011).

Global expansion of CHIKV is instigated by various factors including absence of licensed vaccines and antiviral drugs (Gorcha *et al*., 2014) and extensive geographic spread of the principal vectors *Aedes aegypti* and *Aedes albopictus*. These factors are fueled by globalization of trade and travel and pronounced competence of these vectors in transmitting the virus (Tatem *et al.*, 2006). The risk of transmission of CHIKV however, varies at both local and global scales (Moore *et al*., 2018; Staples *et al.*, 2009). For instance, in Kenya, while human infections and resultant outbreaks are endemic at the Coastal and Northeastern region, not every region is equally affected. This underscores the need for vector competence studies as an important epidemiological risk factor for spread and establishment of CHIKV.

Vector competence is a complex phenotypic trait determined by both biotic and abiotic factors (Lefèvre *et al.*, 2013b). An example of a biotic factor is plant nutrition, which is an understudied in regards to the biology of *Ae. aegypti.* As such, studies in this area may open avenues for control of this vector. While mosquitoes primarily depend on plants for sugars, this essential behavior exposes mosquitoes to a range of plant-produced substances which may potentially influence vector survival, and pathogen transmission dynamics (Cory and Hoover, 2006). For instance, whereas feeding on *Parthenium hysterophorus,* a preferred host plant for the malaria vector *Anopheles gambiae* enhances survival of the vector, its key secondary metabolite parthenin, a sesquiterpene lactone blocks transmission of the malaria parasite *Plasmodium falciparum* (Balaich *et al.*,2016; Manda *et al.*,2007). On the contrary, plant feeding on the invasive shrub *Prosopis juliflora* enhances the malaria transmission potential of *Anopheles* mosquitoes (Muller *et*

al., 2017). Likewise, plant feeding influences the level and transmission of *Leishmania* parasites by sand flies (Schlein and Muller, 2004). However, beyond a few studies linking plant feeding to its survival and reproductive fitness (Nyasembe *et al*., 2021), little is known about the influence of plant feeding on pathogen transmission success in the Aedes-virus interactive system.

Previously, a high degree yet selective plant feeding was observed in nature in both sexes of *Ae. aegypti* amongst them *Pithecellobium dulce* (Nyasembe *et al*., 2018; Olson *et al*., 2020; Nyasembe *et al.*, 2021). *Pithecellobium dulce* is a perennial evergreen tree indigenously grown in America and is cultivated in the Coastal region of Kenya (Srinivas *et al.*, 2018). Locally, *P. dulce* is known by a swahili word "Mkwaju" meaning "Tamarind tree". Phytochemical analysis of different parts of *P. dulce* has revealed the presence of various compounds including alkaloids, tannins, flavonoids, glycosides and triterpenoids (Srinivas *et al*., 2018). For example, the leaves have been reported to possess astringent, emollient, and antidiabetic properties with metabolites such as afzelin, dulcitol and quercetin identified in subsequent studies (Vanitha and Manikandan, 2016). The preference of *Ae. aegypti* for this plant has largely been attributed to plant sugar content and volatile profile (Nyasembe *et al*., 2018) and perhaps the presence of plant metabolites whose role in pathogen transmission dynamics is unknown. Thus, in this study we evaluated the effect of *P. dulce* extract on survival and competence of *Ae. aegypti* to CHIKV.

1.2 Statement of problem

Chikungunya is a re-emerging mosquito borne viral disease of immense public health importance globally. The disease is characterized by both large and small-scale outbreaks as well as inter-epidemic infections that are of social, health and economic concern. The extensive global expansion of CHIKV is due to absence of licensed vaccines and sustainable vector control measures.

Increasing evidence linking plant-nectar feeding to aspects of mosquito vectorial capacity such as increase in survival and fecundity and reduction of biting frequency provides insights into plant-vector interactions that could open avenues for their control (Gu *et al.*, 2011). For instance, in the *Anopheles-Plasmodium* and sand fly-*Leishmania* vectorial systems, plant feeding may expose these vectors to a range of metabolites influencing infection success. (Muller *et al*., 2017; Balaich *et al*., 2016; Schlein and Muller, 2004). Recent studies have demonstrated that plant feeding enhances the survival and reproductive success of *Ae. aegypti* (Nyasembe *et al*., 2021), despite its known preference to feed on humans (Harrington *et al*., 2009). However, little is known about the influence of plant feeding on pathogen transmission success in this vector.

Pithecellobium dulce benth is a preferred host plant fed upon by *Ae. aegypti* in nature (Nyasembe *et al*., 2018), attributed to sugar and amino acid content which the vector ingests to enhance its survival and reproduction success (Nyasembe *et al*., 2021). However, it is conceivable that as demonstrated in *Anopheles-Plasmodium* and sandfly-*Leishmania* vectorial systems (Muller *et al*., 2017; Balaich *et al*., 2016; Schlein and Muller, 1995;), *P. dulce* could be a source of secondary metabolites whose role in the *Aedes*-virus interactive system is unknown. Therefore, we proposed to evaluate the effect of *P. dulce* extract on survival and susceptibility of *Ae. aegypti* to CHIKV infection.

1.3 Justification and significance of the study

Chikungunya virus is increasingly becoming a global concern. This is due to the numerous outbreaks and the inter-epidemics reported at both global and local scale (WHO 2020). Challenges of current vector control strategies coupled with the lack of CHIKV vaccines as well as variation in the global and local transmission of CHIKV has underscored the need to explore and develop novel strategies that can prevent CHIK infection in the vector. Plant feeding is a neglected aspect in the biology of the key vector, *Aedes aegypti* that could open avenues for control.

Mosquitoes solely depend on plants for sugars necessary for their survival and reproductive fitness (Nyasembe *et al*., 2021; Wanjiku *et al.*, 2021; Olson *et al*., 2020 Nyasembe *et al*., 2018). Either subsequent studies have also shown that plant nutrition through the effects of ingested secondary metabolites or nutritional content influences the competence of mosquitoes in transmitting pathogens. (Alaux *et al.*, 2010; Lefèvre *et al*., 2013b). However, the role of secondary metabolites ingested during plant feeding is not known in the *Aedes*-virus interaction.

Building on earlier reports of plant feeding of *Ae. aegypti*, we tested whether this behavior could influence its survival and competence to CHIKV by virtue of ingested metabolites acquired during feeding on *P. dulce* plant. The findings from this study could have epidemiologic importance since identification of metabolites with the potential to regulate mosquito-virus interaction could pave way for transmission blocking.

1.4 Research objectives

1.4.1 Main objective

To determine the effect of *Pithecellobium dulce* stem, leaf and inflorescence extracts on survival and competence of *Ae. aegypti* to chikungunya virus.

1.4.2 Specific objectives

i. To screen the effects of *P. dulce* stem, leaf and inflorescence extracts on survival of *Ae. aegypti*

ii. To identify plant metabolites ingested by *Ae. aegypti* after feeding on *P. dulce* stem, leaf and inflorescence extracts

iii. To determine the effect of *P. dulce* stem, leaf and inflorescence extracts on CHIKV infection, dissemination and transmission potential of *Ae. aegypti*

1.5 Research hypothesis

1. Feeding on *P. dulce* stem, leaf and inflorescence extracts enhances survival of *Ae. aegypti*
2. *Ae. aegypti* ingests secondary metabolites after feeding on *P. dulce* stem, leaf and inflorescence extracts
3. Feeding on *P. dulce* stem, leaf and inflorescence extracts reduces the extrinsic development and competence of *Ae. aegypti* to CHIKV transmission.

CHAPTER 2.0: LITERATURE REVIEW

2.1 Epidemiology, burden and transmission of chikungunya virus

2.1.1 Chikungunya virus discovery and genetic diversity

Chikungunya is mosquito-borne alpha virus belonging to family Togaviridae and the Semliki Forest antigenic complex. The name chikungunya which means "to become contorted" describes the incapacitating arthralgia noted by the locals during the first CHIKV outbreak in 1952-1953 in southern Tanganyika, currently known as Tanzania (Robinson, 1955). The epidemic reported a morbidity rate of 47-50% attributed to lack of pre-existing antibodies in the affected population and container storage of water that served as a reservoir for breeding of the key vector, *Ae. aegypti* (Lumsden 1955; Chretien *et al.,* 2007).

Chikungunya virus is phylogenetically classified into three genotypes namely West Africa (WA), the East, Central and Southern Africa (ECSA) and the Asian genotypes reflecting the geographic location where the respective strains were first identified. The ECSA is the most diverse and competent genotype responsible for major outbreaks of unprecedented magnitude reported globally. In the year 2005-2006, ECSA diverged into a new sub-lineage known as the Indian Ocean lineage (IOL) that developed a pronounced affinity for the second key vector, *Aedes albopictus* due to mutations resulting in major epidemics in the Indian Ocean Islands and the India sub-continent (Phadungsombat *et al*., 2020; Tsetsarkin *et al.*2016). The ECSA-IOL has since spread throughout the world including Kenya causing outbreaks and circulating during the inter-epidemic periods (Fourié *et al*., 2021; Nyamwaya *et al*., 2021; Phadungsombat *et al*., 2020).

2.1.2 Global spread of chikungunya virus

Several CHIKV outbreaks have occurred globally since its discovery during the 1952-53 outbreak in Tanzania (Fig 2.1). The first wave of outbreak occurred in the early 1960's and late 1970's in Thailand, India and Sri Lanka (Kalantri *et al.*, 2006; WHO 2007). The Thailand outbreak in Bangkok (1962) reported an attack rate of 32% and approximately 70,000 infections in children with antibody prevalence that ranged between 10-20% in 1–2-year-olds (Jupp and McIntosh 1989; Halstead *et al*., 1969a; Halstead *et al*., 1969b). In India, high attack rates were reported in Madras (40%) and in Barsi (37%), during the early 1963 and 1973 outbreak respectively (Rao, 1966; Padbidri and Gnaneswar, 1979). The second wave of CHIKV outbreak occurred in the early 1980s and 1990s in Philippines, Thailand, Myanmar and Indonesia and on a small scale in some African countries including Uganda, Congo, South-Africa and Zaire (Zeller *et al*., 2016).

Outbreaks of unprecedented magnitude occurred following the re-emergence of CHIKV in 2004-2005 in Lamu Island and Mombasa, Kenya involving the ECSA genotype. The epidemic reported at least 13,500 human cases and an attack rate of 75% of the Lamu population (Sergon *et al*., 2008) bearing a heavy brunt on the population. The same epidemic extended southwards to Comoros Island, reporting an estimated 215, 000 human cases (Sergon *et al*., 2007). This was followed by the 2005-2006 La Re-union Island outbreak that reported 244,000 estimated cases. The epidemic was associated with a point mutation in the E1-glycoprotein (E1-226V) of *Ae, albopictus,* abundant in the island, that increased its transmission efficiency (Schuffenecker *et al*., 2006). This was the first epidemic to report maternal-neonatal transmission as well as severe symptoms like neurological manifestations, fetal infections and mortality associated with CHIKV. The epidemic further spread to India in 2006, after a 32 year period of no viral activity

(Dash *et al*., 2007), reporting approximately 1.3 million human cases in 13 different states. The expansion continued to South East Asia and Italy marking the first autochthonous transmission in a sub-tropical region with 254 infection cases as well as the ability of the CHIKV to adapt to new ecologies (Angelini *et al*., 2007; Rezza *et al*., 2007). Similar outbreaks were reported in West and South of the Pacific Ocean, the Caribbean island as well the United States of America (USA) resulting in an estimated 1.6 million cases in less than two years aided by travel-related cases (CDC, 2020). The most recent global reported outbreaks include the 2018 outbreak in Sudan, the 2019 outbreak in Yemen and the 2020 outbreak in Chad (WHO 2021).

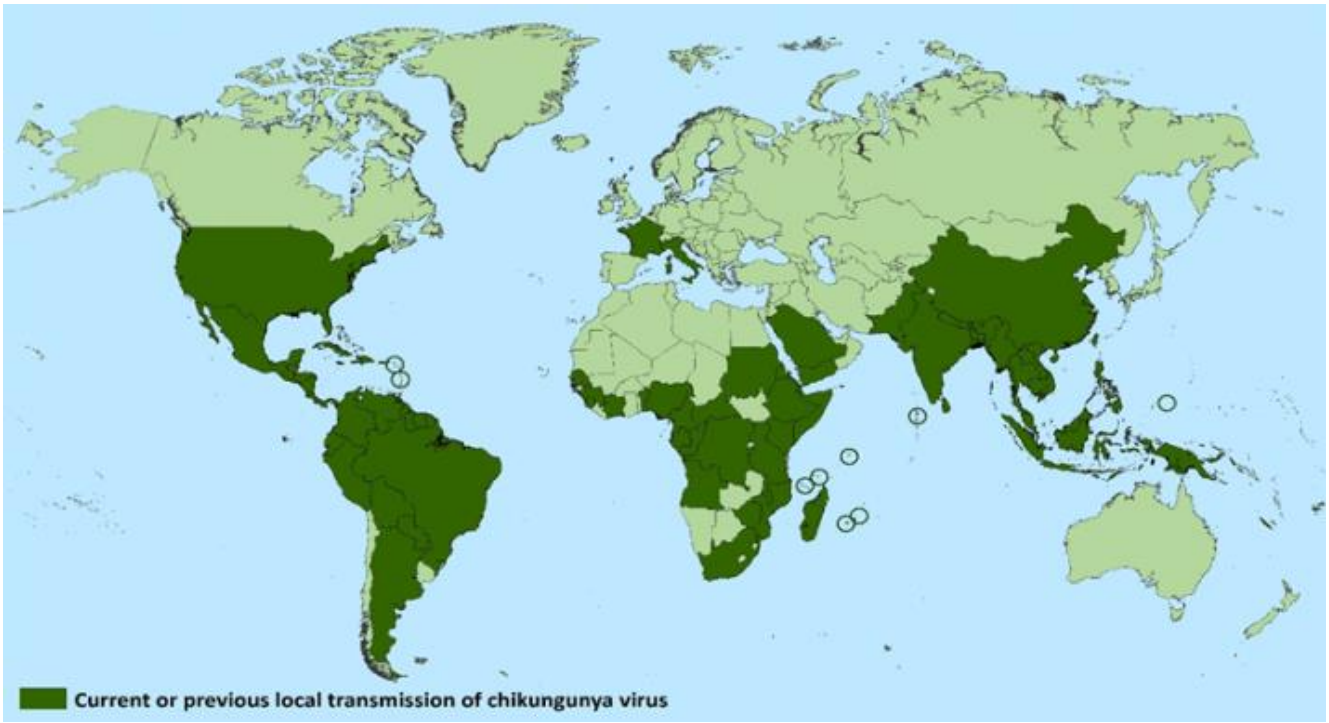


Figure 2.1: Global distribution of CHIKV infections. Areas highlighted dark green indicate current or previous regions of local transmission and those with no reported cases in lime green (CDC, 2020).

2.1.3 Spread of chikungunya virus in Kenya

Chikungunya is a re-emerging arboviral disease in Kenya. Several outbreaks have occurred since the 2004-2005 outbreak in Lamu Island involving the ECSA genotype (Fig 2.2). The outbreaks include the Mandera outbreak that involved 1792 human cases (Konongoi *et al*., 2018), the 2018 Mombasa outbreak involving 40 human cases (Eyase *et al*., 2020). The most recent outbreak occurred in Hagadera Sub-County, Garissa in 2020 (WHO, 2020). High sero-prevalence rates of 59% and 24% have also been reported in Busia and Malindi Counties, Kenya respectively in children and adults (Mease *et al*., 2011). Recent studies have also indicated high CHIKV infection rates among asymptomatic children presenting with febrile illnesses in health facilities in the Coastal and Western regions of Kenya (Nyamwaya 2021; Waggoner 2017).

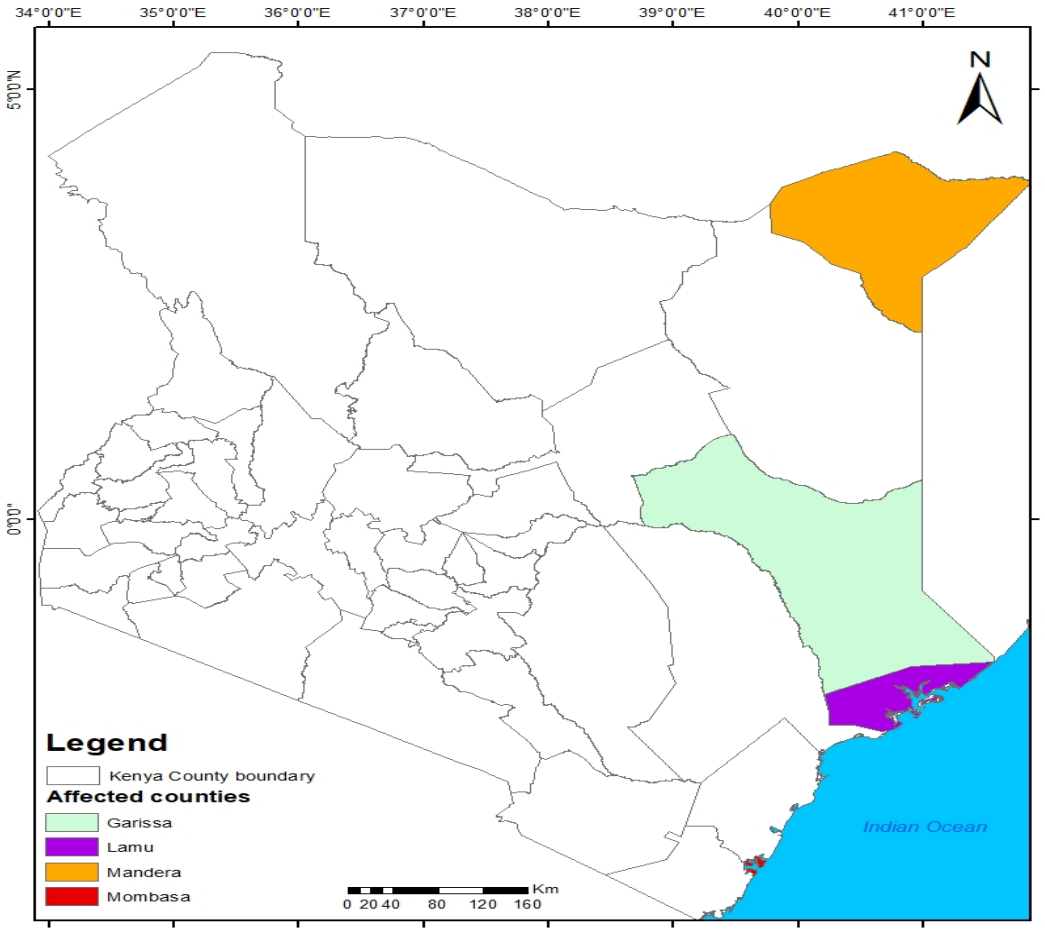


Figure 2.2 Distribution of CHIKV outbreaks in Kenya.

