UNIVERSITY OF NAIROBI

THE EFFECTS OF PLANT INFUSIONS ON THE COLLECTIONS OF

GRAVID *Aedes aegypti* FOR ARBOVIRUS SURVEILLANCE.

ROBERT CHEMJOR

H56/34656/2019

A Thesis Submitted for Examination in partial fulfillment of the requirement for the

Award of the degree of Master of Science in Medical Microbiology of the

University of Nairobi,

Department of Medical Microbiology and Immunology

©December, 2023.

DECLARATION

I hereby confirm 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.

Robert Chemjor

Registration No: H56/34656/2019

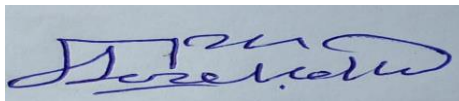Student; Department of Medical Microbiology, University of Nairobi

Signature: Date: 6th Dec, 2023.

This thesis is being submitted with our approval as supervisors;-

Dr. Dunstan Mukoko (Ph.D.)

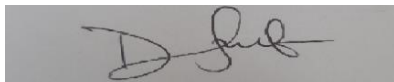Lecturer: Department of Medical Microbiology, University of Nairobi.

Signature: Date: 6th Dec, 2023.

Dr. David P. Tchouassi (Ph.D.)

Senior Scientist: International Centre of Insect Physiology and Ecology

Signature: Date: 6th Dec, 2023.

DEDICATION

I sincerely dedicate this research dissertation to my supportive wife Carolyne Jepkemboi and my children Naomi Jepchumba, Abigael Cherotich, and Nimrod Kibet for their continued encouragement and sacrifice they made during the research period.

To my dad Willy Chesire and my siblings Meshack Chemjor, Ben Chemjor, Nancy Chemjor , Ken Chemjor, Hillary Chemjor, Pius Kibichii, Mercy Jemutai for providing me great support.

Moreover, I dedicate this work to my unit heads, Dr. P. Mandela, Prof. Madadi Obimbo, and colleagues Martin, Maggy Irungu, Jacob Gimongo, Acleus Murunga, Esther Wairimu, Peter Nzioka, Jacinta Waciuri and Judith Machira for their support, cheering, and encouragement during the research period.

Lastly, I dedicate this dissertation to the Almighty God who gives me strength, wisdom, guidance, power of thinking, competence and for giving me good health while doing this. All of these, I offer to you.

ACKNOWLEDGMENT

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS AND ABBREVIATIONS

ANOSIM — Analysis of similarity

BGs — Biogents sentinel

*c*DNA — Complimentary Deoxyribonucleic Acid

CHIKV — Chikungunya virus

Da — Daltons

DENV — Dengue virus

DF — Dengue fever

DHF — Dengue hemorrhagic fever

DNA — Deoxyribonucleic acid

GAT — Gravid *Aedes* trap

GC-MS — Gas Chromatography-Mass Spectrometry

GLM — Generalized linear model

icipe — International Centre of Physiology and ecology

NIST — National Institute of Standards and Technology

NMDS — Non-metric multidimensional scaling analysis

PBS — phosphate-buffered saline

PDMS — Polydimethylsiloxane

RFV — Rift Valley virus

RNA — Ribonucleic acid

RT- PCR — Reverse transcription polymerase chain reaction

SPME — Solid-phase micro-extraction

UoN — University of Nairobi

VOCs — Volatile organic compounds

YF — Yellow fever

YFV — Yellow fever virus

ZIKV — Zika Virus

ABSTRACT

Infusions are commonly used to trap gravid mosquitoes for surveillance and control of arboviral diseases. The nature and plant species can affect attractiveness of infusions likely underpinned by differences in mediating microbe-associated volatile organic compounds. In this study, 4-d old-fermented infusions prepared from leaves of four commonly available plants (mango, banana, cashew, neem) were evaluated on catches of gravid *Aedes aegypti* using Gravid *Aedes* Traps. Field experiment was implemented through a Latin square design in urban Ukunda, an endemic area for dengue in coastal Kenya. Of the infusions, mango recorded 2-7-fold increased captures of gravid *Ae. aegypti* than the other plants; captures decreased in the order banana > cashew > neem. Analysis of the headspace volatiles of the infusions via gas chromatography-mass spectrometry (GC-MS) revealed significant difference in the chemical composition between the plant species (ANOSIM, $R = 0.33$, $p = 0.0003$) belonging to the classes ketones (38.4%), terpenes (26.2%), phenolics (24.7%), alcohols (7.4%), hydrocarbons (2.7%), esters (0.18%), indole (0.4%) and carboxylic acids (0.05%). Qualitative and quantitative variation in the volatile constituents of the plant infusions was observed with those in mango and neem being most and least diverse, respectively. Culturing the infusion water in Luria bertani media recovered bacterial isolates which were identified by PCR and sequencing of the16S rRNA gene. The most attractive infusions (were associated with 9 bacterial isolates, where mango had 5 and and banana 4 bacterial isolates, respectively, belonging to the family *Bacillaceae* and <u>*Moraxellaceae*</u> (e.g., *Acinetobacter* sp). Four-three isolates from cashew belong to the family *Bacillaceae* (2) and *Enterobacteriaceae* (*Klebsiella pneumoniae*, *Citrobacteri koseri*)) similar to neem that in addition exclusively had *Pseudomonadaceae* (e.g., *Pseudomonas mendocina*). The findings indicate that gravid *Ae. aegypti* responded differently to