2.1.3 Burden of chikungunya disease

Chikungunya disease imposes a significant health, economic and social burden in affected countries. Like most febrile illness, the number of Disability Adjusted Life Years (DALYs) measures this impact. For instance, Latin America reported an estimated 25.45 DALYs per 100 000 of population with minimal fatality rate (Cardona-Ospina *et al*., 2015) while in India, the 2006 outbreak was estimated at 25,588 M DALYs, and a total burden of 45.26 DALYs per million human population (Krishna moorthy *et al.*, 2009). In the USA, CHIK disease was estimated at 23.8 DALYs per 100 000 of population with over 90% of the DALYs and 95% of costs being attributed to chronic inflammatory rheumatism, a severe symptom of chikungunya disease (Feldstein *et al*., 2019). The La Reunion Island outbreak experienced severe clinical forms of CHIK disease. Among the cases, maternal-neonatal transmission was reported for the first time leading to cognitive development delays in neonates (Gérardin *et al*., 2014). A subsequent study (Gérardin *et al*., 2016), reported 24 severe encephalitis cases out of the 57 patients diagnosed with central nervous system disease

Chikungunya afflicts all levels of people in the society. However, least developed countries and the poorest segment of the society are the most affected. This is due to absence of modern diagnostic and surveillance techniques, limited research and development funds, and challenges from other endemic diseases such as malaria and dengue virus (Amarasinghe *et al*., 2011). Globally, countries in sub-Saharan Africa, including Kenya bear the heaviest brunt of the disease (WHO, 2014)

2.1.4 Chikungunya virus infection, clinical presentation and diagnosis

Transmission of CHIKV to humans occurs through bites of an infected female *Ae. aegypti* or *Ae. albopictus* mosquito (Schwartz and Albert, 2010b). Within the vector, CHIKV infects the midgut, disseminates into the haemocoel and subsequently infects the salivary glands within a period of 8-10 days (Monteiro *et al*., 2019). The vector then transmits the virus to a susceptible host for amplification. In humans, CHIKV replicates under the skin, disseminates to the liver and joints for a period of 2-4 days before the onset of symptoms (Schwartz and Albert, 2010b). Symptoms of CHIKV occur in 72-97% of patients and they include joint pains, high fever, headache and rashes (Fig 2.3)(Staples *et al.*, 2009). Most CHIKV infections in humans are self-limiting and the acute symptoms resolve within 5-7 days, although with a possibility of the infection recurring in 30-40% of the infected patients (Owino, 2018; Schwartz and Albert, 2010a). Severe cases of CHIKV and resultant deaths are rare except in patients with comorbidities. However, chronic cases such as musculoskeletal disorders and inflammatory rheumatism have been reported (McCarthy and Morrison, 2016).

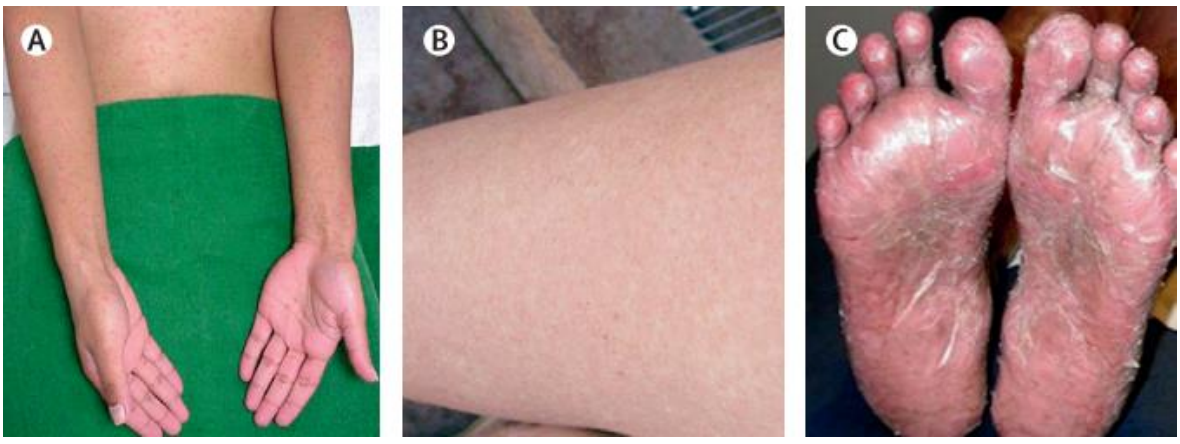


Figure 2.3: Typical rashes common in chikungunya patients a). Maculopapular rash b). Petechial spots and c). Erythroderma of the feet. Sources *www.wikipedia.com*.

2.1.5 Chikungunya virus vectors and transmission cycles

Aedes aegypti and *Ae. albopictus* are the two key vectors responsible for transmitting CHIKV globally (Schwartz and Albert, 2010b). Although these key vectors are considered to have fully adapted to urban cycle, recent studies have attributed sylvatic cycles involving non-human primates (NHPs) and forest dwelling mosquitoes to their circulation (Valentine *et al.*,2019) (Fig 2.4). The sylvatic cycle acts as reservoir enabling re-emergence and development of novel strains of this virus. In addition to *Aedes* species (Fig 2.4), other mosquito species including *Mansonia spp* have been implicated in transmission of CHIKV(Gilotra and Shah, 1967). *Culex quinquefasciatus* was recently identified to be a potential vector of CHIKV during an urban outbreak in Mombasa County, Kenya, 2017–2018 (Lutomiah *et al.*, 2021).

In the sylvatic cycle, mosquitoes acquire infection from infected primates such as chimpanzees and transmit to humans either during host seeking for a blood meal in human habitats or when humans encroach the sylvatic habitat through deforestation, agriculture or urbanization (Weaver *et al.*,2020) (Fig 2.4). Urban cycles on the other hand occurs between an infected vector and a susceptible host or an infected host to a potential host through a vector (Fig 2.4). In the urban cycle, humans serve as amplification hosts for CHIKV. Once a mosquito vector is infected, it carries the infection throughout its lifetime leading to multiple infections to potential hosts on every blood meal taken. *Aedes aegypti* is a dark mosquito with white bands at the base of the tarsal segments and a distinctive lyre-shaped design on the mesonotum. Unlike the white bands, the lyre tends to disappear with age. The vector belongs to the family culicidae, order diptera, class insecta and phylum Arthropoda(Huang and Rueda, 2017). The vector is also a tropical and subtropical mosquito with a high preference for human blood (Harrington *et al.*, 2009) as well as an endophilic vector breeding in containers with stagnant waters within and

around the human habitats. Population and colonization of this vector and resultant urban epidemics are fueled by climatic conditions such as heavy rainfall and temperature (Valdez *et al*, 2018; Nasir *et al.*, 2017; Alto and Juliano, 2001) further implicating outbreaks of the CHIK disease.

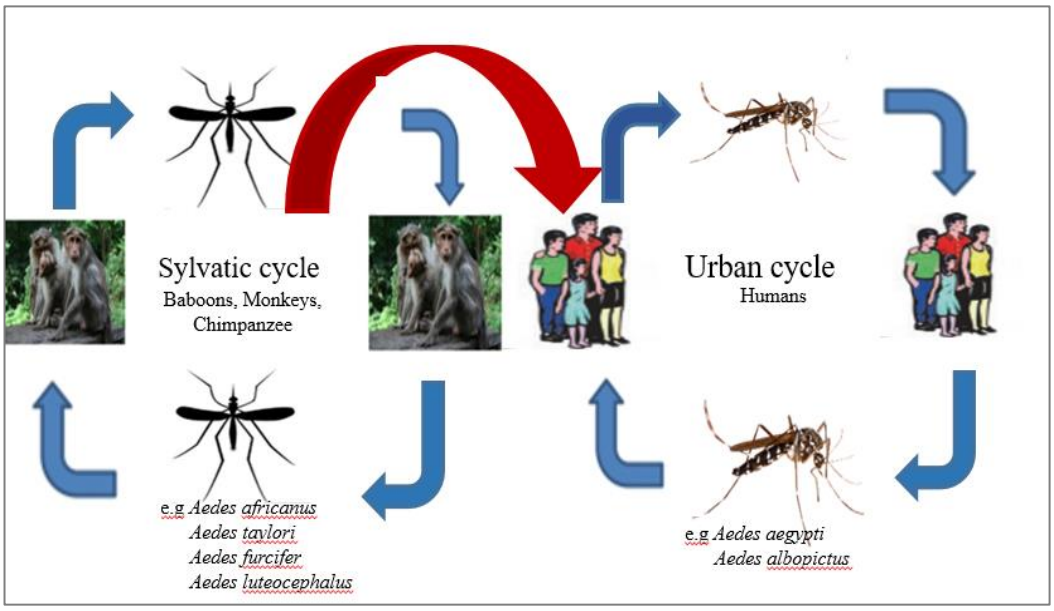


Figure 2.4: Transmission of chikungunya virus in both the sylvatic and urban cycle. Source *www.wikipedia.com*.

Although most CHIKV transmission cases are mosquito borne, vertical transmission between a mother and child during birth (perinatal) have been reported (De Almeida Di Maio Ferreira *et al*., 2021). This transmission often causes neonatal encephalitis and poor development of neurons in the newborn babies (Gérardin *et al*., 2014; Torres *et al*., 2016). Vertical transmission has also been reported in mosquitoes. The transmission occurs transovarially through the mosquito eggs, enhanced by the desiccation resistant nature of *Ae. aegypti* eggs (Honório *et al.*, 2019), further supporting inter-epidemic outbreaks of CHIKV.

2.2 Control of chikungunya disease

Vaccine is usually the first line of prevention for most viral diseases. There is no licensed vaccine or specific therapeutic treatment against CHIKV. Vector control remains the only option to control the disease. Vector control traces back to prehistoric times where different communities burnt plant materials, hanged some plants in the houses and crushed some plant parts to drive mosquitoes away (Pavela and Benelli, 2016; Tisgratog *et al*., 2016; Seyoum *et al*., 2002). Today, vector control is integrated into five disciplines:

i. Effective and focused surveillance

This involves the use of surveillance tools such as risk maps and geographic information system (GIS) to determine vector density and breeding sites of *Ae. aegypti.*

ii. Environmental management and community-based campaigns

Modification of building designs such as roof gutters, covering mosquito breeding containers and the use of ovitraps that target the aquatic stages of mosquitoes have contributed immensely to the reduction of vector populations (Vanlerberghe *et al*.,2009). Community based programs such as educating the public on control strategies on the other hand, has provided a platform for inclusive control (Elsinga *et al*., 2017; Hierlihy *et al*., 2019).

iii. Chemical control

The use of chemical control is effective but has other demerits. For instance, development of resistance to Dichlorodiphenyltrichloroethane (DDT), the first chemical used in *Ae. aegypti* control, and cross-resistance to other insecticide classes such as carbamates and organophosphates have contributed to outbreaks of diseases involving *Ae. aegypti* vector (Curtis and Lines, 2000). Other limitations associated with use of synthetic chemicals include carcinogenic and teratogenic effects in humans as well as residual toxicity to the environment.

iv. Biological control

This involves the use of natural enemies and biological organisms such as larvivorous fish, copepods and bacterial agents. *Copepod mesocyclops* was shown to be effective in control of *Ae. aegypti* in Vietnam and Thailand (Kittayapong *et al*., 2006; Nam *et al*., 2005; Kay *et al*., 2002).The wMel Wolbachia strain, a natural enemy of *Ae. aegypti* and *Ae. albopictus* populations, contributed in CHIKV reduction through the vector, attributed to the natural cytoplasmic incompatibility (Blagrove *et al.*, 2013; Van den Hurk *et al*., 2012).

v. Genetic-based strategies

These involves the incorporation of various techniques that either reduce or replace vector populations in their natural habitat. Sterile insect technique (SIT) involves the production of genetically modified mosquitoes using x-ray or gamma ray radiation to induce random mutation. This technique has progressively been applied in agriculture, contributing to control of over 20 insect species pests. In Italy, SIT was successfully implemented in the control of *Ae. albopictus* (Bellini *et al.*, 2013). Lucantoni *et al*., 2011 describes other genetic techniques such as the use of photosensitizers, nanotechnology and micro emulsion. These techniques are however, expensive and difficult to sustain and implement at global and local scale.

Challenges of current vector control strategies coupled with the lack of CHIKV vaccines as well as variation in the global and local transmission of CHIKV has underscored the need to explore and develop novel strategies that can prevent chikungunya infection in the vector. Vector competence is one of the most promising approaches in relation to assessment of transmission risk and spread of CHIKV.

2.3 Vector competence.

Vector competence is the potential of a vector to acquire an infection, disseminate and transmit the infection to a susceptible host. It is a component of vectorial capacity (VC). Vectorial capacity is a parameter that mathematically links mosquito's behavior and biological activities to pathogen transmission (Smith *et al*., 2012).

Ross-Mac-Donald defines vectorial capacity as:

$$Vectorial\ capacity = \frac{ma^2bpn}{\ln(p)}$$

Where: ***m*** is density of vectors in relation to the host;

a probabili*y* of the vector feeding on the potential host,

b transmission rate compared to the original infection rate (vector competence)

p daily survival rate of a vector

n time it takes for a pathogen to move from point of entry to point of exit, also known as the extrinsic incubation period (EIP) and

1/ln (probability of the vector surviving the EIP.

Genetic and environmental factors as well as the interaction between these factors as outlined below determine the competence:

2.3.1 Influence of genetic factors on vector competence

Studies on genetic factors have revealed a number of ways (phenotypes) in which mosquitoes limit virus development. These phenotypes encompass various mechanisms that are specific to each pathogen and they include mosquito barriers, immune pathways, intracellular processes and digestive enzymes (Tabachnick, 2013; Bennett *et al*., 2002). After an adult female mosquito acquires an infectious blood meal, the virus first encounters the midgut infection barrier (MIB). The MIB blocks attachment of virus to the cell receptors preventing its replication and

further development. If the virus overcomes the MIB, it escapes the midgut epithelial cells to the mosquito hemolymph replicating in other secondary tissues such as nerves and muscles overcoming the midgut escape barrier (MEB) to develop a disseminated infection (Schwartz and Albert, 2010b). The MEB is the barrier that blocks the infection from disseminating to secondary tissues and this process is influenced by genes within multiple quantitative trait loci (QTL), although, the particular genes involved in this action are yet to be identified (Chen *et al.*, 2008). Suppose the virus overcomes the MEB, it escapes to the peripheral tissues infecting the salivary glands hence escaping the salivary gland infection barrier (SGIB). Hence, SGIB is the barrier that protects the vector from transmitting the pathogens to a susceptible host. Presence of virus in the saliva makes the vector competent to transmit the virus to a potential host upon biting.

Viral development in mosquito disease vectors is also influenced by various antimicrobial pathways such as Toll and the Janus kinase signal transducer and activator of transcription (JAK-STAT) signaling pathway (Ramirez and Dimopoulos, 2010; Souza-Neto *et al.*, 2009). Similarly, limitation of viral development has been influenced by genes that control trypsin and serine proteases and other proteins which bind to the virus preventing transmission (Brackney *et al.*, 2008; Molina-Cruz *et al*., 2005). Genetics of mosquito can also affect vector competence as well as virus strain (Dickson *et al.*, 2014). However, accepted that interaction of genotype x environment factors modulates outcome of vector competence. For instance, a mosquito population can better transmit a virus yet refractory to another strain of the same virus (Dickson *et al*., 2014).

2.3.2 Influence of environmental factors on vector competence

Environmental factors including changes in temperature, microbial gut flora, larval and adult diets, predation and exposure to pesticides play a major role in modulating mosquito's

competence to pathogens (Lefèvre *et al.*, 2013). For instance, while high temperatures > 30˚c enhances malaria parasite development and infectivity of malaria vector *An. gambiae,* the competence of the vector is greatly reduced (Okech *et al*., 2004). In *Ae. aegypti,* high temperatures enhanced competence to CHIKV and dengue virus respectively (Agha *et al*., 2017; Chepkorir *et al*., 2014). Similarly, high bacterial load in the midgut of *An. gambiae* and diversity of the microbiota has been shown to reduce competence influencing disease transmission dynamics (Boissière *et al*., 2012; Meister *et al*., 2009). In addition, chromo bacterium Csp_P reduces malaria and dengue virus infection in *An. gambiae* and *Ae. aegypti* vectors respectively (Jose Luis Ramirez *et al*., 2014).

The influence of mosquito diet on competence of mosquito disease vectors to pathogens has been majorly attributed to plant produced substances acquired during plant feeding and differences in nutritional value (Lefèvre *et al.*, 2010). Effects of nutritional value on competence are well documented for malaria vectors *An. gambiae* and *An. stephensi* larval stages (Koella and Sorensen, 2002; Suwanchaichinda and Paskewitz, 1998). However, the underlying mechanisms as to how mosquito diet affect competence of *Ae. aegypti* to viral pathogens remains elusive.

2.4 Plant feeding and influence on vector competence

Plant feeding is a common biological trait in the life cycle of mosquito disease vectors. Adult mosquitoes selectively depend on plants for sugars vital for survival, metabolic actions and reproductive fitness (Beier *et al*., 2007; Nyasembe *et al*., 2021; Olson *et al*., 2020; Wanjiku *et al*., 2021). The male adult mosquitoes solely depend on the preferred host plants while their female counterparts intermittently depend on these plants (Takken *et al.*, 2013b). Abundance and availability of suitable host plants modulates vector competence. For instance, availability of *An.*

gambiae and *An. sergentii*, host plants promoted completion of their sporogonic cycle, hence increasing their survival while abundance of the host plants reduced human bites (Müller *et al.*, 2017; Nyasembe *et al*., 2015; Muller *et al*., 2011). In the course of plant feeding, mosquitoes ingest a range of plant metabolites that affect vector survival, and pathogen transmission dynamics. For instance, *Parthenium hysterophorus,* suitable host plant for malaria vector *An. gambiae* promotes survival and blocks development of malaria parasite *P. falciparum* in the mosquito midgut through the action of metabolite parthenin, a sesquiterpene lactone (Balaich *et al.*,2016; Manda *et al.*,2007). In contrast, plant feeding influences the level and transmission of *Leishmania* parasites by sand flies (Schlein and Muller, 2004). Similarly, Muller *et al.*, (2017) found that plant feeding on the invasive shrub *Prosopis juliflora* enhances the malaria parasite transmission capacity in *Anopheles* mosquitoes. Even though previous studies have linked plant feeding to the survival of *Ae. aegypti* (Vincent *et al*., 2021), about the nutritional contribution of plants in modulating pathogen transmission success in *Aedes*-virus system.

2.4.1 *Pithecellobium dulce* and *Aedes aegypti* interaction

Aedes aegypti is a highly anthropophilic and endophilic mosquito species, widely thought to prefer human blood for metabolic processes and reproduction (Harrington *et al.,* 2009). A high degree of plant feeding has however been demonstrated to be an important trait in the life cycle of wild *Ae. aegypti* (Olson *et al*., 2020; Wanjiku *et al*., 2021). Through the application of deoxyribonucleic acid (DNA) barcoding, *P. dulce* was identified as the most suitable host plant of *Ae. aegypti* based on detection plant DNA detection of field collected samples (Nyasembe *et al.*, 2018). Subsequent analysis using coupled gas chromatographic and electrophysiological assays identified constituents of *P. dulce* odors detected by the antennae of the female *Ae. aegypti* mosquito(Nyasembe *et al*., 2018).

Pithecellobium dulce benth is an evergreen perennial tree (Fig 2.5) indigenously grown in America and in Kenya, its cultivated at the Coastal region (Srinivas *et al.*, 2018). The fruits and seeds are edible while the leaves and bark are used in treatment of various ailments(Kulkarni *et al.*, 2018). In natural environment, *Ae. aegypti* imbibes on the fruits, leaves and inflorescence of this plant (Nyasembe *et al*., 2021). In Kenya, the tree is known by a swahili name "mkwaju" meaning Tamarind tree.