infusions of the plants whose variation in bacterial composition and volatile emission profiles could account for the observed differential attraction. Identifying the active constituents in most- and least-attractive substrates can be explored in the development of push-pull strategy for controlling this container-breeding mosquito. The potential use of the microbes and associated volatiles in the vector management needs to be explored. The *Aedes aegypti* mosquito is the primary vector of several viral diseases including dengue and chikungunya which pose a significant threat to human health globally. Outbreaks and inter-epidemic transmissions are associated with the absence of sustainable vector control measures, vaccines and antiviral drugs. Surveillance of this vector for disease risk assessment is an important strategy which is currently being undertaken using the Biogent sentinel traps (BGs) baited with carbon dioxide (CO_2. Thus, there is need for development of tools that increase possibility of detecting infected mosquitoes. Gravid *Aedes* Trap (GAT) is a monitoring tool known to exclusively target blood-fed mosquitoes and whose efficiency can be optimized using lures. This study aimed at elucidating the attractiveness of infusions from leaves of locally available plants including mango, cashew, banana and neem and their associated microbial population, targeting gravid *Ae. aegypti* for improved arbovirus surveillance.

Results: The GAT baited with mango infusion attracted significantly higher number of mosquitoes while the trap baited with neem infusion attracted the least number of mosquitoes (p<0.001). The attraction could be attributed to the volatile organic compounds (VOCs) including phenol, guiacol and spathulenol among others. Similarly, the classes of microbial population including Bacillus, Acinetobacter, Citrobacter identified could be have played a significant role in influencing the attraction of these gravid mosquitoes.

Conclusions/recommendation: This study lays the foundation for the development of potent oviposition lures which will contribute significantly in the improvement of arbovirus surveillance tools and subsequently control of *Aedes*-borne viral disease. we recommend further laboratory bioassays such as Coupled gas chromatography electroantennographic Detection (GC-EAD) of the individual volatile compounds in order to understand the mosquito antennal response to the distinct chemicals in this identified by GC-MS.

Keywords: *Aedes aegypti*, gravid mosquito, plant leaf infusions, volatile organic compounds, cultured bacteria, Arbovirus surveillance and control, *Aedes* gravid Trap

CHAPTER 1:0 INTROD[i]UCTION

1:1 Background

Aedes aegypti is a principal vector of arboviruses such as dengue (DENV), yellow fever (YF), chikungunya (CHIKV), and Zika (ZIKV) viruses globally (Gubler, 2002). Over the past decades, incidences of diseases caused by these pathogens have increased with frequent outbreaks and inter-epidemic transmissions reported in various parts of the globe (Gubler, 2002) the mortality rates of 20% of untreated cases reported(Ochieng *et al*., 2015) . In Kenya, outbreaks of both DENV and CHIKV are prevalent at the Coastal and North-Eastern frontiers(Konongoi *et al*., 2018; Lutomiah *et al*., 2016; Obonyo *et al*., 2018; Sang *et al*., 2022a) The prevalence is majorly attributed to ability of this vector to adapt to urban landscapes fueled by globalization of trade and travel, which support its prolific breeding in and around human dwellings, as well as its specialization in feeding primarily on human hosts and a small portion of other vertebrates (Tchouassi *et al.*, 2022). In addition, the spread is associated with the absence of licensed vaccines and antiviral drugs (Bhatt et al., 2013a) as well as lack of sustainable vector control and management techniques.

Vector surveillance is a key strategy of arboviral disease risk assessment and control. It entails field trapping and screening of vectors for viral infections in order to predict health risks to humans in a given population (Tchouassi *et al*., 2013). Currently, adult *Ae. aegypti* is monitored using Biogents Sentinel (BGS) traps, commonly baited with chemical attractants of such as carbon dioxide (CO_2), and/other host-derived attractants (e.g., BG-lure, linalool oxide, hexanol among others(Agha *et al*., 2021; Owino *et al*., 2015; Tchouassi *et al*., 2021). While this monitoring tool does increase mosquito catches, it primarily targets newly emerged and host-seeking mosquitoes; because these mosquitos have never been in contact with a host, they reduce the sensitivity of arboviral detection while consuming limited resources (Crepeau *et al*., 2013). The development of tools that target

mosquitoes with a history of contact with a host during the blood meal (i.e., gravid females) is considered cost-effective and sensitive and this approach is essential for disease screening in order to pre-empt health risks (Farlow *et al*., 2020).

Gravid *Aedes* traps (GAT) were developed to monitor adult gravid *Ae. aegypti* seeking egg-laying sites(Barrera, 2022) The efficacy of GAT is related to the type of infusion and thus identifying the constituents that attract mosquitoes to infusions can be a selective way to target specific species in surveillance/control programs (Mulatier *et al*., 2023). In addition infusions use in GAT or other lethal traps are cumbersome, produce offensive smell. Thus, there is an urgent to develop user-friendly chemically based lures or attractants that attract gravid mosquitoes. Extracts such as infusions or juices have been tested in the laboratory and field experiments and have been reported to contain volatile organic compounds that are potentially attractive to gravid *Ae. aegypti* as well as induce oviposition (Wooding et al., 2020). Compounds such as cedrol, linalool oxide, butanone and phenol identified from various plant types have been found to be attract mosquitoes in laboratory bioassay(Milugo *et al.*, 2021a*)*. However, there is limited knowledge to validate the implementation of odor-based lures in GAT in field experiments, moreover chemical signature of infusions are either poorly characterized or in few instances identified compounds confined to lab evaluation only e.g., Bermuda grass infusion-associated compounds skatole, *p*-cresol, 4-ethylphenol, phenol, and indole (Mulatier *et al*., 2023)

 Microbial physiological activities and their by-products have been directly linked to the production of olfactory cues that influence mosquitoes including *Ae. aegypti* oviposition behavior, bacterial species in bamboo leaf infusions are sources of the odorants carboxylic acids and methyl esters found to stimulate egg-laying in *Ae. Aegypti* (Ponnusamy *et al*., 2008a, 2010a). Similarly, volatiles of bacteria origin (e.g. geosmin) and from plant infusions have been implicated in the attraction of

other gravid mosquito species (Melo *et al*., 2020; Trexler *et al*., 2003). However, the volatile profile of a given oviposition substrate may depend on the inhabiting microbial species.

 In this study, the attractiveness of leaf infusions of four commonly available plants (mango, banana, cashew, and neem) was on field captures of *Ae. aegypti* in BG-GAT was investigated. The chemical signature of each infusion was analysed. In addition, the bacterial composition associated with each infusion type was cultured and isolates identified by molecular method including Sanger-Sequencing.

1:2 Statement of problem

Aedes-borne viral diseases including dengue and chikungunya are increasingly becoming a global health threat. The absence of sustainable vector control tools given the rapid expansion and colonization of the key vector, *Ae. aegypti,* coupled with the absence of vaccines and antiviral drugs poses a further social and economic challenge especially in Africa.

Surveillance of this life-threatening vector and their associated pathogens is an important approach, which is currently being implemented by use of BGs traps. The BGs trap employs the use of attractants such as CO_2 which enables massive catches of host seeking mosquitoes including the adults, males, juveniles as well as the gravid. Although, this would be sustainable for comprehensive studies it's often labor intensive and decreases the chances of detection of viral infected mosquitoes. Thus, there is need to develop and or modify a trap that can only focus on previously blood-fed female mosquitoes hence increasing the chances of viral detection.

Plant infusions and bacteria found in mosquito breeding sites have previously been shown to attract collections of gravid mosquitoes to egg-laying sites (Wooding et al., 2020);Wang *et al*., 2018). Although, the effects of organic volatile chemicals (VOCs) produced by these plant infusions are plant specific, little is also known on microbial diversity of bacteria found in these infusions. Therefore, we proposed to elucidate the attractiveness of infusions from leaves of locally available plants including mango, cashew, banana and neem and their associated microbial population, targeting gravid *Ae. aegypti* for improved arbovirus surveillance.