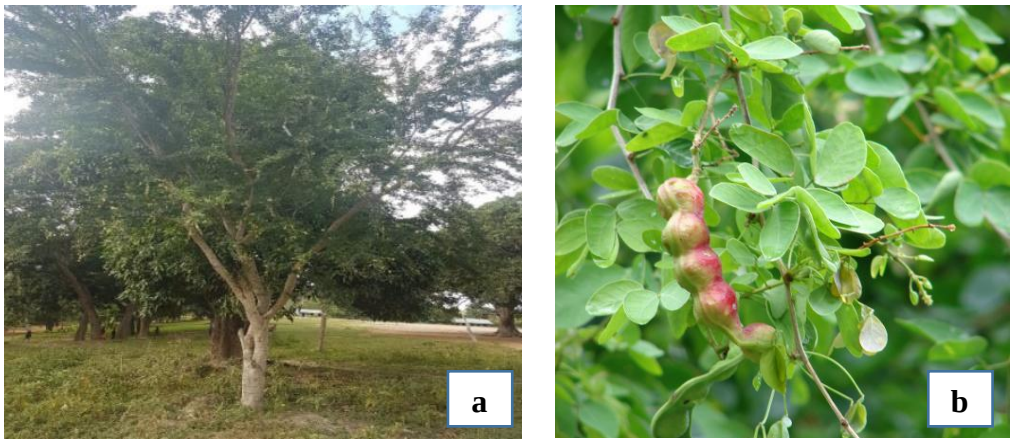


Figure 2.5: *Pithecellobium dulce* tree (**a**) and its leaves, fruits and seed pod (**b**) Source Brenda

Phytochemical analysis of different plant parts of *P. dulce* have revealed presence of various compounds including alkaloids, tannins, flavonoids, glycosides and triterpenoids among others(Srinivas *et al*., 2018). For example, the leaves have been reported to possess astringent, emollient, and antidiabetic properties with metabolites such as afzelin, dulcitol and quercetin identified in subsequent studies (Vanitha and Manikandan, 2016). These phyto-compounds are also used to protect plants from external forces as well as providing a wide spectrum of biological activities on other organisms (Kessler and Baldwin, 2002). Plant feeding of *Ae. aegypti* on *P. dulce* could be related to nutrient content, volatile profiles and perhaps secondary metabolites with yet unknown functions (Nyasembe *et al*., 2018).

CHAPTER 3.0: MATERIALS AND METHODS

3.1 Plant collection and preparation of extracts

Leaves, fruit and inflorescence of the plant *P. dulce* (previously morphologically and molecularly identified)(Nyasembe *et al*., 2018) were collected from Rabai, Kilifi County, Kenya (3˚ 37 49.62" S, 39˚ 50 59.71" E) and transported to ICIPE laboratories. The leaves, fruit and inflorescence of the plant were air dried at room temperature for three weeks and powdered together using an electrical grinder (Retsch GmbH, Haan, Germany). The powdered *P. dulce* weighing 1.9kg was extracted in 2.5L of methanol solvent (Analytical grade, Fluka) three times for one week. The extract solution was filtered through a Buchner funnel lined with a filter paper (Whatman No. 1). The extract was then evaporated to dryness under reduced pressure using a rotary vacuum evaporator to obtain a crude extract. The crude extract weighed 247.2g and was stored at -20˚C until further analysis.

3.2 Mosquitoes collection and rearing

Adult female *Ae. aegypti* used in this experiment were obtained from a colony maintained at icipe and previously obtained as eggs from Rabai, Kilifi County, Kenya in 2018. The eggs were dispensed in rearing trays (25 cm ×20cm ×14cm) filled with distilled water. The larvae were maintained in densities of 150-200 per tray and fed on fishmeal (Tetramin1, Melle, Germany). The pupae were picked and placed in rearing cages (50×50×50 cm) for pupation into First filial generation (F0) adults. The adults were fed on 6% glucose solution placed in a glass vial in the middle of the cage using filter paper wicks (Whatman No. 1). The rearing conditions were maintained at a mean temperature and relative humidity (RH) of 31°C and 80% respectively, and under a 12:12 light and dark photoperiod.

3.3 Determination of optimal concentration of dimethyl sulfoxide (DMSO) for use in survival assays

Eight batches of 200 female mosquitoes (5-7 days old) per replicate were released into the experimental cages (50cm×50cm×50cm) and left to acclimatize for 1 hour. The mosquitoes were fed on different concentrations of DMSO in 6% glucose solution (Table 3.1). The positive control was 6% glucose solution while the negative control was distilled water only (Table 3.1). The feeding was done using filter paper wicks (Whatman No. 1) placed in the middle of the cage. The mosquitoes were maintained under the same insectary conditions previously described and the experiment monitored for 21 days (to cover 14 days required for extrinsic development of CHIKV). The mortality was recorded daily as well as replacement of the test solutions. The experiment was replicated six times for each treatment.

Table 3.1: Different doses of DMSO in 6% glucose solution and controls tested for survival of *Aedes aegypti*

Cage No	Volume of 6% glucose solution (mL)	Volume of DMSO solution (mL)	Total volume (mL)
1	18	2	20
2	19	1	20
3	19.5	0.5	20
4	19.75	0.25	20
5	19.875	0.125	20
6	19.938	0.0625	20
7	Positive control	-	20
8	Negative control	-	20 (distilled water)

3.4 Survival assays

Eight batches of 200 female mosquitoes (5-7 days old) per replicate were released into the experimental cages (50cm×50cm×50cm) and left to acclimatize for 1 hour. The mosquitoes were allowed access to different concentrations of the plant extract in 19.897mL of 6% glucose solution and 0.103mL of DMSO (Table 3.2). The positive control was 19.897mL in 6% glucose solution while the negative control was 19.897mL distilled water in 0.103ml of DMSO (Table 3.2). The test solutions were placed in a glass vial and the feeding done using filter paper wicks (Whatmann No 1). The mosquitoes were maintained under the same insectary conditions previously described and monitored for 21 days. The mortality was recorded daily as well as replacement of the test solutions. The experiment was replicated six times for each treatment.

Table 3.2: Different concentrations of *Pithecellobium dulce* extract in 6% glucose solution and 0.103mL of DMSO and controls tested for survival of *Aedes aegypti*

Cage No	Volume of 6% glucose solution (mL)	Volume of DMSO solution (mL)	Total volume (mL)	Concentration of *P. dulce* extract(mg/mL)
1	19.897	0.103	20	400
2	19.897	0.103	20	200
3	19.897	0.103	20	100
4	19.897	0.103	20	50
5	19.897	0.103	20	25
6	19.897	0.103	20	12.5
7	Positive control	0.103	20	-
8	Negative control	0.103	20	-

3:5 Chemical analysis of *Pithecellobium duce* extract and mosquito midguts

Three batches of 400 mosquitoes (4-7 days old) were released into the experimental cages (50cm×50cm×50cm) and left to acclimatize for 1 hour. The mosquitoes were fed to 25.8mg/mL of *P. dulce* extract (optimal plant extract concentration) diluted in 0.103ml of DMSO and 19.897mL of 6% glucose solution. The control involved mosquitoes exclusively fed on glucose solution. The mosquitoes were maintained under insectary conditions previously described. On day 7, the midguts of the mosquitoes were dissected and pooled (n=400) and then extracted overnight in 1 mL of methanol (LC-MS LiChrosolv®, Merck ≥99.97%). Thereafter, the midguts were vortexed for 10 seconds, sonicated for 15 minutes and centrifuged at 14,000 revolutions per minute (rpm) for 10 minutes.

To determine the chemical composition of plant extract, 0.1545mg of the plant extract was dissolved in 1.5mL of LC-MS grade methanol (LC-MS LiChrosolv®, Merck ≥99.97%) and the sample prepared as described above for the pooled midguts. All the supernatants were filtered using glass wool, diluted to 1 mg/mL, transferred into a sample vial and 0.1 µl of the sample analyzed using an Ultra Performance Liquid Chromatography coupled to a triple quadrupole tandem mass spectrometry (UPLC-QqQ-MS/MS). Chromatographic separation was performed on a ACQUITY UPLC I-class system (Waters Corp., Milford, 151 MA) fitted on an ACQUITY UPLC BEH C18 column (2.1 mm × 50 mm, 1.7 µm particle size; Waters Corp., Wexford, Ireland), that was heated to 45°C. The auto sampler tray was cooled to 5°C. The mobile phase comprised of water acidified with 0.01% formic acid (solvent A) and methanol (solvent B) and followed a gradient system. The gradient system used was 0–5 min, 5% B, 10–15 min, 40% B, 15–20 min, 40% B, 25–30 min 60% B, 30–35 min 60% B, 35–40 min, 80% B, 40–45 80% B, 45–50 min, 100% B, 50–55min B. The flow rate was held constant at 0.1 mL/min. The UPLC was interfaced

with an electrospray ionization (ESI) Waters Xevo TQ-S operated in full scan MS in both positive and negative ionization modes. The data was acquired over the *m/z* range 100–1500 with a capillary voltage of 3 kV, sampling cone voltage of 40V, source temperature 150°C and desolvation temperature of 250°C. The nitrogen desolvation flow rate was 600 L/h.

Data was acquired using Mass Lynx version 4.1 SCN 712 (Waters). Potential assignments of compounds were determined after the generation of the mass spectrum for each peak, establishing the molecular ion peaks using adducts, common fragments, literature and confirmed with authentic standards where available.

One milligram of the plant extract was re-extracted in 1mL dichloromethane (DCM) as well as the pools of the mosquito midgut (n=400) fed on either the plant extract or glucose solution (control). The samples were then prepared as previously described. An aliquot (1µl) of each sample and similar volume of the blank were injected into a 7890 gas chromatograph (Agilent Technologies, CA, USA) coupled to an inert XL EI/CI with Triple-Axis mass selective detector (MSD) mass spectrometer (5975C, electron energy 70 eV, Agilent) in a split less injection mode. The GC instrument was fitted with a HP-5MS (5% phenyl-methylpolysiloxane) column (30 mm x 0.25 µm x 0.25 µm film thickness). Helium was used as a carrier gas and flow rate maintained at 1.2ml min^{-1}. Both the injector and the detector were maintained at ion source temperature of 230℃ and a quadrupole temperature of 150℃. The oven temperature was held at 35℃ for 5 min^{-1} and programmed to increase at 10˚C min−1 to 280℃ for 10.5 mins, then 50˚C min^{-1} to 285℃ for 9.9 mins and the sample programmed to run for 50 mins. Data was obtained over 38–550 *m/z* mass range in the full scan mode. The filament delay time was set at 2 min. The detected compounds were identified either based on their mass spectra or compared with spectra of

reference libraries (National Institute of Standards and Technology (NIST-2008). (C8-C26) blend of alkanes were used to calculate the retention index (RI)(Lucero *et al.*, 2009) using the formula:

$$RTx = 100n0 + 100(\frac{(RTx - RTnO)}{(RT\ n1 - RTnO)})$$

RT_X is the retention time of the sample, *RT(n0)* is the retention time of the carbon that eluted before the sample(x) and *RT(n1)* is the retention of the carbon that eluted after the sample(x). Compounds identified in the control were excluded in the analysis.

3.6 Effect of *Pithecellobium dulce* extract on chikungunya virus infection, dissemination and transmission success in *Aedes aegypti*

3.6.1 Mosquito rearing and identification

Mosquitoes used in this experiment were obtained as eggs collected using oviposition cups lined with oviposition papers from Rabai, Kilifi County, Kenya in December, 2019. The eggs were dispensed in rearing trays (25 cm ×20cm ×14cm) filled with distilled water. The larvae, pupae and adults were maintained under the same insectary conditions as previously described. The F0 adult mosquitoes were identified morphologically after knocking them down at -20˚C for 30 seconds (Huang and Rueda, 2017). The identified *Ae. aegypti* was allowed to fed for an hour on a laboratory mouse, strained in a cage (ICIPE, Animal house), to acquire blood vital for egg production. The engorged females were picked and transferred into separate cages and allowed to lay eggs in petri dish cushioned with cotton wool and a filter paper. The laid eggs were reared into adults and maintained insectary conditions as described previously. F1 and F2 adult female mosquitoes were used for the infection assays.

3.6. 2 Virus amplification and quantification

3.6.2.1 Virus amplification

Infection assays were performed using the ECSA lineage of chikungunya virus (CHIKV 006/02/2018) isolated from a patient during the Mombasa outbreak in 2018. The virus (passage 3) was amplified in a T-25cell culture flask (Corning incorporated, USA) containing confluent monolayer of Vero cells (ATT-CCL-81). The cells were previously grown in a growth media consisting of Minimum Essential Medium (MEM) (Sigma-Aldrich, St. Louis, MO) with Earle's salts and reduced $NaHCO_3$, supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Sigma-Aldrich), 2% L-glutamine (Sigma-Aldrich), and 2% antibiotic/anti-mycotic solution with 10,000 units penicillin, 10 mg streptomycin and 25µg amphotericin B per ml (Sigma-Aldrich). 300µl of the CHIKV was inoculated in confluent Vero cells, placed in an incubator for an hour to allow for virus adsorption and maintained using 5mL of maintenance media (MEM supplemented with 2% FBS with Earle's salts and reduced $NaHCO_3$, 2% L-glutamine (Sigma-Aldrich), and 2% antibiotic/anti-mycotic solution with 10,000 units penicillin.) The inoculated flask was incubated at 37˚C in a 5% CO_2 incubator and the cytopathic effect (CPE), estimated at 80-90%, observed after a 24-hour period. The virus showing CPE was harvested and 1mL of the aliquot placed in each cryovial and stored at -80˚C until use for infection assay.

3.6.2.2 Virus quantification

Chikungunya virus was quantified by plaque assay. 10-fold serial dilutions using 50µl of the amplified virus were inoculated in a 12-well plate with a confluent monolayer of Vero cells. The cells were previously cultured in growth media prepared as described above for virus amplification. Each well was inoculated with 100 µl of the respective CHIKV dilution, negative control (maintenance media) and positive control (CHIKV). The inoculated plate was incubated

at 37˚C in a 5% CO_2 incubator for 1 hour with frequent rocking after every 15 minutes to allow for virus adsorption.

The infected cells were maintained using 2.5% methylcellulose mixed with 2X maintenance medium (MEM, GIBCO Invitrogen corporation, Carlsbad, California) and incubated at 37˚C with 5% CO2 for 4 days; fixed for 24 hours with 10% formalin, stained for 24 hours with 0.5% crystal violet, washed and the plaques counted to determine the virus titer.

The virus titer was quantified using the formula:

$$Plaque\ forming\ unit = \frac{Number\ of\ plaques}{Dilution\ factor\ \times Volume\ of\ diluted\ virus}$$

3.6.3 Oral infection of the mosquitoes

Four batches consisting of 100 *Ae. aegypti* mosquitoes per replicate aged 7-10 days old were released into experimental cages (50cm×50cm×50cm) and left to acclimatize for one hour. Previously starved mosquitoes for 6-8 hours, were orally infected with either freshly grown or frozen CHIKV isolate mixed with defribrinated sheep blood (Central laboratories, Kabete), Kenya in one to one ratio (1:1) for one hour. The oral infection was performed using a Hemotek membrane feeding system (Discovery Workshops, Accrington, United Kingdom), covered with a freshly prepared mouse skin (ICIPE, Animal House). Hemotek membrane feeding system maintains the blood meal at 37˚C.

Fully engorged mosquitoes were selected using a mouth aspirator modified with high efficiency particulate air (HEPA) filter for subsequent feeding assays using 25.8mg/ml of *P. dulce* extracts (see result section). The selected mosquitoes were maintained under the same insectary conditions previously described. The effect of the extracts was evaluated among four treatments:

i. Pre-exposed- those fed on the extract before infection and maintained on glucose solution after infection. Mosquitoes were fed on the plant extract immediately after emergence until the infection period, which was done when mosquitoes were 7-10 days old.

ii. Post-exposed- those fed on glucose solution before infection then plant extract after infection. Mosquitoes were fed on the plant extract after infection until 5, 7 and 10 days post infection (dpi).

iii. Pre and post exposed- those fed on plant extract before and after infection

iv. Control group-those fed on glucose solution before and after infection without the plant extract.

100 µl of the blood-virus suspension were aliquoted from the Hemotek membrane feeding system at the start and end of the experiment to quantify the respective feeding concentrations. The blood virus suspension was added to 400µl of homogenization media (MEM, and supplemented with 15% FBS, 2% L-glutamine, and 2% antibiotic/ antimycotic), and stored at -80˚C until assayed by virus culture. The unfed mosquitoes were immediately destroyed. Mosquito mortality in the experimental treatments was monitored on daily basis and the data recorded. The experiment for each cohort was done in three replicates to obtain an adequate sample size.

3.7.2 Virus screening for infection and dissemination

A representative sample (33%) of the orally infected mosquitoes (for each experimental treatment) were randomly picked on day 5, 7 and 10 post-feeding, placed in small plastic cups (covered with a fine netting material and secured with rubber bands), and knocked down at -20˚C for 40 seconds. The wings and the legs of each mosquito were carefully removed using sterilized

forceps and body placed on a sticky tape. The mosquito's proboscis was inserted into a capillary tube containing 200µl of homogenization media. The mosquitoes were allowed to salivate in the capillary tubes for 30 minutes and the saliva stored at -80˚C until assayed by virus culture. The body, wings/legs were placed separately in 1.5mL micro centrifuge tubes (Eppendorf) containing 450µl of homogenization media.

The mosquito body in each of the experimental treatment were screened for infection. The mosquito bodies were homogenized using a mini bead beater (Bio Spec Products Inc, Bartlesville, OK 74005 USA) with the aid of a copper bead (BB-caliber airgun shot) and centrifuged at 14,000 rpm (Eppendorf centrifuge 5417R) for 10 minutes, previously fast cooled to 4˚C. Supernatants of each mosquito sample was inoculated in a 24-well plate containing confluent monolayer of Vero cells as previously described. The same procedure was repeated for the wings and legs of the positive mosquito bodies.

Only the samples that showed CPE were quantified using plaque assay as previously described. Plaques ranging from 10-100 were counted from a suitable well and the virus titer determined using the previously PFU described formula.

Only the legs of the positive mosquito bodies were homogenized and the virus titer quantified as described above for virus quantification. Presence of CHIKV in both the mosquito body and the legs was scored as evidence of successful infection and dissemination respectively. Absence of CHIKV in the mosquito legs was considered as non-disseminated infection limited to the midgut (Turrell *et al*., 1992).

3.7.3: Virus screening for transmission potential

100 µl saliva of the respective positive mosquito leg samples were inoculated in a 24-well plate containing confluent monolayer of Vero cells as described above. The inoculated cells were

placed incubated for 1 hour to allow for virus adsorption, with frequent agitation after every 15 minutes. The inoculated cells were maintained using 1mL maintenance media for each well and incubated at 37˚C with 5% CO2 for 5 days. The supernatant of the mosquito sample that showed CPE was harvested and the virus titer quantified by plaque assay as described previously. Detection of CHIKV in the saliva was considered as transmission success.

3.8. Ethical statement

Scientific and ethical approval to carry out this study was obtained from the Kenya Medical Research Institute; Scientific Ethical Review Unit (KEMRI-SERU) (Project number **3312**). Student research permit to conduct this study was obtained from the National Commission for Science, Technology and Innovation (NACOSTI), license number **NACOSTI/P/21/8629**.