1:3 Justification and significance of study

Arbovirus surveillance is vital vector control strategy that is currently used in monitoring and prediction of circulation of disease pathogens. The use of BGs traps plays a key role in surveillance of *Aedes*-borne viral diseases such as dengue. However, there is need for a sensitive, cost effective and target specific and or modified trap to enable improved catches and accurate detection of virus in infected gravid and/or female mosquitoes.

The ability of GAT to only target mosquitoes with a previous blood meal offers a suitable alternative as it increases the likelihood of identifying infected mosquitoes within a short time and with limited resources. Building on previous studies that showed improved catches in traps baited with attractants from plant infusions and their associated microbial population, we tested whether this behavior could influence the attractiveness of gravid female *Ae. aegypti* to infusions from leaves of Mango, Cashew, Banana and Neem as well as their respective bacterial populations. Findings from this study lays the foundation for the development of potent oviposition attractants which will contribute significantly in the improvement of arbovirus surveillance tools and subsequently control of *Aedes*-borne viral diseases.

1:4 Research objectives

1:4:1 Main objective

To elucidate the attractiveness of infusions from leaves of locally available plants; Mango, Cashew, Banana and Neem targeting gravid *Aedes aegypti* for improved arbovirus surveillance.

1:4:1:1: Specific objectives

1. To compare catches of gravid *Ae. aegypti* in Gravid *Aedes* traps (GAT) baited with infusions from the different plant types: Mango, Neem, Banana, and Cashew.
2. To compare the volatile chemical profiles collected from prepared infusions of selected plants.
3. To Identify cultured bacterial species associated with the different plant infusions

CHAPTER 2:0 LITERATURE REVIEW

2:1 History, Distribution and Ecology of the arboviruses

Arthropod-borne viral diseases (arboviruses) including dengue and chikungunya, pose a global threat to human and animal health, infecting millions of individuals and causing a heavy social and economic burden. Least developed countries and the poorest segment of the society are the most affected, with countries in sub-Saharan Africa bearing the heaviest brunt (WHO, 2016, 2009)

Global expansion of these arboviral diseases is preceded by various factors. For instance, absence of effective vaccines and antiviral drugs, extensive geographic spread and colonization of key vectors, *Aedes aegytpi* and *Aedes albopictus,* fueled by increased global trade and travel (Bonizzoni *et al*., 2012; Brown *et al*., 2011; Charrel *et al*., 2007; Tatem *et al*., 2006) as well as the efficiency

6

of these vectors in transmitting the virus (Charrel *et al*., 2007) .*Aedes aegypti* is believed to have originated in Africa where its ancestral form is a zoophilic tree hole mosquito named *Aedes aegypti formosus* (Brown *et al*., 2011).As a result of changes in climatic conditions coupled with slave trade, *Ae. aegypti* was introduced into the new world and has since spread to different parts of the world. Currently, *Ae. aegypti* populations are exclusively found in close association with humans in the domestic environment, breeding in artificial containers (Brown *et al*., 2011; Tabachnick, 2013) and with a high preferential for human blood.

Subsequent studies on distribution of these vectors, determining their ecological requirements and habitat suitability, have predicted that out of 250 countries, 215 countries are suitable for their existence, further intensifying the threat from spread of arboviruses. (See figure 1).(Brady & Hay, 2020; Cianci *et al*., 2015; Khormi & Kumar, 2014; Kramer & Ciota, 2015; Tongaonkar & Ghosh, 1973). In Kenya, mosquito-borne viruses are a major component of re-emerging infectious diseases; in 2011, dengue virus outbreaks occurred in northern Kenya (Mandera), followed by coastal towns in Mombasa, Lamu, and Kwale counties in 2013 and 2017, with mortality rates of 1% in treated cases and 20% in untreated cases (Ndenga *et al*., 2017a).

2.2 Transmission and burden of Arboviruses.

2:1:1 Dengue Virus

Dengue virus (DENV) is a mosquito-borne virus that causes Dengue fever (DF), a febrile illness characterized by arthralgia and fever that can progress to dengue hemorrhagic fever (DHF) DENV has four antigenically distinct serotypes (DENV1,2,3,&4). The virus is spread by the bite of a female *Ae. aegypti* mosquito (Demanou *et al*., 2014). For decades, the incidences have increased 30 times, which is attributed to vector-friendly factors such as climate changes and rapid urbanization (WHO, 2009). According to recent research, there are 200 million asymptomatic infections worldwide each year, with over 50,000 requiring hospitalization (Bhatt *et al*., 2013b)

2:1:2 Chikungunya

Chikungunya virus (CHIKV) is a positive single-strand RNA virus of the genus *Alphavirus* that was discovered in a febrile patient in Tanzania in 1953. CHIKV illness is characterized by fever, severe arthralgia, rash, headache, and malaise; rare complications include neurological problems, which are most common in the elderly (Sergon *et al*., 2008). Chikungunya outbreaks have been reported in Kenya, with seroprevalence indicating that the disease is endemic; however, from 2004 to 2011, CHIKV affected 75% of the population in Lamu and Mombasa (Owino, 2018).

2:3 Biology and behavior of *Aedes aegypti*

Aedes aegypti (stegomyia) belongs to the family; *Culicidae*, sub family; *Culinae,* genus; *Aedes*. The adult *Ae. aegypti* is a small to medium-sized mosquito approximately 4 – 7mm, adults have white scales on the dorsal surface of the thorax that form the shape of a violin, and female is always larger than males (Arslan *et al.,* 2016). *Aedes aegypti* is holometabolic, which means it goes through a complete metamorphosis that includes egg, larvae, pupa, and adult stages.

Once the mosquitoes emerge from pupation, the females look for compatible partners to mate. After mating, the females will look for a blood meal from a human host or any other vertebrate in a diverse ecological context, which is a source of nutrients used to synthesize yolk proteins, the females feed multiple times(Sang *et al*., 2022b). Female *Ae. aegypti* feeds mainly on human blood and a small portion of other non-human vertebrates (Ndenga *et al*., 2017b)

Aedes aegypti distribution in Kenya has not been extensively studied; however, it is common in lowland areas and exists in two forms, domestic and sylvatic, with the domestic form common on the Kenyan coast (Ndenga *et al*., 2017b). The feeding patterns of the domestic form of *Aedes aegypti* have adapted to rapid urbanization by breeding in man-made containers around the home and specializing in biting humans for blood.

2:4 Vector-based surveillance

Dengue and chikungunya virus circulate in mosquito populations days prior to a symptomatic human case, allowing for early vector control actions and outbreaks mitigation is a crucial strategy (Arslan *et al.*, 2016). Effective surveillance tool/method deployed is essential in disease assessment; in this case, it entails monitoring vectors and screening for arboviral infections. The sensitivity of

the trapping method used can have an effect on the success rate of the monitoring tool (Omondi *et al.*, 2019). Vector density, rapid urbanization, and climatic changes are among the factors that have rapidly increased frequent (re-) emerging cases of arbovirus in coastal Kenya and around the world, these factors have contributed to the biological adaptation of the *Ae. aegypti* (Mesquita *et al.*, 2015).

2:5 Plant infusions in vector-based surveillance

Previous evaluation of the plant infusions on the attraction of the mosquito species, *Ae. aegypti* and *Ae. albopictus* revealed that fermented leaf mass improved vector responses (Ponnusamy *et al.*, 2010b). Experiments conducted previously with senescent bamboo (*Arundinaria gigantea*) and white oak (*Quercus alba*) as cues successfully attracted gravid female mosquitoes. (Ponnusamy *et al.*, 2010b). The experiment did not identify any specific organic volatile compounds that contributed to the attractiveness of the infusion.

The field evaluation of ovitraps in Cambodia using 100% hay infusion and 10% of the same infusion was found to greatly improve oviposition indices, with 100% hay infusion attracting more than 10% infusion. (A Polson *et al.*, 2002). It was reported in the experiment that the grass infusion (*Panicum maximum*) increased oviposition responses compared to 15-20-day old infusions, with older infusions receiving more mosquitoes (Sant'ana *et al.*, 2006).

The current evaluation of vector monitoring methods emphasizes the significance of using precise, and sensitive tools to study the chemical ecology of mosquitoes (*Ae. aegypti*). Testing and determining the effectiveness of particular organic compounds of plant origin that entice and encourage gravid mosquitoes to lay their eggs is an ideal endeavor (Logan *et al.*, 2010). The findings will be useful for mosquito and virus surveillance, as well as other interventions to combat mosquito-borne diseases.

2:5.1 Gravid *Aedes* trap

The gravid *Aedes* trap (GAT) was designed to monitor adult female *Aedes aegypti* seeking egg-laying sites, rather than pupal and larval surveys, it was also intended to be used in areas where there is a limited source of power, GAT utilizes visual and olfactory cues to attract the gravid *Aedes aegypti* (Eiras *et al*., 2014). Biogents sentinel traps (BGS) that are currently deployed in the field require a source of power and it targets host-seeking mosquitoes.