3.9. Statistical analysis

All statistical analyses were performed in R version 4.1.1 (R core and team, 2021). Survival of *Ae. aegypti* on DMSO extracts of *P. dulce* was analyzed using Kaplan-Meier model. Response of *Ae. aegypti* to difference concentrations of DMSO extracts of *P. dulce* was analyzed using logit analysis(Finney, 1978) and a two parameter logistic regression model (Jeske *et al.,* 2009) fitted to determine the optimal concentration. Logistic regression model by generalized linear model (GLM, binomial errors, logit link; *lsmeans* package) was used to determine the infection, dissemination and transmission rates of orally infected female *Ae. aegypti* with CHIKV before and after feeding on *P. dulce* extract. GLM (Anova, HSD test) was a used to determine the effect of treatments and days post infection (dpi) on the mean titers for infection, dissemination and transmission. Statistical significance was considered for ($P < 0.05$).

CHAPTER 4.0: RESULTS

4.1 Survival analysis

4.1.1 Optimal DMSO dose.

The optimal dose of dimethyl sulfoxide (DMSO), a solvent often used as a carrier of secondary metabolites in living cells was determined prior to survival analysis. Figure 4.1 shows the dose-response survival activity of DMSO in 6% glucose solution against adult female *Ae. aegypti* after a 21-day exposure period. Relative to the control, there were significant differences across all doses (Log rank=4734, df=7, P<0.0001) except for 0.0625ml (Fig 4.1). On day 21, DMSO had an estimated LD_{10} and LD_{50} of 0.037ml (0.033-0.041ml) and 0.103ml (0.099-0.108 ml) at 95% CI respectively. However, the LD_{10} value was lower than the lowest dose tested (0.0625ml) and it was not significantly different from the positive control. The optimal dose was therefore estimated at LD_{50} at 0.103ml (Fig 4.2).

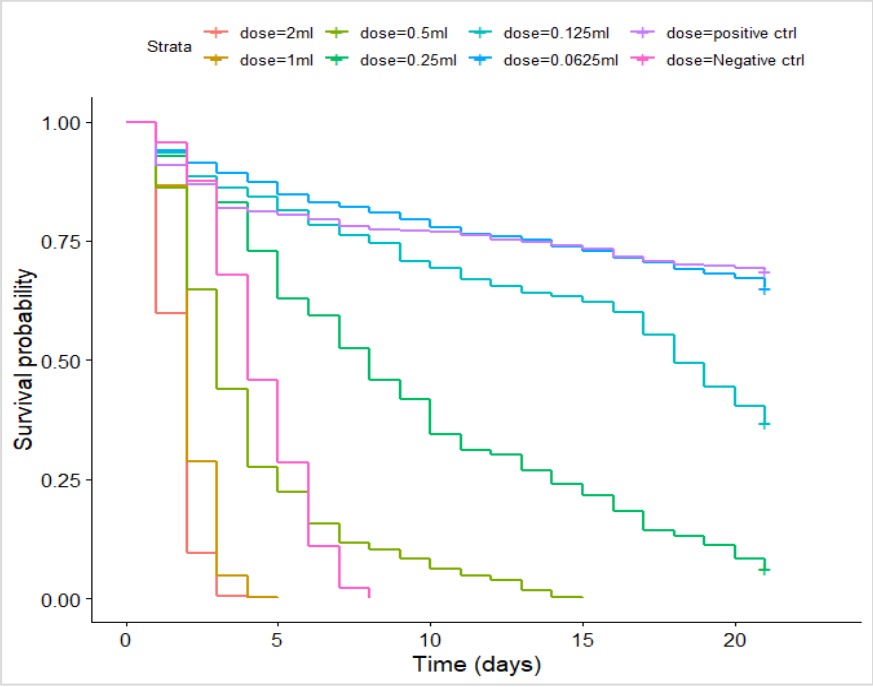


Figure 4.1 Kaplan-Meier survival curves for *Ae. aegypti* orally fed on different dose of DMSO compared to positive control (6% glucose) and negative control (distilled water).

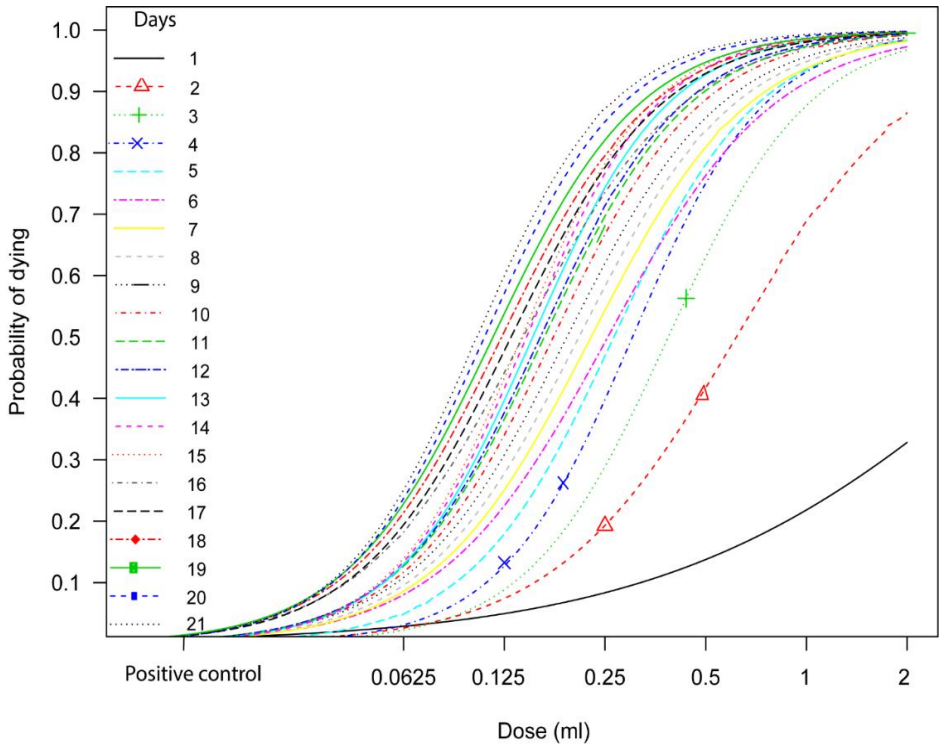


Figure 4.2: Estimated dose-response curves showing the probability of *Ae. aegypti* dying against dose level for a period of 21 days.

4.1.2 Survival analysis using *Pithecellobium dulce* extract

Similar survival assays using the optimal DMSO (0.103ml) dose determined above were set. Survival of female *Ae. aegypti* on extracts of *P. dulce* was tested for a period of 21 days in a dose dependent manner. The probability of *Ae. aegypti* survival decreased with increase in *P. dulce* extract concentration (Log rank=4916, df=7, P<0.0001) (Fig 4.3). The median survival times of female *Ae. aegypti* fed on *P. dulce* extracts and associated 95% confidence limits are described in Table 4.1. The hazard ratios, which quantify the difference in survival curves compared to the positive, control (6% glucose solution), and are shown in Fig 4.4. Relative to the controls, survival significantly varied across the concentrations tested except 12.5mg/ml (P<0.0001).

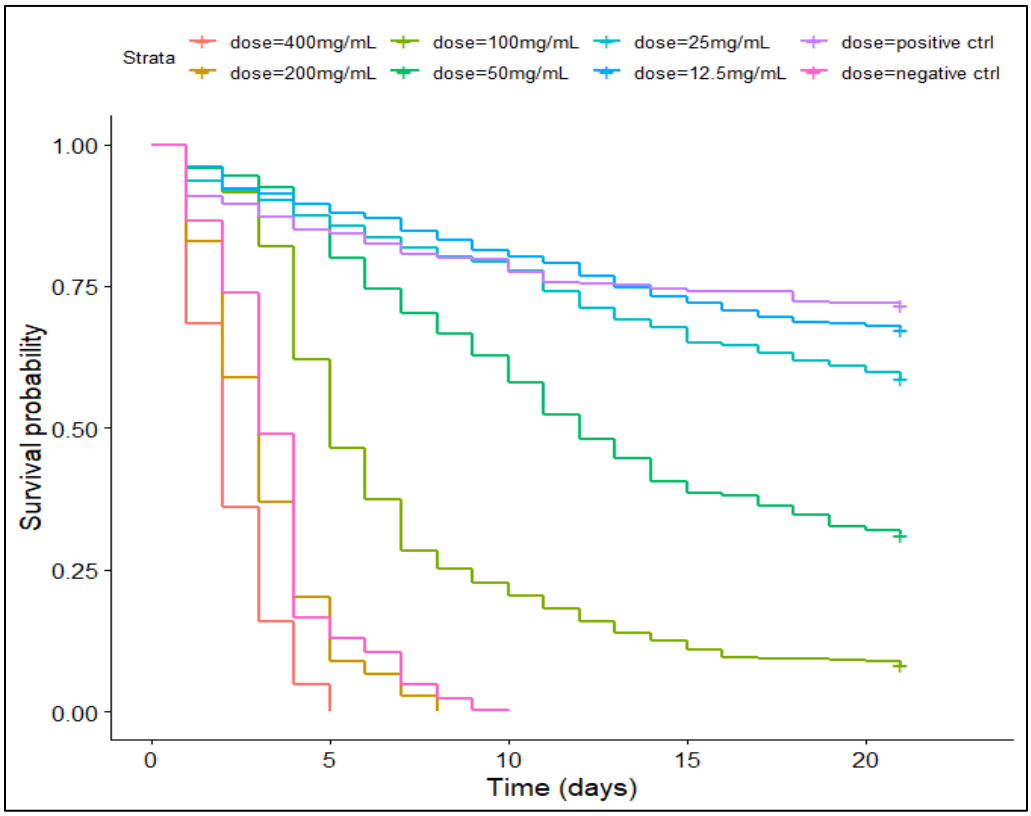


Figure 4.3 Kaplan-Meier survival curves for *Ae. aegypti* orally fed on different concentrations of *P. dulce* extracts compared to a positive control (6% glucose) and negative control (distilled water).

Table 4.1: Median survival times of female *Ae. aegypti* fed on *P. dulce* extracts and associated 95% confidence limits (CL).

Dose (mg/mL)	Median time (days)	Lower 95% CL	Upper 95% CL
400	2	2	2
200	3	3	3
100	5	5	6
50	12	11	13
25	–	–	–
12.5	–	–	–
Positive control (6% glucose solution)	–	–	–
Negative control (distilled water)	3	3	4

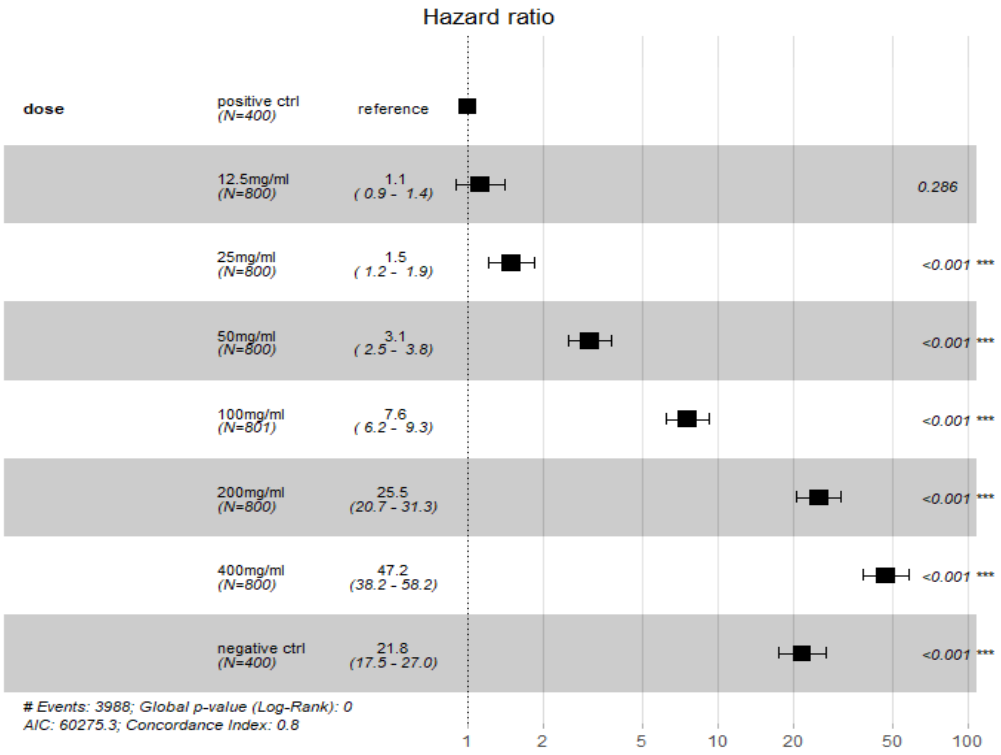


Figure 4.4: Hazard ratios for survival analysis of female *Ae. aegypti* on *P. dulce* extract.

4.1.3 Dose-response analysis for *Pithecellobium dulce* extract.

Pithecellobium dulce extract had an estimated LD_{10} of 5.5mg/ml (95%CI 4.7-6.3mg/ml) and LD_{50} of 25.8mg/ml (95%CI 24.3-27.3 mg/ml). The LD_{10} allowed maximum survival of *Ae. aegypti,* however, the value was lower than the lowest dose tested (12.5mg/ml) and it was not differ significantly different from the positive control. Thus, the LD_{50} of 25.8mg/ml was considered the optimal concentration (Fig 4.5)

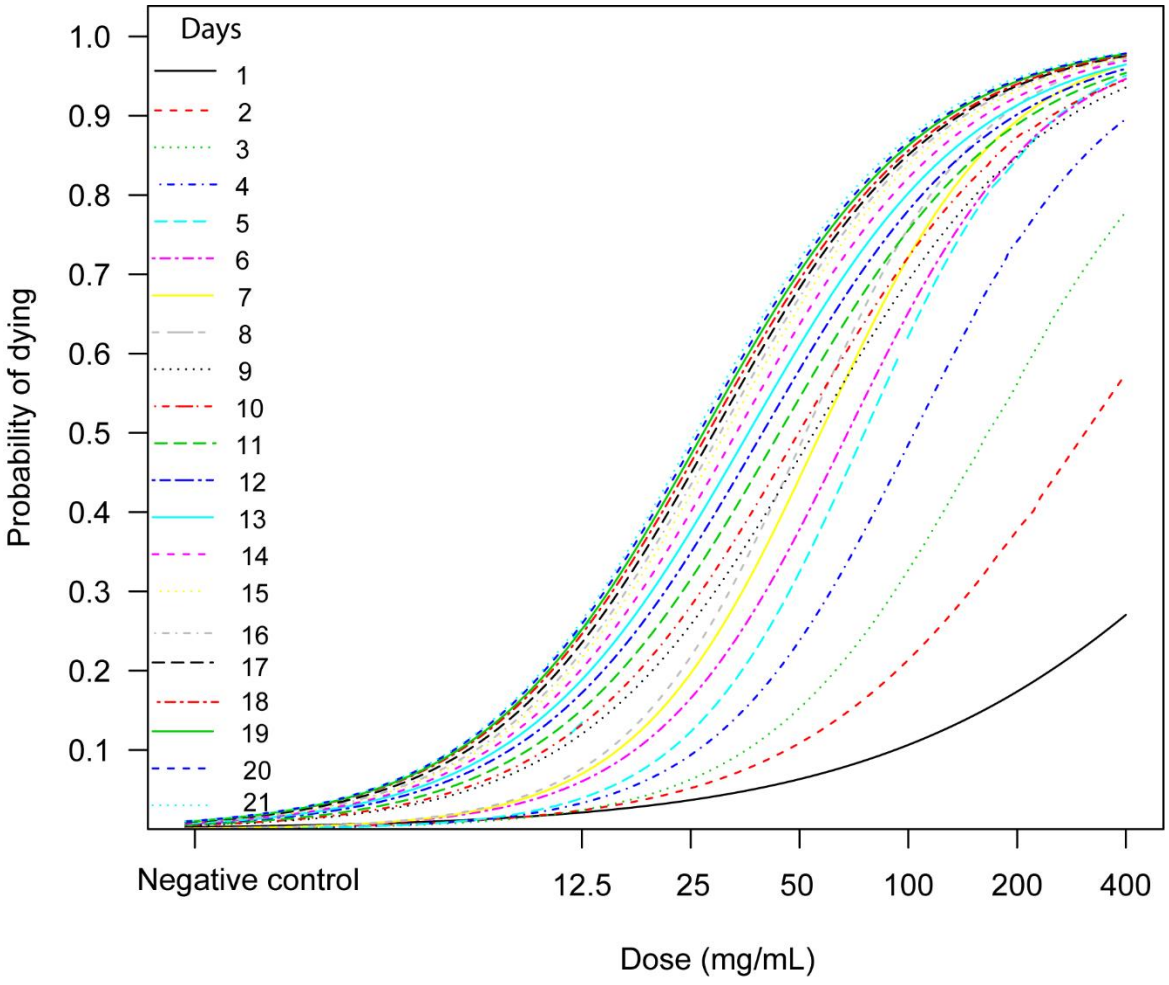
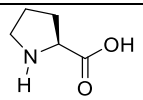
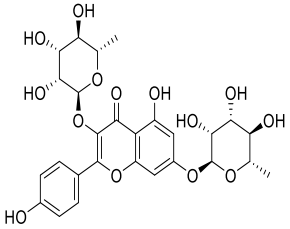
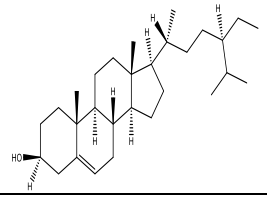


Figure 4.5: Estimated dose-response curves showing the probability of *Ae. aegypti* dying against dose level for a period of 21 days. At day 21, *P. dulce* extract had an estimated LD_{10} and $LD_{50\ o}$ of 5.5mg/mL and 25.8mg/ml respectively.

4.2 Chemical analysis of the *Pithecellobium dulce* extract and the mosquito midgut

Three classes of compounds including amino acids, glycosylated flavonoid and phyto-sterol were tentatively identified in both the plant and the midgut extract by LC-QqQ MS, based on molecular ion peaks, mass fragmentation and literature data. Proline eluted at 1.66 mins with a molecular ion peak $[M + H]^+$ at *m/z* 116.1. Kaempferol 3-0 Rhamnoside (Afzelin) eluted at 21.31 mins and had a molecular ion peak $[M + H]^+$ at *m/z* 433.5 with two key characteristic aglycone fragment ions at m/z 285.1 and at *m/z* 149.1 (Table 4.2; Fig 4.6). Unlike in the mosquito midgut, the detection of kaempferol 3-0 Rhamnoside was associated with the presence of double mass plus a sodium adduct at *m/z* 888.2137, **$2[M + H]^+ + Na$** in the plant extract. β sitosterol eluted at 29.69 mins and had a molecular ion peak $[M + H]^+$ at *m/z* 415.7. An evidence of double mass plus a sodium adduct at *m/z* 852.2537 **$2[M + H]^+ + Na$** was also reported in the mosquito midgut. Two unidentified compounds with molecular ion peaks $[M + H]^+$ at *m/z* 187.9 and 201.3 were found in both the plant extract and mosquito midgut.

Table 4.2: LC-QqQ MS fragments of identified compounds in the plant and the midgut extract (pool of 400 midguts).