Ovitraps collections have been used for many years and as a result, the representative used to calculate adult abundance in an area or region, faced challenges because of the skip oviposition behavior observed in the *Ae. aegypti*, (Eiras *et al*., 2014). It affected the sensitivity and specificity of such monitoring tool which necessitated the development of the GAT.

The gravid *Aedes* trap is a passive trap (Fig 1.0), it evaluates a favorable ecological and aquatic habitat that is suited for egg laying and supports following developmental phases. These are the physicochemical qualities that an insect takes into account (Milugo *et al*., 2021b). The GAT with water or treatment allows the mosquito to enter the jar. It is separated from the net to prevent the insect from drowning. As a result, the insect will be trapped by a sticky pad placed sideways of the funnel.

Currently, there's a lack of clarity regarding how the GAT can be made attractive by using efficiently prepared organic infusions. A crucial factor to take into account during preparation is infusion fermentation. Prior research has associated the length of fermentation with increased effectiveness, and it has been shown that fermented organic matter can improve the efficacy of GAT treated with organic matter (Ponnusamy *et al*., 2010b).Fiji Republic, a South Pacific country, has all dengue mosquito vectors *Ae. Aegypti* and *Ae. albopictus* with a history of Zika transmission; different plant infusions tested in the field showed varying degrees of attraction when compared to

water, in this experiment it was reported that prepared organic infusions from Mango, grass, and chicken feeds were effective in attracting gravid *Aedes aegypti* (Harwood *et al*., 2018).

Gravid *Aedes* looking oviposition site

Adhesive pad
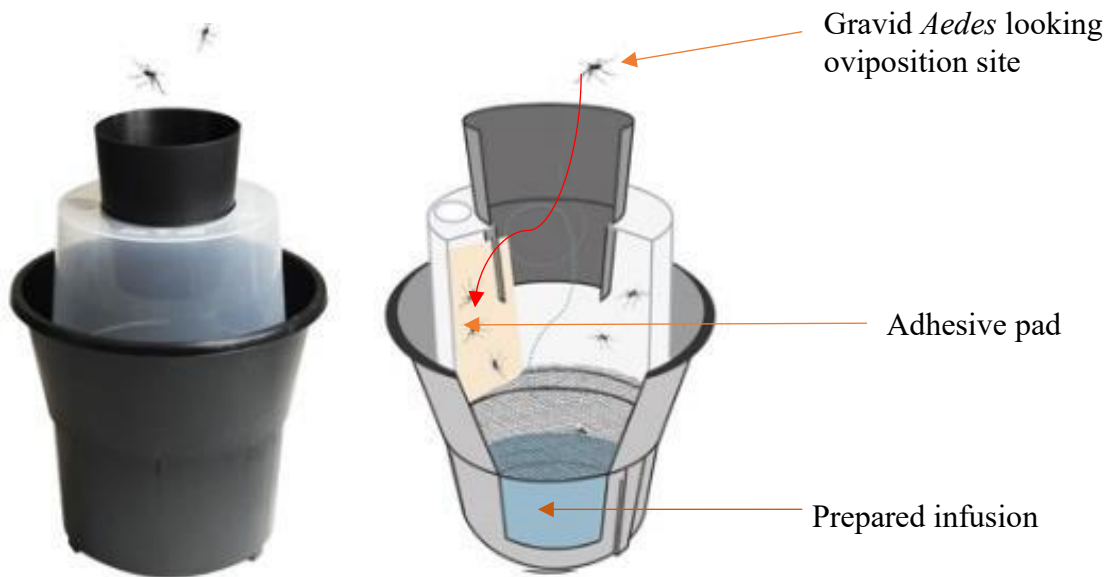
Prepared infusion

Figure: 1.0. Picture of the Gravid *Aedes* trap (GAT) impregnated with the prepared

2:4 Bacterial diversity in plant infusions

Recent decades have experienced a rapid expansion and distribution of mosquito-borne viral diseases across borders, these has been attributed by the situations such as rapid urbanization and environmental changes which have forced the primary vector (*Ae. Aegypti)* to adapt to human-inhabited areas, by feeding and breeding in man-made containers found in the domestic environment and tree holes for the sylvatic forms (Ndenga *et al.*, 2017b). Experiments with prepared bamboo infusions were conducted previously; one was filtered through 0.22 μm filter membrane to remove bacteria, while the other was left unfiltered to study how bacteria extracted from the infusions affected oviposition stimulation and attractiveness. According to the findings, the filtered infusion had less eggs than the unfiltered infusion (Ponnusamy *et al.,* 2008a). Other bioassays on gravid *Aedes aegypti* revealed that the organic fermenting leaves in the infusion-augmented traps attracted and persuaded the insects to lay their eggs there. Bacterial cultures, bioassay-guided fractionation of bacterial cultures, and chemical analysis have all been used to demonstrate that certain carboxylic acids and methyl esters of bacteria are effective oviposition booster's kairomones for gravid *Ae. aegypti* (Ponnusamy *et al*., 2008a).

CHAPTER 3:0 MATERIALS AND METHODS

3:1 Study site description

The study was conducted in three sites in Kenya, all urban areas: Ukunda and Diani (4˚16'038.8992"S, 39 ˚34'09.0012"E, altitude 23 m.a.s.l), and Msambweni, ($4^0$27'58.4382"S, 39˚28'017.8716"E, altitude 20 m.a.s.l.) in Kwale County (Fig: 2.0) Urban sites describe sampling areas around premises and residential areas within the town at the study sites. The climate for the area is tropical, with long rains and short rains. The average annual rainfall in the region varies between 400 mm and 1680 mm, with June receiving the highest amount of rainfall (131.95 mm) in

the county, a humidity of 60–80%, and an annual temperature of $24^0C – 34°C$ (Ndenga *et al.*, 2017). In addition, the study area is characterized by unplanned construction, a poor sewage system, and the absence of proper water supply (Ndenga *et al.*, 2017). The common mosquito-borne diseases in this county are arboviruses (DENV &CHIKV), malaria, and lymphatic filariasis. The selection of this study site was based on its endemicity to arboviral diseases and seroprevalence rates among the population (Sergon *et al.*, 2008).
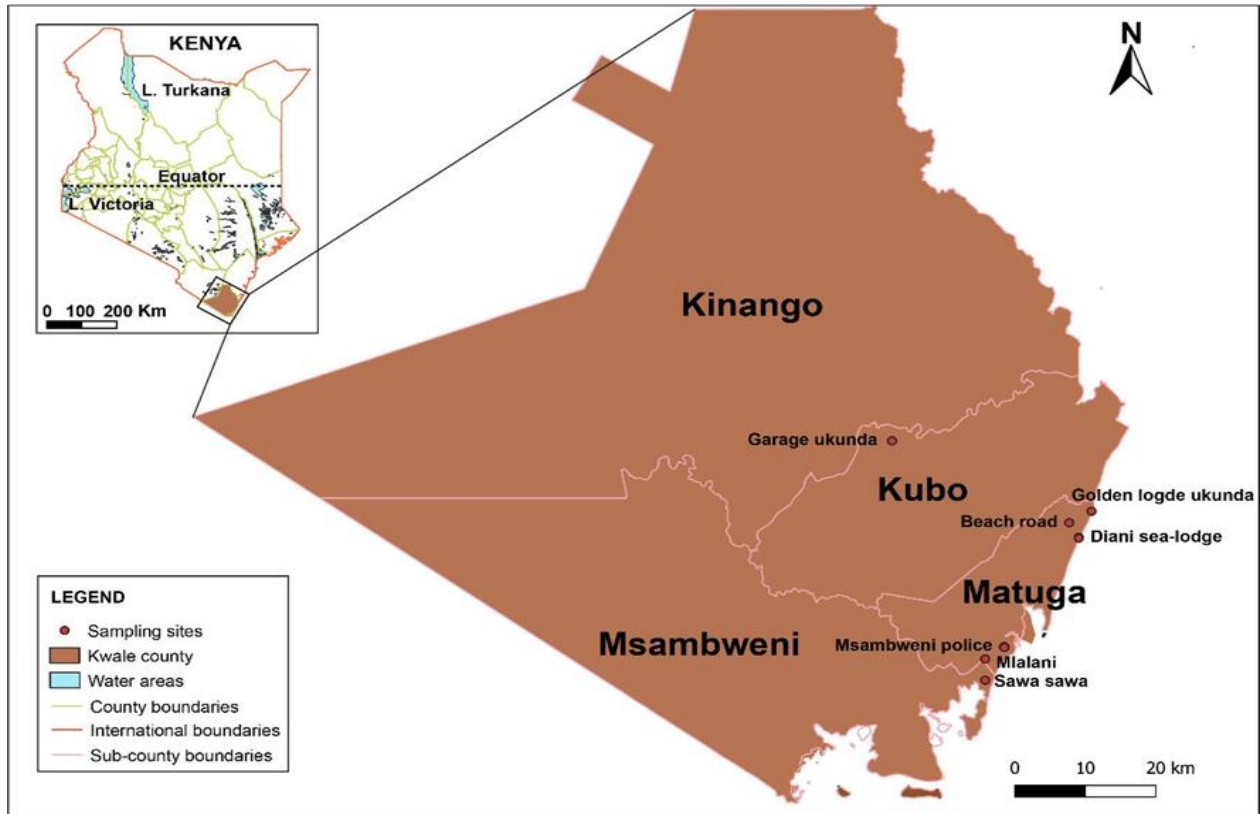
Figure: 2.0. Map showing sampling areas within Kwale County