Peak No	T_R (min)	Compound	Structure	Class of compound	$[M+H]^+$	$[M-H]^-$	Positive mode fragmentation	Reference
1.	1.661	Proline		Amino acid	116.1	115.1	-	-
2.	21.311	Kaempferol 3-O Rhamnoside		Flavanoid	433.4	431.4	285.1, 273.1, 257.6	Jang *et al*., 2018; Murugesan *et al*, 2019
3.	29.696	β sitosterol		Phyto-sterol	415.7	413.7	396.1, 303.2	Azeez *et al.*,2018

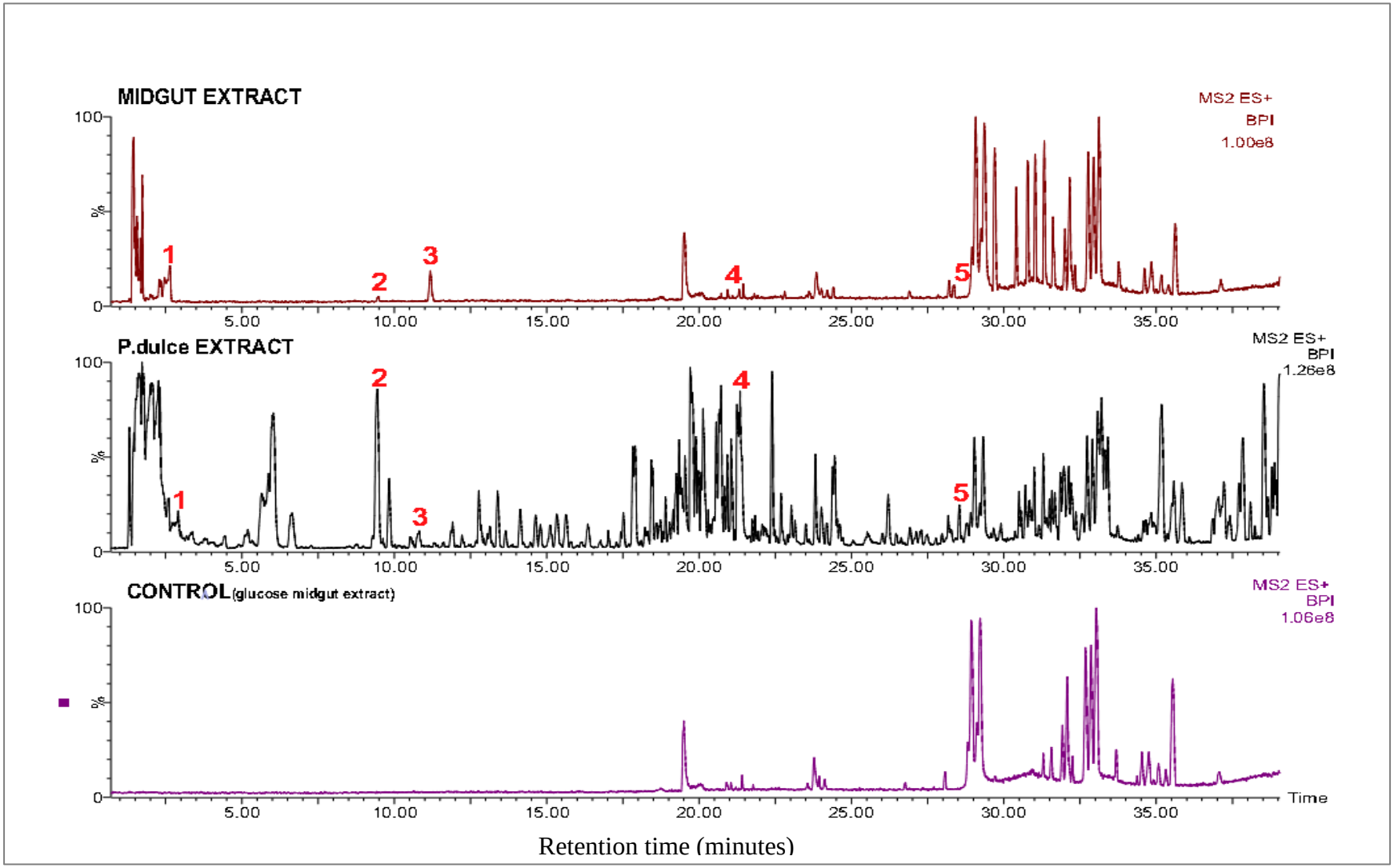


Figure 4.6: LC-QqQ-MS profile of the *P. dulce* and the mosquito midgut extract. Compound **1.** Proline, **2 & 3** unidentified **4.** Kaempferol 3-O- rhamnoside **5.** β sitosterol

4.2.1 GC-MS analysis of the *Pithecellobium dulce* extract and the mosquito midgut extract

GC-MS analysis of *P. dulce,* pools of mosquito midgut after feeding on *P. dulce* extract and pools of mosquito midgut exclusively fed on glucose solution (control) identified several metabolites including hexadecanoic acid, octadecanoic acid, caryophyllene oxide, octadecenoic acid, 9, 12 octadecadienoic acid (z,z), 9, 12, 15 octadecatrienoic acid and 4,8,12,16 tetramethyl-heptadecan-4-olide among others. Out of these metabolites, oleic acid was common in midgut of mosquitoes exclusively fed on glucose and the plant extract eluting at 25.35 mins. Hexadecanoic acid was detected in the plant extract, mosquito midgut fed on extract and mosquito midgut exclusively fed on glucose. In addition to the aforementioned metabolites, phytol was detected in the plant extract, it eluted at 25.14 mins, and dl-alpha tocopherol that eluted at 35.05 mins as illustrated in the appendix. Interestingly, linoleic acid, caryophyllene oxide, palmitoleic acid and octadecanoic acid were the only unique metabolites detected in both the plant extract and the midgut of mosquitoes fed on the extract (Fig 4.7).

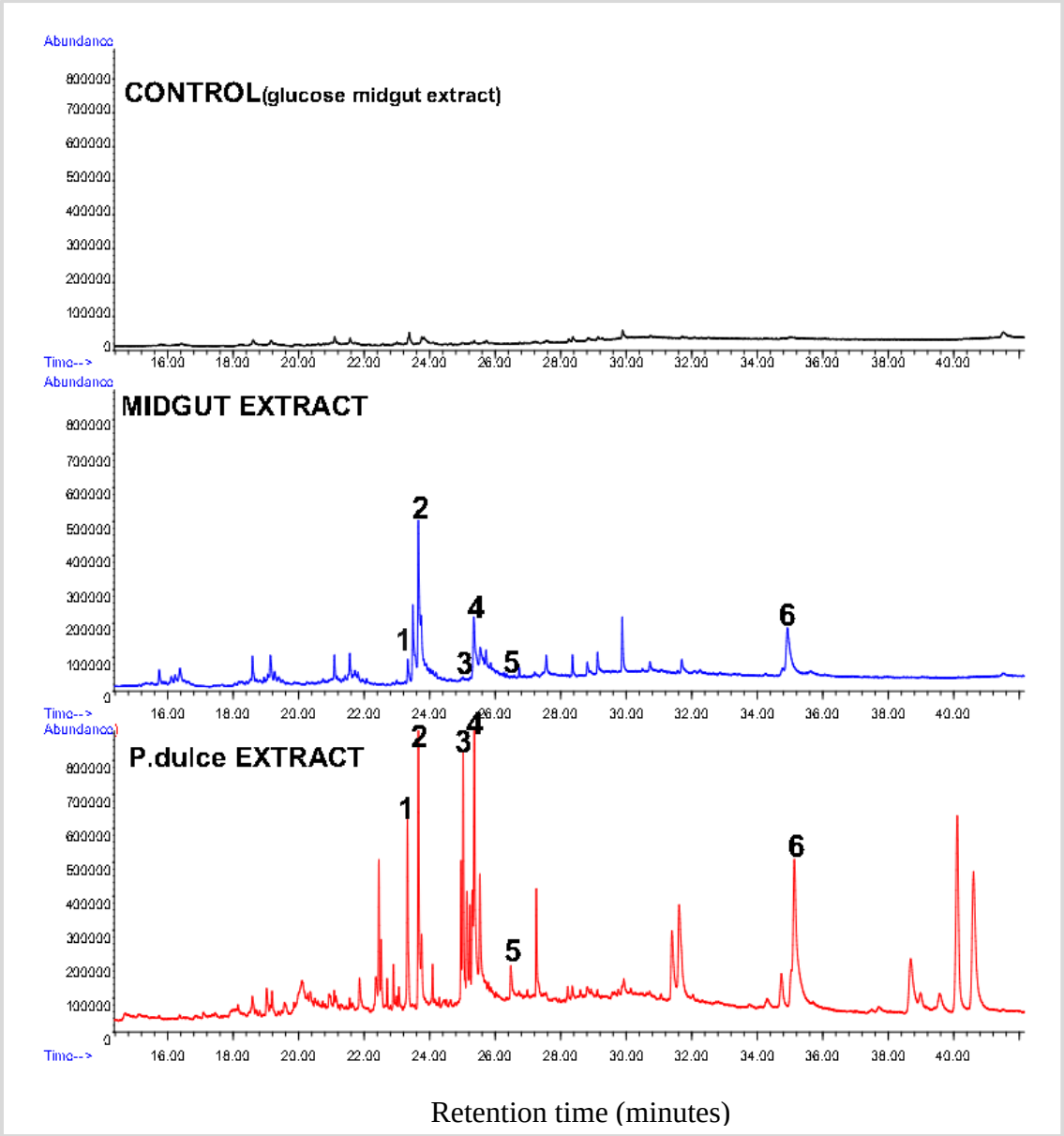


Figure 4.7: GC-MS of identified metabolites in the plant extract and the mosquito midgut. Compound **1.** Palmitoleic acid **2**. Hexadecanoic acid **3**. Caryophyllene oxide **4**. Linoleic acid **5**. Octadecanoic acid and **6**. Cholesterol

4.3 Effect of *Pithecellobium dulce* extract on infection success.

4.3.1 Proportion rate of infection by *Ae. aegypti* post-infection with CHIKV before and after feeding on *P. dulce* extract.

Aedes aegypti feeding success rate among the different treatments was high ranging from 70-90%. The infectious blood using the frozen and freshly cultured virus had a mean titer of Log $10^{(5.9542)}$ and Log $10^{(8.173)}$ plaque forming units (PFU) respectively. The pre- and post-feeding blood meal titers were nearly the same in virus titer. High infection rates were observed when mosquitoes were exposed to freshly cultured virus compared to the frozen virus. Using the freshly cultured virus, the pre-and post-exposed recorded significantly high infection rate compared to other treatments ($p<0.001$) (Figure 4.8A). The post-exposed recorded the lowest infection rate (Figure 4.8A). Despite the low infection rate observed in the control and pre-exposed treatments, these treatments recorded significantly high dissemination rates ($p<0.001$) (Figure 4.8B). Similar to the infection rates, the post-exposed recorded significant low dissemination rates compared to other treatments ($p<0.001$), (Figure 4.8B). Transmission was observed in the pre-exposed at 7.14%, the pre and post-exposed at 7.84% and the control at 30.48%. The post-exposed treatment recorded no transmission.

Infection using the frozen virus similarly recorded an interesting trend despite the sample size. The control treatment recorded highly significant infection rates compared to the plant extract treatments ($p<0.001$) (Figure 4.8 C). No significant difference was observed between the pre-exposed and post exposed treatments. The pre and post-exposed on the other hand recorded the lowest infection rate. Significant dissemination rates were observed in the control and the pre-exposed treatments ($p<0.001$). The post-exposed

recorded low dissemination rate while no dissemination was observed in the pre and post-exposed treatments (Figure 4.8 D). No transmission was observed among the treatments.

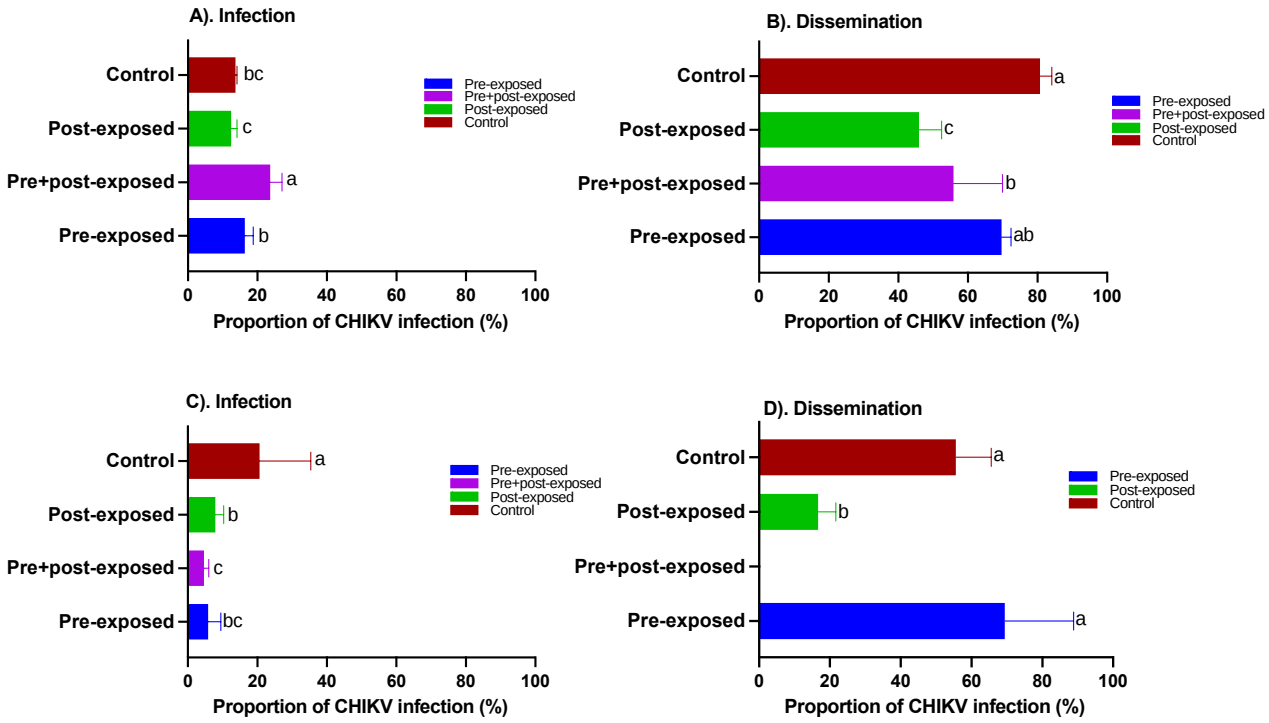


Figure 4.8 Proportion of infection and dissemination by *Ae. aegypti* post-infection with either freshly cultured CHIKV (A and B) or frozen virus (C and D) before and after feeding on *P.dulce* extract. Same small letters represent no significant difference while different letters represent significant difference among the treatments.

4.3.2: Replication dynamics of chikungunya virus in *Aedes aegypti* among the different treatments days post infection.

The bodies, legs and saliva of individual *Ae. aegypti* maintained on the different treatments were analyzed using plaque assay to determine the viral titers. The type of virus used during infection and days post infection (dpi) recorded no significant difference in the mean titers of the treatments ($F=2.85$, $df=1$, $P=0.1$) and ($F=0.71$, $df=2$, $P=0.5$) respectively. However, highly significant difference was observed among the treatments ($F=6.56$, $df=3$, $P<0.001$). During infection, the post-exposed treatment varied

significantly from the control (odds ratio 0.134, 95% CI 0.0364-0.03595, P<0.001) while there was no significant difference between the pre-exposed and the pre- and post-exposed treatments compared to the control (Fig 4.9 A).

Dissemination of CHIKV in *Ae. aegypti* recorded interesting results. Despite having observed no significant difference in mean titers of the treatments in either of the type of virus used, dissemination using frozen virus was only observed in the pre-exposed and control on day 5 and 10 post infection. Irrespective of dpi, the pre-exposed and control recorded mean log titers of 3.86PFU/mL and 3.54PFU/mL respectively. The post-exposed recorded relatively reduced log titer of 2.18PFU/mL while no dissemination was observed in the pre and post-exposed treatment. In the freshly cultured virus, highly significant difference was observed among the treatments (F=16.5, df=3, P<0.001) (Figure 8.0 B). The post-exposed treatment was highly significant compared to the control as well as the pre-exposed treatment (P<0.001) (Figure 8.0 B). No significant difference was observed between the pre-exposed and pre + post-exposed treatments attributing the effect to the pre-exposure to the plant extract (Figure 4.9 B).

Transmission of CHIKV in *Ae. aegypti* was only observed while using the freshly cultured virus. The control treatment recorded mean titers of 3.94PFU/mL while the pre-exposed and the pre-and post-exposed treatments recorded mean titers of 3.77PFU/mL and 2.00PFU/mL respectively. No transmission was observed in the post-exposed treatment.

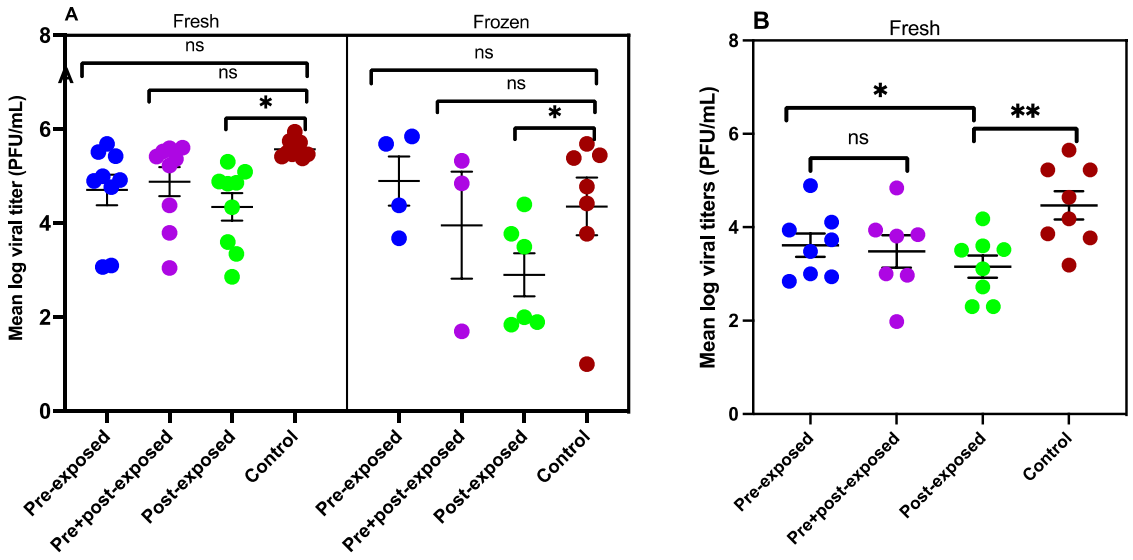


Figure 4.9: Chikungunya virus replication dynamics in *Ae. aegypti* before and after feeding on *P. dulce* extract. Infection using both freshly cultured and frozen virus (A), dissemination in freshly cultured virus (B). The dots and error bars represent individual mean titers among the different treatments days post infection ($p<0.05$).

CHAPTER 5.0: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

Determining the effect of plant feeding on pathogen transmission dynamics in mosquito disease vectors is a vital step in the development of novel control strategies. Findings from this study demonstrated that DMSO extracts of *P. dulce* orally fed to *Ae. aegypti* reduced survival of this vector in a dose dependent manner. Dose-response activity of plant extracts on mosquito mortality is well documented in the malaria vector *An. gambiae* (Wachira *et al*., 2014). The estimated optimal dose of the plant extract that enhanced survival of *Ae. aegypti* beyond 21 days was 25.8 mg/mL far beyond the value (13.1 ± 0.8 days), reported by Nyasembe *et al*., (2021) that assessed survival after feeding the mosquito on fresh *P. dulce* cuttings. The disparity in the optimal survival dose could be attributed to the difference in study design likely resulting in varied amount of the plant content ingested. Nonetheless, the present finding confirms the impact on mosquito survival following a meal on this plant. Further evidence of higher plant feeding by this vector and repeated detection of this plant in the midgut of wild caught *Ae. aegypti* (Wanjiku *et al.,* 2021; Olson *et al*., 2020) lends support for this view suggesting that host plants could in fact play an important role in regulating the population dynamics of this vector in nature.

Further chemical analysis of the midgut content in pools after ingestion of the plant extract identified metabolites namely Proline, β sitosterol, Kaemperol 3-O-rhamnoside and Linoleic acid. Proline is an amino acid often utilized as an energy substrate during flight by *Ae. aegypti* females as well as during reproduction (Nyasembe *et al*., 2021; Scaraffia and Wells, 2003). β Sitosterol is a phyto sterol important in the growth,

development, metabolic actions as well as reproduction success of mosquitoes (Perera and Wijerathna, 2019). It has also been found to exhibit larvicidal effect against *Ae. aegypti* (Ali *et al.*, 2018; Sigamani *et al*., 2020). Kaempferol 3-O-rhamnoside is a glycosylated flavonoid that protects plants from oxidative, nuclear DNA damage and fungal infections (Rashid *et al*., 2019). In humans, Kaempferol 3-O-rhamnoside has been reported to possess anti-cancer, anti-oxidant and anti-microbial activities (Diantini *et al*., 2012; Yang *et al.*, 2014). Linoleic acid on the other hand, is a poly-saturated fatty acid (PUFA's) common in plant oils. In insects, linoleic acid is acquired through feeding on diet and it is used in the biosynthesis of prostaglandins (PGs) and cell membranes. Prostaglandins have been shown to stimulate egg-laying behavior and improve reproduction in some insect species as well as mediates host immune responses impacting host immunity (Stanley-Samuelson and Loher, 1986; Ahmed *et al.*, 2018; Kim and Stanley, 2021). In *Ae. aegypti*, prostaglandins have been show to control the amplitude of immune responses, influencing susceptibility of this vector to pathogens. Overall, these findings confirm ingestion of metabolites in meals on this plant. The presence of these metabolites, which exhibit a wide range of biological activities in the plant diet, suggest their potential influence on the susceptibility of *Ae. aegypti* to viral pathogens.

Indeed our investigations established that *P. dulce* metabolites had an influence on *Ae. aegypti* susceptibility to CHIKV. We observed the highest infection rates in mosquitoes that were fed on the extract before infection and maintained on glucose solution after infection (pre-exposed), and in those fed on plant extract before and after infection (pre and post exposed), however, with a corresponding subsequent decrease in the dissemination rates in the latter cohort. Those fed on glucose solution before infection then plant extract after infection (post-exposed) and control treatments on the other hand,

had low infection rates with subsequent increase in dissemination rates especially in the latter. The potential negative effect on the midgut microbiota (Dennison *et al.*, 2014; Jupatanakul *et al.*, 2014; Gabrieli *et al*., 2021) or via immune suppression (Tanabe *et al*., 2018) could account for the increased infection rate imposed by the pre-exposed treatment. Alternatively, the immune response mounted in response to the infection challenged could synergise with metabolites contained in the plant to help limit virus infection as observed in the post-exposed treatment. In either case, the lower dissemination rates observed in the post-exposed treatments suggest potential influence of the plant extract on the virus replication success and subsequent transmission. The absence of transmission in the post-exposed further lends support for this hypothesis.

Midgut infection days post feeding was reported by day 5, similar to previous studies that demonstrated that infection by CHIKV in East Africa regions occurs within 2-9 days after infection (Rudolph *et al.*, 2014). The viral titers increased progressively days post feeding when using either the freshly cultured or the frozen virus. Marked vector competence was however, observed while using freshly cultured virus compared to frozen virus, a trend similar to that shown while using Zika virus (Azar and Weaver, 2019). Presence of CHIKV in the saliva was observed in the control, pre-exposed and pre-and post-exposed treatments, although with significantly reduced titer in the latter treatment. This could potentially be attributed to the effect of *P. dulce* extract. The absence of transmission of CHIKV in the post-exposed treatments further supports possibility of *P. dulce* extract influencing viral activity in the vector. Further analysis with the tentatively identified metabolites singly and in blends is needed to ascertain their effect on competence of this mosquito to the CHIKV.

5.2 Conclusion

In conclusion, oral feeding of *Ae. aegypti* on extracts of *P. dulce* affected survival and modulated infection success with pronounced increase at the dissemination stage. Ingestion of metabolites occurred during plant feeding and chemical analyses of the midgut identified compounds namely Proline, β sitosterol, Kaemperol 3-O-rhamnoside and Linoleic acid. These compounds are known to influence survival and reproduction of the insects as well as anti-pathogen activities influencing host immunity. Their contribution to the observed anti-CHIKV effect in *Ae. aegypti* when orally fed on extracts of this plant, requires further elucidation. Overall, these findings demonstrate not only the effect of plant diet on the survival of this mosquito vector but also its impact on modulating transmission success and thus competence to CHIKV.

5.3 Recommendations

Transmission blocking could be envisaged if the detected metabolites are found to inhibit viral replication in the mosquito through attractive targeted sugar baits (ATSBs) or provide leads into development antiviral strategies targeting plant-feeding behavior.

REFERENCES

Agha, S. B., Chepkorir, E., Mulwa, F., Tigoi, C., Arum, S., Guarido, Ambala P; Chelangat B; Lutomiah, J., Tchouassi, D and Rosemary Sang: Vector competence of populations of *Aedes aegypti* from three distinct cities in Kenya for chikungunya virus.*PLoSNeglectedTropicalDiseases* https://doi.org/10.1371/journal.pntd.0005860.

Ahmed, S., Stanley, D., and Kim, Y. (2018). An Insect Prostaglandin E2 Synthase Acts in Immunity and Reproduction. *Frontiers in Physiology*, *9*, 1231. https://doi.org/10.3389/fphys.2018.01231

Alaux, C., Ducloz, F., Crauser, D., and Le Conte, Y. (2010). Diet effects on honeybee immunocompetence. *Biology Letters*. https://doi.org/10.1098/rsbl.2009.0986

Ali, S. I., Gopalakrishnan, B., and Venkatesalu, V. (2018). Evaluation of larvicidal activity of Senecio laetus Edgew. against the malarial vector, *Anopheles stephensi*, dengue vector, *Aedes aegypti* and Bancroftian filariasis vector, *Culex quinquefasciatus*. *South African Journal of Botany*, *114*, 117–125. https://doi.org/10.1016/j.sajb.2017.10.018

Alto, B. W., and Juliano, S. A. (2001). Precipitation and temperature effects on populations of Aedes albopictus (Diptera: Culicidae): implications for range expansion. *Journal of Medical Entomology*, *38*(5), 646–656. https://doi.org/10.1603/0022-2585-38.5.646

Amarasinghe, A., Kuritsky, J. N., William Letson, G., and Margolis, H. S. (2011). Dengue virus infection in Africa. *Emerging Infectious Diseases*, *17*(8), 1349–1354. https://doi.org/10.3201/eid1708.101515

Angelini, R., Finarelli, A. C., Angelini, P., Po, C., Petropulacos, K., Macini, P., Fiorentini C, Fortuna C, Venturi G, Romi R, Majori G, Nicoletti L, Rezza G, Cassone, A. (2007). An outbreak of chikungunya fever in the province of Ravenna, Italy. *Euro Surveillance : Bulletin Europeen Sur Les Maladies Transmissibles = European Communicable Disease Bulletin*, *12*(9), E070906.1. https://doi.org/10.2807/esw.12.36.03260-en

Azar, S. R., and Weaver, S. C. (2019). Vector competence: What has Zika virus taught us? *Viruses*, *11*(9), 1–24. https://doi.org/10.3390/v11090867

Azeez, R. A., Abaas, I. S., and Kadhim, E. J. (2018). Isolation and characterization of β-sitosterol from *Elaeagnus angustifolia* cultivated in iraq. *Asian Journal of Pharmaceutical and Clinical Research*, *11*(11), 442–446. https://doi.org/10.22159/ajpcr.2018.v11i11.29030

Beier, J. C., Hassanali, A., Jackson, R. R., Githure, J. I., Manda, H., Gouagna, L. C., and Foster, W. A. (2007). Effect of discriminative plant-sugar feeding on the survival and fecundity of *Anopheles gambiae*. *Malaria Journal*. https://doi.org/10.1186/1475-2875-6-113

Bellini, R., Medici, A., Puggioli, A., and Balestrino, F. (2013). Pilot Field Trials With *Aedes albopictus* Irradiated Sterile Males in Italian Urban Areas, 317–325.

Bennett, K. E., Olson, K. E., Muñoz, M. de L., Fernandez-Salas, I., Farfan-Ale, J. A., Higgs, S and Beaty, B. J. (2002). Variation in vector competence for dengue 2 virus among 24 collections of Aedes aegypti from Mexico and the United States. *American Journal of Tropical Medicine and Hygiene*, *67*(1), 85–92. https://doi.org/10.4269/ajtmh.2002.67.85

Boissière, A., Tchioffo, M. T., Bachar, D., Abate, L., Marie, A., Nsango, S. E and Morlais, I. (2012). Midgut Microbiota of the Malaria Mosquito Vector *Anopheles gambiae* and Interactions with Plasmodium falciparum Infection. *PLOS Pathogens*, *8*(5), 1–12. https://doi.org/10.1371/journal.ppat.1002742

Brackney, D. E., Foy, B. D., and Olson, K. E. (2008). The effects of midgut serine proteases on dengue virus type 2 infectivity of *Aedes aegypti*. *American Journal of Tropical Medicine and Hygiene*, *79*(2), 267–274.

Chen, X. G., Mathur, G., and James, A. A. (2008). *Chapter 2 Gene Expression Studies in Mosquitoes*. *Advances in Genetics* (Vol. 64). Elsevier Masson SAS. https://doi.org/10.1016/S0065-2660(08)00802-X

Chepkorir, E., Lutomiah, J., Mutisya, J., Mulwa, F., Limbaso, K., and Orindi, B. (2014). Vector competence of *Aedes aegypti* populations from Kilifi and Nairobi for dengue 2 virus and the influence of temperature, 1–8.

Chretien, J. P., Anyamba, A., Bedno, S. A., Breiman, R. F., Sang, R., Sergon, K and Linthicum, K. J. (2007). Drought-associated chikungunya emergence along coastal East Africa. *American Journal of Tropical Medicine and Hygiene*, *76*(3), 405–407.

https://doi.org/10.4269/ajtmh.2007.76.405

Cory, J. S., and Hoover, K. (2006). Plant-mediated effects in insect-pathogen interactions. *Trends in Ecology and Evolution*. https://doi.org/10.1016/j.tree.2006.02.005

Dash, P., Parida, M., S R, S., Verma, S., Tripathi, N., Shrivastava, A., and Krishnamurthy, S. (2007). East Central South African genotype as the causative agent in reemergence of Chikungunya outbreak in India. *Vector Borne and Zoonotic Diseases (Larchmont, N.Y.)*, *7*, 519–527. https://doi.org/10.1089/vbz.2007.7272

De Almeida Di Maio Ferreira, F. C. P., Da Silva, A. S. V., Recht, J., Guaraldo, L., Moreira, M. E. L., Siqueira, A. M. De, and Brasil, P. (2021). Vertical transmission of chikungunya virus: A systematic review. *PLoS ONE*, *16*(4 April), 1–19. https://doi.org/10.1371/journal.pone.0249166

Dennison, N. J., Jupatanakul, N., and Dimopoulos, G. (2014). The mosquito microbiota influences vector competence for human pathogens. *Current Opinion in Insect Science*, *3*, 6–13. https://doi.org/10.1016/j.cois.2014.07.004

Diantini, A., Subarnas, A., Lestari, K., Halimah, E., Susilawati, Y., Supriyatna, … Abdulah, R. (2012). Kaempferol-3-O-rhamnoside isolated from the leaves of Schima wallichii Korth. inhibits MCF-7 breast cancer cell proliferation through activation of the caspase cascade pathway. *Oncology Letters*, *3*(5), 1069–1072. https://doi.org/10.3892/ol.2012.596

Dickson, L. B., Sanchez-Vargas, I., Sylla, M., Fleming, K., and Black IV, W. C. (2014). Vector Competence in West African *Aedes aegypti* Is Flavivirus Species and Genotype Dependent. *PLOS Neglected Tropical Diseases*, *8*(10), 1–11. https://doi.org/10.1371/journal.pntd.0003153

Elsinga, J., Van Der Veen, H. T., Gerstenbluth, I., Burgerhof, J. G. M., Dijkstra, A., Grobusch, M. P., and Bailey, A. (2017). Community participation in mosquito breeding site control: An interdisciplinary mixed methods study in Curaçao. *Parasites and Vectors*, *10*(1), 1–14. https://doi.org/10.1186/s13071-017-2371-6

Eyase, F., Langat, S., Berry, I. M., Mulwa, F., Nyunja, A., Mutisya, Samuel Owaka, Samson L, Victor O, Hellen K, Edith K, Joel L, Richard G, Sang, R. (2020). Emergence of a novel chikungunya virus strain bearing the E1:V80A substitution, out of the Mombasa, Kenya 2017-2018 outbreak. *PLoS ONE*, *15*(11 November), 1–

14. https://doi.org/10.1371/journal.pone.0241754

Feldstein, L. R., Ellis, E. M., Rowhani-Rahbar, A., Hennessey, M. J., Staples, J. E., Halloran, M. E., and Weaver, M. R. (2019). Estimating the cost of illness and burden of disease associated with the 2014–2015 chikungunya outbreak in the U.S. Virgin Islands. *PLoS Neglected Tropical Diseases*, *13*(7), 1–14. https://doi.org/10.1371/journal.pntd.0007563

Finney, D. J. (1952). Statistical method in biological assay. *Statistical Method in Biological Assay*.

Foster, W. A., and Takken, W. (2004). Nectar-related vs. human-related volatiles: behavioural response and choice by female and male *Anopheles gambiae* (Diptera: Culicidae) between emergence and first feeding. *Bulletin of Entomological Research*. https://doi.org/10.1079/ber2003288

Fourié, T., Dia, A., Savreux, Q., Pommier de Santi, V., de Lamballerie, X., Leparc-Goffart, I., and Simon, F. (2021). Emergence of Indian lineage of ECSA chikungunya virus in Djibouti, 2019. *International Journal of Infectious Diseases*, *108*, 198–201. https://doi.org/10.1016/j.ijid.2021.03.090

Gabrieli, P., Caccia, S., Varotto-Boccazzi, I., Arnoldi, I., Barbieri, G., Comandatore, F., and Epis, S. (2021). Mosquito Trilogy: Microbiota, Immunity and Pathogens, and Their Implications for the Control of Disease Transmission . *Frontiers in Microbiology* . Retrieved from https://www.frontiersin.org/article/10.3389/fmicb.2021.630438

Gérardin, P., Couderc, T., Bintner, M., Tournebize, P., Renouil, M., Lémant, J., Boisson, V; Borgherini, G; Frédérik; S, Frédéric; Lecuit, M; and Michault, A (2016). Chikungunya virus–associated encephalitis. *Neurology*, *86*(1), 94 LP – 102. https://doi.org/10.1212/WNL.0000000000002234.

Gérardin, P., Sampériz, S., Ramful, D., Boumahni, B., Bintner, M., Alessandri, J. L., Carbonnier; M, Tiran-Rajaoefera, I; Beullier, G; Boya; I, Noormahomed; B, Okoı; J, Rollot; O, Cotte;L; Marie-Christine Jaffar-Bandjee, Michault; A, Favier, F; Kaminski, M; Fourmaintraux; A; and Fritel, X. (2014). Neurocognitive Outcome of Children Exposed to Perinatal Mother-to-Child Chikungunya Virus Infection: The CHIMERE Cohort Study on Reunion Island. *PLoS Neglected Tropical Diseases*,

8(7). https://doi.org/10.1371/journal.pntd.0002996

Gilotra, S. K., and Shah, K. V. (1967). Laboratory studies on transmission of chikungunya virus by mosquitoes12. *American Journal of Epidemiology*, *86*(2), 379–385. https://doi.org/10.1093/oxfordjournals.aje.a120748

Gorchakov, R., Adams, A. P., Vinet-Oliphant, H., Weaver, S. C., Wang, E., Plante, K., … Seymour, R. L. (2014). Chikungunya Vaccine Candidate Is Highly Attenuated and Protects Nonhuman Primates Against Telemetrically Monitored Disease Following a Single Dose. *Journal of Infectious Diseases*. https://doi.org/10.1093/infdis/jiu014

Grossi-Soyster, E. N., Cook, E. A. J., de Glanville, W. A., Thomas, L. F., Krystosik, A. R., Lee, J., and LaBeaud, A. D. (2017). Serological and spatial analysis of alphavirus and flavivirus prevalence and risk factors in a rural community in western Kenya. *PLoS Neglected Tropical Diseases*, *11*(10), 1–16. https://doi.org/10.1371/journal.pntd.0005998

Gu, W., Müller, G., Schlein, Y., Novak, R. J., and Beier, J. C. (2011). Natural plant sugar sources of Anopheles mosquitoes strongly impact malaria transmission potential. *PLoS ONE*, *6*(1). https://doi.org/10.1371/journal.pone.0015996

Harrington, L. C., Edman, J. D., and Scott, T. W. (2009). Why Do Female <I>Aedes aegypti</I> (Diptera: Culicidae) Feed Preferentially and Frequently on Human Blood? *Journal of Medical Entomology*. https://doi.org/10.1603/0022-2585-38.3.411

Honório, N. A., Wiggins, K., Eastmond, B., Câmara, D. C. P., and Alto, B. W. (2019). Experimental vertical transmission of chikungunya virus by brazilian and florida *Aedes albopictus* populations. *Viruses*, *11*(4). https://doi.org/10.3390/v11040353

Huang, Y. M., and Rueda, L. M. (2017). Pictorial keys to the sections, groups, and species of the Aedes (Finlaya) in the Afrotropical Region (Diptera: Culicidae). *Zootaxa*, *4221*(1), 131–141. https://doi.org/10.11646/zootaxa.4221.1.7

Jang, G. H., Kim, H. W., Lee, M. K., Jeong, S. Y., Bak, A. R., Lee, D. J., and Kim, J. B. (2018). Characterization and quantification of flavonoid glycosides in the Prunus genus by UPLC-DAD-QTOF/MS. *Saudi Journal of Biological Sciences*, *25*(8), 1622–1631. https://doi.org/10.1016/j.sjbs.2016.08.001

Jaime Andres Cardona-Ospina, Fredi Alexander Diaz-Quijano, Alfonso J, and Rodriguez-

Morales (2015). International Journal of Infectious Diseases, *38*, 60–61. https://doi.org/10.1016/j.ijid.2015.07.015.

Jeske, D. R., Xu, H. K., Blessinger, T., Jensen, P., and Trumble, J. (2009). Testing for the equality of EC50 values in the presence of unequal slopes with application to toxicity of selenium types. *Journal of Agricultural, Biological, and Environmental Statistics*, *14*(4), 469–483. https://doi.org/10.1198/jabes.2009.07088

Jupatanakul, N., Sim, S., and Dimopoulos, G. (2014). The insect microbiome modulates vector competence for arboviruses. *Viruses*, *6*(11), 4294–4313. https://doi.org/10.3390/v6114294

Kalantri, S. P., Joshi, R., and Riley, L. E. E. W. (2006). Chikungunya epidemic : An Indian perspective, 315–322.

Kessler, A., and Baldwin, I. T. (2002). Plant responses to insect herbivory : The Emerging Molecular Analysis . *Annual Review of Plant Biology*. https://doi.org/10.1146/annurev.arplant.53.100301.135207

Kim, Y., and Stanley, D. (2021). Eicosanoid signaling in insect immunology: New genes and unresolved issues. *Genes*, *12*(2), 1–15. https://doi.org/10.3390/genes12020211

Kittayapong, P., Chansang, U., Chansang, C., and Bhumiratana, A. (2006). Community Participation and Appropriate Technologies for Dengue Vector Control at Transmission Foci in Thailand. *Journal of the American Mosquito Control Association*, *22*(3), 538–546. Retrieved from https://doi.org/10.2987/8756-971X(2006)22[538:CPAATF]2.0.CO

Konongoi, S. L., Nyunja, A., Ofula, V., Owaka, S., Koka, H., Koskei, E.,Fredrick, E; Daniel L; James,M; Joel, L; and Sang, R. (2018). Human and entomologic investigations of chikungunya outbreak in Mandera, Northeastern Kenya, 2016. *PLoS ONE*, *13*(10), 1–13. https://doi.org/10.1371/journal.pone.0205058

Krishnamoorthy, K., Harichandrakumar, K. T., Kumari, A. K., and Das, L. K. (2009). Burden of Chikungunya in India: Estimates of disability adjusted life years (DALY) lost in 2006 epidemic. *Journal of Vector Borne Diseases*, *46*(1), 26–35.

Kulkarni, K. V, Kaushik, C., Kulkarni, V., and Jamakhandi, V. R. (2018). Medicinal uses of *Pithecellobium dulce* and its health benefits. *~ 700 ~ Journal of Pharmacognosy and Phytochemistry*, *7*(2), 700–704.

Lefèvre, T., Oliver, L., Hunter, M. D., and De Roode, J. C. (2010). Evidence for trans-generational medication in nature. *Ecology Letters*. https://doi.org/10.1111/j.1461-0248.2010.01537.x

Lefèvre, T., Vantaux, A., Dabiré, K. R., Mouline, K., and Cohuet, A. (2013a). Non-Genetic Determinants of Mosquito Competence for Malaria Parasites. *PLoS Pathogens*. https://doi.org/10.1371/journal.ppat.1003365

Lefèvre, T., Vantaux, A., Dabiré, K. R., Mouline, K., and Cohuet, A. (2013b, June). Non-Genetic Determinants of Mosquito Competence for Malaria Parasites. *PLoS Pathogens*. https://doi.org/10.1371/journal.ppat.1003365

Lucantoni, L., Magaraggia, M., Lupidi, G., Ouédraogo, R., Coppellotti, O., Esposito, F., and Habluetzel, A. (2011). Novel, Meso-Substituted Cationic Porphyrin Molecule for Photo-Mediated Larval Control of the Dengue Vector Aedes aegypti. *PLoS Neglected Tropical Diseases*, *5*, e1434. https://doi.org/10.1371/journal.pntd.0001434

Lucero, M., Estell, R., Tellez, M., and Fredrickson, E. (2009). A retention index calculator simplifies identification of plant volatile organic compounds. *Phytochemical Analysis*, *20*(5), 378–384. https://doi.org/10.1002/pca.1137

Mason, P. J., and Haddow, A. J. (1957). An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952–1953. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *51*(3), 238–240. https://doi.org/10.1016/0035-9203(57)90022-6

McCarthy, M. K., and Morrison, T. E. (2016). Chronic chikungunya virus musculoskeletal disease: What are the underlying mechanisms? *Future Microbiology*, *11*(3), 331–334. https://doi.org/10.2217/fmb.15.147

Mease, L. E., Coldren, R. L., Musila, L. A., Prosser, T., Ogolla, F., Ofula, V. O., and Adungo, N. (2011). Seroprevalence and distribution of arboviral infections among rural Kenyan adults: A cross-sectional study. *Virology Journal*, *8*(July). https://doi.org/10.1186/1743-422X-8-371

Meister, S., Agianian, B., Turlure, F., Relógio, A., Morlais, I., Kafatos, F. C., and Christophides, G. K. (2009). Anopheles gambiae PGRPLC-mediated defense against bacteria modulates infections with malaria parasites. *PLoS Pathogens*, *5*(8).

https://doi.org/10.1371/journal.ppat.1000542

Molina-Cruz, A., Gupta, L., Richardson, J., Bennett, K., Black IV, W., and Barillas-Mury, C. (2005). Effect of mosquito midgut trypsin activity on dengue-2 virus infection and dissemination in Aedes aegypti. *American Journal of Tropical Medicine and Hygiene*, *72*(5), 631–637.

Monteiro, V. V. S., Navegantes-Lima, K. C., de Lemos, A. B., da Silva, G. L., de Souza Gomes, R., Reis, J. F., Junior, L.C., Silva, O.S., Romao, P, R, T and Monteiro, M. C. (2019). Aedes–Chikungunya Virus Interaction: Key Role of Vector Midguts Microbiota and Its Saliva in the Host Infection. *Frontiers in Microbiology*, *10*, 492. https://doi.org/10.3389/fmicb.2019.00492

Moore, S. M., ten Bosch, Q. A., Siraj, A. S., Soda, K. J., España, G., Campo, A., Gomez, S., Raybaud, B., Salas, D., Wenger, E., Welkhoff, P and Perkins, T. A. (2018). Local and regional dynamics of chikungunya virus transmission in Colombia: The role of mismatched spatial heterogeneity. *BMC Medicine*, *16*(1), 1–16. https://doi.org/10.1186/s12916-018-1127-2.

Mostowy, W. M., and Foster, W. A. (2004). *Antagonistic effects of energy status on meal size and egg-batch size of Aedes aegypti (Diptera: Culicidae)*. *Journal of Vector Ecology*.

Muller, G. C., Junnila, A., Traore, M. M., Traore, S. F., Doumbia, S., Sissoko, F., and Beier, J. C. (2017). The invasive shrub Prosopis juliflora enhances the malaria parasite transmission capacity of Anopheles mosquitoes : a habitat manipulation experiment. *Malaria Journal*, 1–9. https://doi.org/10.1186/s12936-017-1878-9

Müller, G. C., Xue, R.-D., and Schlein, Y. (2011). Differential attraction of *Aedes albopictus* in the field to flowers, fruits and honeydew. *Acta Tropica*, *118*(1), 45–49. https://doi.org/10.1016/J.ACTATROPICA.2011.01.009

Mulwa, F., Lutomiah, J., Chepkorir, E., Okello, S., Eyase, F., Tigoi, C., K, Michael and Sang, R. (2018). Vector competence of *Aedes bromeliae* and *Aedes vitattus* mosquito populations from Kenya for chikungunya virus. *PLoS Neglected Tropical Diseases*, *12*(10), e0006746. https://doi.org/10.1371/journal.pntd.0006746

Murugesan, S., Arumugam, V., Kumar, D., and Anuf, A. (2019). *Nutritional and therapeutic benefits of medicinal plant Pithecellobium dulce (Fabaceae): A review*.

https://doi.org/10.13140/RG.2.2.18703.33443

Nam, V. S., Yen, N. T., Phong, T. V., Ninh, T. U., Mai, L. Q., Lo, L. V.,Le, A.B., Briscombe,A., Aaskoz, J,G., Ryan., P.A and Kay, B. H. (2005). Elimination of dengue by community programs using Mesocyclops (copepoda) against *Aedes aegypti* in central Vietnam. *American Journal of Tropical Medicine and Hygiene*, *72*(1), 67–73. https://doi.org/10.4269/ajtmh.2005.72.67.

Nasir, S., Jabeen, F., Abbas, S., Nasir, I., and Debboun, M. (2017). Effect of climatic conditions and water bodies on population dynamics of the dengue vector, *Aedes aegypti* (Diptera: Culicidae). *Journal of Arthropod-Borne Diseases*, *11*(1), 50–59.

Nyamwaya, D. K., Otiende, M., Omuoyo, D. O., Githinji, G., Karanja, H. K., Gitonga, J. N., Laurent, Z; Otieno, J.R., Sang, R., Kamau, E., Cheruiyot, S., Otieno, E.,Agoti, C, N., Bejon, P., Thumbi, S.M and Warimwe, G. M. (2021). Endemic chikungunya fever in Kenyan children: a prospective cohort study. *BMC Infectious Diseases*, *21*(1), 1–10. https://doi.org/10.1186/s12879-021-05875-5.

Nyasembe, Vincent O., Tchouassi, D. P., Pirk, C. W. W., Sole, C. L., and Torto, B. (2018). Host plant forensics and olfactory-based detection in Afro-tropical mosquito disease vectors. *PLoS Neglected Tropical Diseases*. https://doi.org/10.1371/journal.pntd.0006185

Nyasembe, Vincent Odhiambo, Tchouassi, D. P., Muturi, M. N., Pirk, C. W. W., Sole, C. L., and Torto, B. (2021). Plant nutrient quality impacts survival and reproductive fitness of the dengue vector *Aedes aegypti*. *Parasites & Vectors*, 1–10. https://doi.org/10.1186/s13071-020-04519-y

Okech, B. A., Gouagna, L. C., Kabiru, E. W., Walczak, E., Beier, J. C., Yan, G., and Githure, J. I. (2004). Resistance of early midgut stages of natural *Plasmodium falciparum* parasites to high temperatures in experimentally infected *Anopheles gambiae* (Diptera: Culicidae). *Journal of Parasitology*, *90*(4), 764–768. https://doi.org/10.1645/GE-135R1

Olson, M. F., Garcia-luna, S., Juarez, J. G., Martin, E., Harrington, L. C., Eubanks, M. D.,Ismael E.B and Hamer, G. L. (2020). Vector Control , Pest Management , Resistance , Repellents Sugar Feeding Patterns for *Aedes aegypti* and *Culex quinquefasciatus* (Diptera : Culicidae) Mosquitoes in South Texas, *57*(February),

1111–1119. https://doi.org/10.1093/jme/tjaa005

Owino, E. A. (2018). Aedes spp mosquitoes and emerging neglected diseases of Kenya. *~ 1 ~ International Journal of Mosquito Research*, *5*(5), 1–11.

Padbidri, V. S., and Gnaneswar, T. T. (1979). Epidemiological investigations of chikungunya epidemic at Barsi, Maharashtra state, India. *Journal of Hygiene, Epidemiology, Microbiology, and Immunology*, *23*(4), 445—451. Retrieved from http://europepmc.org/abstract/MED/575900

Pavela, R., and Benelli, G. (2016). Ethnobotanical knowledge on botanical repellents employed in the African region against mosquito vectors - A review. *Experimental Parasitology*, *167*, 103–108. https://doi.org/10.1016/j.exppara.2016.05.010

Perera, H., and Wijerathna, T. (2019). Sterol Carrier Protein Inhibition-Based Control of Mosquito Vectors: Current Knowledge and Future Perspectives. *Canadian Journal of Infectious Diseases and Medical Microbiology*, *2019*. https://doi.org/10.1155/2019/7240356

Phadungsombat, J., Imad, H., Rahman, M., Nakayama, E. E., Kludkleeb, S., Ponam, T.,and Shioda, T. (2020). A novel sub-lineage of chikungunya virus east/central/south african genotype indian ocean lineage caused sequential outbreaks in Bangladesh and Thailand. *Viruses*, *12*(11). https://doi.org/10.3390/v12111319

Pierre, Â., and Curie, M. (2002). Effect of adult nutrition on the melanization immune response of the malaria vector *Anopheles stephensi*, 316–320.

Powers, A. M., and Logue, C. H. (2007). Changing patterns of chikungunya virus: Re-emergence of a zoonotic arbovirus. *Journal of General Virology*, *88*(9), 2363–2377. https://doi.org/10.1099/vir.0.82858-0

Ramirez, Jose L., and Dimopoulos, G. (2010). The Toll immune signaling pathway control conserved anti-dengue defenses across diverse *Aedes aegypti* strains and against multiple dengue virus serotypes. *Developmental and Comparative Immunology*, *34*(6), 625–629. https://doi.org/10.1016/j.dci.2010.01.006

Ramirez, Jose Luis, Short, S. M., Bahia, A. C., Saraiva, R. G., Dong, Y., Kang, S and Dimopoulos, G. (2014). Chromobacterium Csp_P Reduces Malaria and Dengue Infection in Vector Mosquitoes and Has Entomopathogenic and In Vitro Anti-pathogen Activities. *PLoS Pathogens*, *10*(10).

https://doi.org/10.1371/journal.ppat.1004398

Rao, T. R. (1966). Recent epidemics caused by Chikungunya virus in India, 1963–1965. *Scientific Culture*, *32*, 215.

Rashid, M. I., Fareed, M. I., Rashid, H., Aziz, H., Ehsan, N., Khalid, S and Hakeem, K. R. (2019). Flavonoids and Their Biological Secrets. *Plant and Human Health, Volume 2*, *2*, 579–605. https://doi.org/10.1007/978-3-030-03344-6_24

Renault, P., Solet, J. L., Sissoko, D., Balleydier, E., Larrieu, S., Filleul, L and Pierre, V. (2007). A major epidemic of chikungunya virus infection on Réunion Island, France, 2005-2006. *American Journal of Tropical Medicine and Hygiene*, *77*(4), 727–731. https://doi.org/10.4269/ajtmh.2007.77.727

Rezza, G., Nicoletti, L., Angelini, R., Romi, R., Finarelli, A. C., Panning, M and Cassone, A. (2007). Infection with chikungunya virus in Italy: an outbreak in a temperate region. *The Lancet*, *370*(9602), 1840–1846. https://doi.org/10.1016/S0140-6736(07)61779-6

Robinson, Marion C. (1955). An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952-53. I. Clinical features. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *49*(1), 28–32. https://doi.org/10.1016/0035-9203(55)90080-8

Rudolph, K. E., Lessler, J., Moloney, R. M., Kmush, B., and Cummings, D. A. T. (2014). Review article: Incubation periods of mosquito-borne viral infections: a systematic review. *American Journal of Tropical Medicine and Hygiene*, *90*(5), 882–891. https://doi.org/10.4269/ajtmh.13-0403

Scaraffia, P. Y., and Wells, M. A. (2003). Proline can be utilized as an energy substrate during flight of *Aedes aegypti* females. *Journal of Insect Physiology*, *49*(6), 591–601. https://doi.org/10.1016/S0022-1910(03)00031-3

Schlein, Y., and Muller, G. (1995). Assessment of plant tissue feeding by sand flies (Diptera: Psychodidae) and mosquitoes (Diptera: Culicidae). *Journal of Medical Entomology*, *32*(6), 882–887. https://doi.org/10.1093/jmedent/32.6.882

Schuffenecker, I., Iteman, I., Michault, A., Murri, S., Frangeul, L., Vaney, M. C and Brisse, S. (2006). Genome microevolution of chikungunya viruses causing the Indian Ocean outbreak. *PLoS Medicine*, *3*(7), 1058–1070.

https://doi.org/10.1371/journal.pmed.0030263

Schwartz, O., and Albert, M. L. (2010a). Biology and pathogenesis of chikungunya virus. *Nature Reviews Microbiology*. https://doi.org/10.1038/nrmicro2368

Schwartz, O., and Albert, M. L. (2010b). Biology and pathogenesis of chikungunya virus. *Nature Reviews Microbiology*, *8*(7), 491–500. https://doi.org/10.1038/nrmicro2368

Sergon, K., Njuguna, C., Kalani, R., Ofula, V., Onyango, C., Konongoi, L. S and Breiman, R. F. (2008). Seroprevalence of Chikungunya virus (CHIKV) infection on Lamu Island, Kenya, October 2004. *American Journal of Tropical Medicine and Hygiene*.

Sergon, K., Yahaya, A. A., Brown, J., Bedja, S. A., Mlindasse, M., Agata, N and Breiman, R. F. (2007). Seroprevalence of Chikungunya virus infection on Grande Comore Island, Union of the Comoros, 2005. *American Journal of Tropical Medicine and Hygiene*, *76*(6), 1189–1193. https://doi.org/10.4269/ajtmh.2007.76.1189

Seyoum, A., Pålsson, K., Kung'a, S., Kabiru, E. W., Lwande, W., Killeen, G. F., … Knols, B. G. J. (2002). Traditional use of mosquito-repellent plants in western Kenya and their evaluation in semi-field experimental huts against *Anopheles gambiae*: Ethnobotanical studies and application by thermal expulsion and direct burning. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *96*(3), 225–231. https://doi.org/10.1016/S0035-9203(02)90084-2

Sigamani, S., Chinnasamy, R., Dharmaraj, R. K., Ramamurthy, D., Devarajan, N., Narayanasamy, M., and Natarajan, H. (2020). Larvicidal potency of the extracts from Chlorella sp. against *Aedes aegypti*. *Biocatalysis and Agricultural Biotechnology*, *27*, 101663. https://doi.org/10.1016/j.bcab.2020.101663

Smith, D. L., Battle, K. E., Hay, S. I., Barker, C. M., Scott, T. W., and McKenzie, F. E. (2012). Ross, Macdonald, and a theory for the dynamics and control of mosquito-transmitted pathogens. *PLoS Pathogens*, *8*(4). https://doi.org/10.1371/journal.ppat.1002588

Souza-Neto, J. A., Sim, S., and Dimopoulos, G. (2009). An evolutionary conserved function of the JAK-STAT pathway in anti-dengue defense. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(42), 17841–17846. https://doi.org/10.1073/pnas.0905006106

Srinivas G, Jn, S., Hp, G., and Champawat. (2018). A review on *Pithecellobium dulce*: A

potential medicinal tree. *540 ~ International Journal of Chemical Studies*, *6*(2), 540–544.

Stanley-Samuelson, D. W., and Loher, W. (1986). Prostaglandins in Insect Reproduction. *Annals of the Entomological Society of America*, *79*(6), 841–853. https://doi.org/10.1093/aesa/79.6.841

Staples, J. E., Breiman, R. F., and Powers, A. M. (2009). Chikungunya Fever : An Epidemiological Review of a Re-Emerging Infectious Disease, *49*(Figure 1). https://doi.org/10.1086/605496

Styer, L. M., Kent, K. A., Albright, R. G., Bennett, C. J., Kramer, L. D., and Bernard, K. A. (2007). Mosquitoes inoculate high doses of West Nile virus as they probe and feed on live hosts. *PLoS Pathogens*, *3*(9), 1262–1270. https://doi.org/10.1371/journal.ppat.0030132

Suwanchaichinda, C., and Paskewitz, S. M. (1998). Effects of Larval Nutrition, Adult Body Size, and Adult Temperature on the Ability of *Anopheles gambiae* (Diptera: Culicidae) to Melanize Sephadex Beads. *Journal of Medical Entomology*. https://doi.org/10.1093/jmedent/35.2.157

Tabachnick, W. J. (2013). Nature, nurture and evolution of intra-species variation in mosquito arbovirus transmission competence. *International Journal of Environmental Research and Public Health*, *10*(1), 249–277. https://doi.org/10.3390/ijerph10010249

Takken, W., Koenraadt, C. J. M., Stone, C. M., and Foster, W. A. (2013a). *Plant-sugar feeding and vectorial capacity*. *Ecology of parasite-vector interactions*. https://doi.org/10.3920/978-90-8686-744-8_3

Takken, W., Koenraadt, C. J. M., Stone, C. M., and Foster, W. A. (2013b). Plant-sugar feeding and vectorial capacity. In *Ecology of parasite-vector interactions*. https://doi.org/10.3920/978-90-8686-744-8_3

Tanabe, I. S. B., Tanabe, E. L. L., Santos, E. C., Martins, W. V., Araújo, I. M. T. C., Cavalcante, M. C. A., and Bassi, Ê. J. (2018). Cellular and Molecular Immune Response to Chikungunya Virus Infection. *Frontiers in Cellular and Infection Microbiology*, *8*(October), 345. https://doi.org/10.3389/fcimb.2018.00345

Tarimo, B. B., Tao, D., Cheseto, X., Jackson, B. T., Ebrahimi, B., Torto, B., Bryan, T.,

Cheseto, X., Woodbridge A., Foster, R., Balaich, J. N. (2016). The Nonartemisinin Sesquiterpene Lactones Parthenin and Parthenolide Block *Plasmodium falciparum* Sexual Stage Transmission. *Antimicrobial Agents and Chemotherapy*. https://doi.org/10.1128/aac.02002-15

Tatem, A. J., Hay, S. I., and Rogers, D. J. (2006). Global traffic and disease vector dispersal. *Proceedings of the National Academy of Sciences*. https://doi.org/10.1073/pnas.0508391103

Teal, P. E. A., Tumlinson, J. H., Kaplan, F., Cheseto, X., Foster, W. A., Torto, B.,and Borgemeister, C. (2015). The Invasive American Weed *Parthenium hysterophorus* Can Negatively Impact Malaria Control in Africa. *PLOS ONE*. https://doi.org/10.1371/journal.pone.0137836

Thiberville, S. D., Moyen, N., Dupuis-Maguiraga, L., Nougairede, A., Gould, E. A., Roques, P., and de Lamballerie, X. (2013). Chikungunya fever: Epidemiology, clinical syndrome, pathogenesis and therapy. *Antiviral Research*, *99*(3), 345–370. https://doi.org/10.1016/j.antiviral.2013.06.009

Tisgratog, R., Sanguanpong, U., Grieco, J. P., Ngoen-Kluan, R., and Chareonviriyaphap, T. (2016). Plants traditionally used as mosquito repellents and the implication for their use in vector control. *Acta Tropica*, *157*, 136–144. https://doi.org/10.1016/j.actatropica.2016.01.024

Torres, J. R., Falleiros-Arlant, L. H., Dueñas, L., Pleitez-Navarrete, J., Salgado, D. M., and Castillo, J. B.-D. (2016). Congenital and perinatal complications of chikungunya fever: a Latin American experience. *International Journal of Infectious Diseases : IJID : Official Publication of the International Society for Infectious Diseases*, *51*, 85–88. https://doi.org/10.1016/j.ijid.2016.09.009

Tsetsarkin, K. A., Chen, R., and Weaver, S. C. (2016). Interspecies transmission and chikungunya virus emergence. *Current Opinion in Virology*, *16*, 143–150. https://doi.org/https://doi.org/10.1016/j.coviro.2016.02.007

Valdez, L. D., Sibona, G. J., and Condat, C. A. (2018). Impact of rainfall on *Aedes aegypti* populations. *Ecological Modelling*, *385*(November 2017), 96–105. https://doi.org/10.1016/j.ecolmodel.2018.07.003

Valentine, M. J., Murdock, C. C., and Kelly, P. J. (2019). Sylvatic cycles of arboviruses

in non-human primates. *Parasites and Vectors*, *12*(1), 1–18. https://doi.org/10.1186/s13071-019-3732-0

Van den Hurk, A. F., Hall-Mendelin, S., Pyke, A. T., Frentiu, F. D., McElroy, K., Day, A., Higgs, S., L, Scott and O'Neill. Impact of Wolbachia on Infection with Chikungunya and Yellow Fever Viruses in the Mosquito Vector *Aedes aegypti*. *PLoS Neglected Tropical Diseases*, *6*(11). https://doi.org/10.1371/journal.pntd.0001892

Vanitha, V., and Manikandan, K. (2016). Bio-activity guided determination of active compounds in the leaves of *Pithecellobium dulce. Rasayan Journal of Chemistry*.

Vanlerberghe, V., Toledo, M. E., Rodríguez, M., Gomez, D., Baly, A., Benitez, J. R., and Van der Stuyft, P. (2009). Community involvement in dengue vector control: cluster randomised trial. *BMJ (Clinical Research Ed.)*, *338*, b1959–b1959. https://doi.org/10.1136/bmj.b1959

Wachira, S. W., Omar, S., Jacob, J. W., Wahome, M., Alborn, H. T., Spring, D. R.,Masiga,D.K and Torto, B. (2014). Toxicity of six plant extracts and two pyridone alkaloids from Ricinus communis against the malaria vector Anopheles gambiae. *Parasites and Vectors*. https://doi.org/10.1186/1756-3305-7-312.

Wanjiku, C., Tchouassi, D. P., Sole, C. L., Pirk, C., and Torto, B. (2021). Plant sugar feeding patterns of wild-caught *Aedes aegypti* from dengue endemic and non-endemic areas of Kenya. *Medical and Veterinary Entomology*. https://doi.org/10.1111/mve.12514

Watson, R. (2007). Europe witnesses first local transmission of chikungunya fever in Italy. *BMJ (Clinical Research Ed.)*, *335*(7619), 532–533. https://doi.org/10.1136/bmj.39332.708738.db

Weaver, S. C., Chen, R., and Diallo, M. (2020). Chikungunya Virus: Role of Vectors in Emergence from Enzootic Cycles. *Annual Review of Entomology*, *65*(1). https://doi.org/10.1146/annurev-ento-011019-025207

WHO. (2018). Weekly Bulletin on Outbreaks, (September), 15–21.

Yactayo, S., Staples, J. E., Millot, V., Cibrelus, L., and Ramon-Pardo, P. (2016). Epidemiology of chikungunya in the americas. *Journal of Infectious Diseases*, *214*(Suppl 5), S441–S445. https://doi.org/10.1093/infdis/jiw390

Yang, S. M., Han, S. H., Kim, B. G., and Ahn, J. H. (2014). Production of kaempferol 3-

O-rhamnoside from glucose using engineered *Escherichia coli*. *Journal of Industrial Microbiology and Biotechnology*, *41*(8), 1311–1318. https://doi.org/10.1007/s10295-014-1465-9

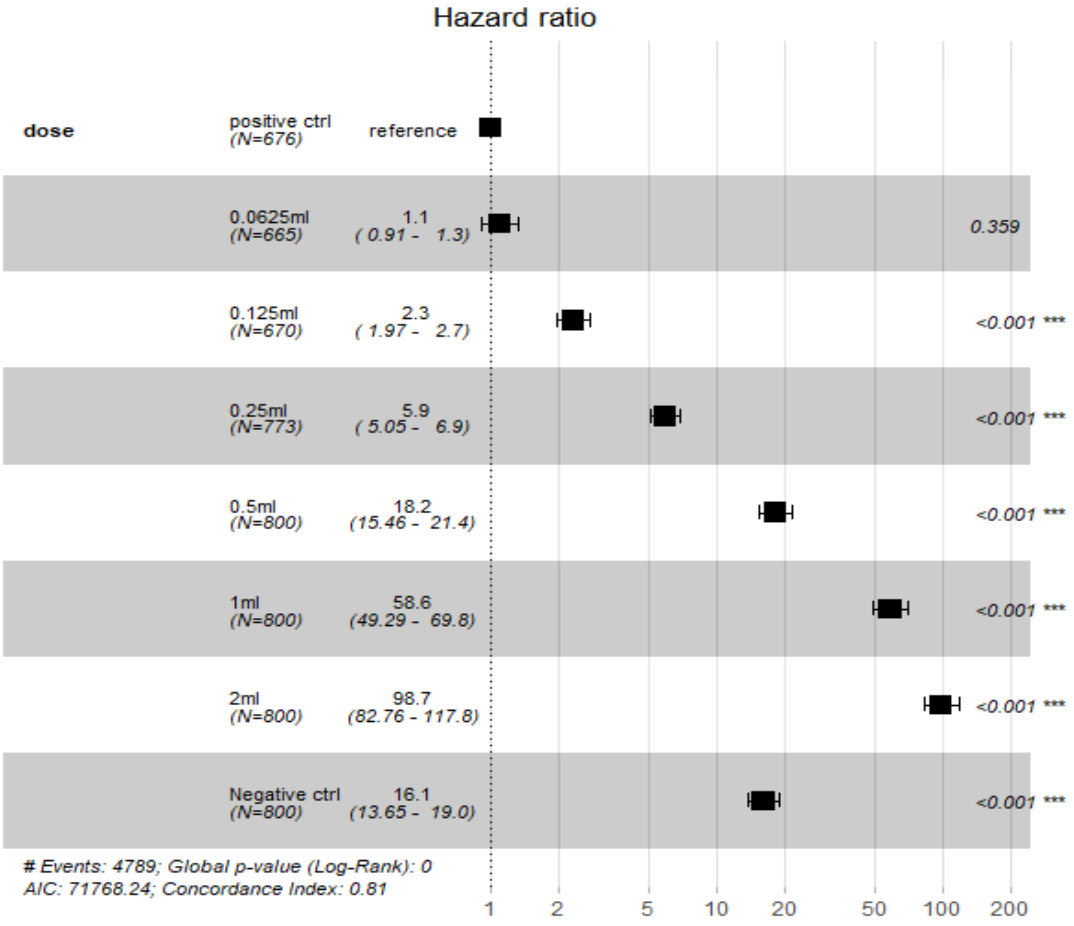
Zeller, H., Van Bortel, W., and Sudre, B. (2016). Chikungunya: Its history in Africa and Asia and its spread to new regions in 2013-2014. *Journal of Infectious Diseases*, *214*(Suppl 5), S436–S440. https://doi.org/10.1093/infdis/jiw391

APPENDICES

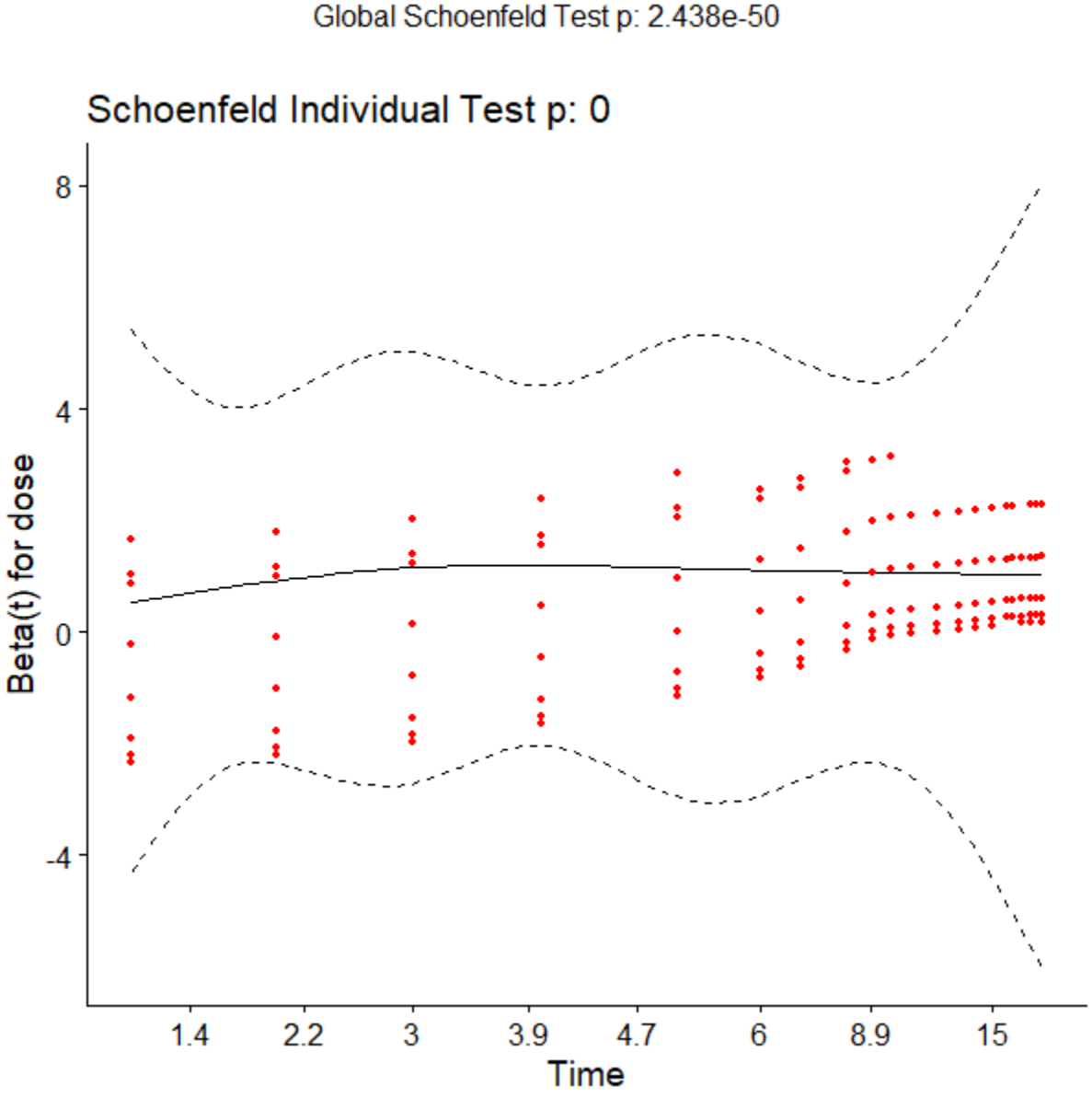
Appendix1.0: Estimated median survival time for each dimethyl sulfoxide dose

Dose (mL)	Median time (Days)	95% Confidence interval	
		Lower limit	Upper limit
2ml	2	2	2
1ml	2	2	2
0.5ml	3	3	3
0.25ml	8	7	8
0.125ml	18	18	19
0.0625ml	–	–	–
Positive control	–	–	–
Negative control	4	4	4

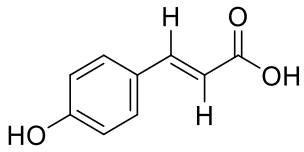
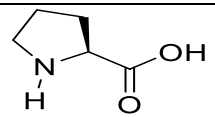
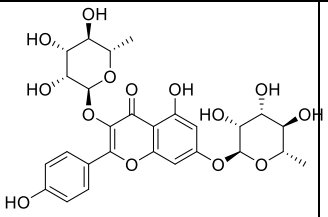
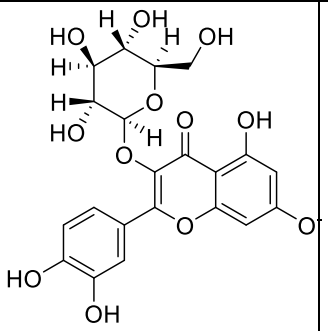
Appendix 2.0: Forest plot of hazard ratios for dimethyl sulfoxide

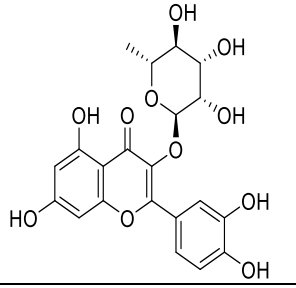
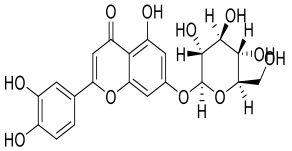
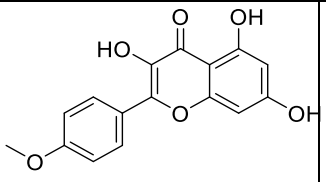
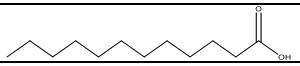
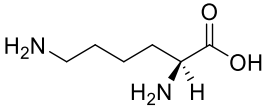
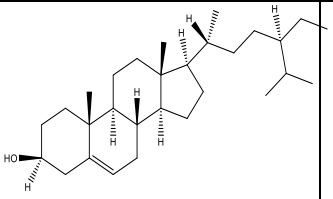


Appendix3.0: Schoen field assessment for proportional hazards assumption illustrating that the model used for survival analysis fitted well.



Appendix 4.0: LC-QqQ MS fragments of identified compounds in the plant extract

Peak No	T_R (min)	Compound	Structures	Class of compound	$[M+H]^+$	$[M-H]^-$	Positive mode fragmentation	Reference
1.	3.189	Coumaric acid		Phenol	165.1	163.1	147.1, 119.2	Megala and Geetha., 2009
2.	4.071	Proline		Amino acid	116.1	114.1		
3.	21.311	Kaempferol 3-0 rhamnoside		Flavanoid	433.1	431.1		
4.	20.133	Quercetin 3-0 glucoside		Flavanoid	463.1	461.1		

5.	20.668	Quercetin 3-O-rhamnoside		Flavanoid	449.1	447.1	285.1, 273.6 257.1	Nigam *et a.*,2011
6.	20.541	Luteolin 7-O-glucoside (cynaroside)		449.5	447.5		300.3, 271.5	
7.	20.126	Kaempferide		300.5	299.5			
8.	2.308	Lauric acid		201.2	199.5			
9.	3.932	Lysine		146.95				
10.	29.536	β sitosterol		Phyto-sterol				

Appendix 5.0 Compounds identified in *Pithecellobium dulce* plant extract

No	R_T (mins)	Compound	Quality	RI calculated	RI Literature	Peak area
1.	8.305	p-Xylene	30	863.3	870	188637
2.	22.5204	2-Pentadecanone, 6,10,14-trimethyl-	98	1843.7	1847	7607770
3.	23.6553	n-Hexadecanoic acid	99	1956.5	1964	24078286
4.	25.0242	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	99	2100.6	2105	21546887
5.	25.1412	Phytol	96	2113.7	2122	12438502
6.	25.2992	9,12-Octadecadienoic acid (Z,Z)-	99	2131.4	2134	9257166
7.	25.3694	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	99	2139.2	2143	34521284
8.	25.5331	Octadecanoic acid	99	2157.5	2172	22160995
9.	27.2589	4,8,12,16-Tetramethylheptadecan-4-olide	98	2362.6	2364	16234081
10	33.7524	.beta.-Tocopherol	64	-	3076	1696123
11	35.0452	dl-.alpha.-Tocopherol	96	-	3149	6028258
12	38.6839	Stigmasta-7,16-dien-3-ol, (3.beta.,5.alpha.)-	94	-	3401	20222083
13	39.5672	.beta.-Amyrin	93	-	3337	8187619
14	40.1113	Lup-20(29)-en-3-one	99	-	3384	38853793

Appendix 6.0 Compounds identified in mosquito midgut after ingestion of *Pithecellobium dulce* extract

No	R_T (mins)	Compound	Quality	RI calculated	RI Literature	Peak area
1.	19.3439	31.47 Dodecanoic acid	86	1557	1568	11271913
2.	21.6253	Tetradecanoic acid	72	1758	1763	55752719
3.	21.7365	Methanone (4methylphenyl)phenyl	30	1769	-	58257
4.	23.1522	Methyl hexadec-9-enoate	99	1905	1907*	2242884
5.	23.5032	Palmitoleic acid	86	1941	1941	46007505
6.	23.6962	n-Hexadecanoic acid	99	1960	1963	1.54E+08
7.	24.6264	Heptadecanoic acid	83	2055	2077	3767417
8.	25.3055	9, 12 Octadecadienoic acid(z,z)	55	2131	2134	1296123
9.	25.3693	9-Octadecenoic acid, (E)-	99	2139	2141	74450280
10	25.5682	Octadecanoic acid	99	2161	2177	1.1E+08
11	34.922	Cholesterol	99	-	-	34647284

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Date of Issue: **25/January/2021**

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