3:2 Selection locally available plants

The study site is covered by vegetation which includes trees, the selected trees mango (*Magnifera indica*), banana (*Musa acuminata),* cashew (*Anacardium occidentale*) and neem (*Azadirachta indica)* are widely distributed in Kwale County and coastal Kenya at large. Tree holes and banana important anthills plays important role providing the *Aedes* spp and other mosquitoes favorable breeding sites (Lehmann & Kioko, 2005)

3:3 Preparation of plant leaf infusions from locally selected types

Fresh leaves from the selected plants; mango, banana, cashew, and neem, were harvested and air dried on a clean canvas mat for approximately 5 days. The leaves from the respective plants were then chopped into small portions using a kitchen knife and weighed using a digital weighing scale. We measured 33.6 grams of each plant leaf infusions and placed in a plastic jar with 1 liter of previously autoclaved water, sealed tightly to allow for fermentation for a period of 7 days as previously described by (Trexler *et al*., 1998). Aged infusions (at least 7-day) have been found to be most attractive to *Ae. aegypti* (Ponnusamy *et al.*, 2008a; Trexler *et al.*, 2003)

3:4 Evaluation of different infusions on gravid *Ae. aegypti* captures in Gravid *Aedes* Traps

A Latin Square design (4x4) was utilized for field evaluation and placement of GAT and BGs traps baited with infusions prepared from leaves of the aforementioned plants. Each trap covered an area of 40m by 40m per treatment per day. The traps were randomized

3:5 Mosquito processing and identification to species

The prepared leaf infusion was evaluated for six months, from January 2022 to June 2022, with 256 replicates. We deployed traps impregnated with the leaf infusion, and the trapped mosquitoes were retrieved every 24 hours for 3 consecutive days. We recorded the number of gravid mosquitoes trapped and pooled them per infusion type, site of collection, and collection date, using a pair of sharp forceps mosquitoes were identified to the species level using morphological features and published taxonomic keys (Huang & Rueda, 2016). The mosquitoes were then stored at temperatures before transferring them to *icipe*, Duduville campus for further analysis

16

3:6:0 Chemical analysis of the headspace volatiles of the different plant leaf infusions

The dry leaves from selected trees were transported to the laboratory at *icipe* Nairobi, and then we prepared the infusion according to the Trexler protocol,(Trexler *et al.,* 1998) where we measured 33.6g of dry (each plant leaf) chopped leaves to 1 liter of autoclaved water. The aliquots were subjected to headspace volatile trapping, and another was used for bacterial culture

3:6:1 Headspace collection of volatiles

To collect headspace volatiles, 500 mL of the prepared plant leaf infusions were placed in air-tight glass jars and activated charcoal-filtered and humidified air passed over it (Fig: 3.0). The volatiles were collected for 24 h on pre-cleaned (dichloromethane (DCM) and oven-dried) Super Q adsorbent filters (30 mg each, Analytical Research System, Gainesville, Florida, USA) at a flow rate of 170 mL/ min. The four Super-Q filters (each treatment), were each eluted with 200 µL GC-grade DCM (Sigma Aldrich, St. Louis, Missouri, USA) into 2 mL clear glass vials, each containing 250 µL conical point glass inserts (Supelco, Bellefonte, PA, USA)

Figure: 3.0. Image of the air-tight trapping jars for headspace trapping of the chemical volatiles at *icipe* chemistry laboratory

3:6:2 Gas chromatography-mass spectrometry analysis

To analyze and identify the constituent compounds of the plant leaf volatiles, an aliquot (200 μl) of each sample was injected into a gas chromatograph (Agilent technologies-7890) coupled to an inert XL EI/CI mass spectrophotometer (5975C, EI, 70eV, Agilent, Palo Alto, Califonia, USA) (GC/MS) in a splitless injection mode. The GC was equipped with an HP-5 column (30 m x 0.25 mm ID x 0.25 μm film thickness, Agilent, Palo Alto, California, USA), with helium as the carrier gas at a flow rate of 1.2 ml/min. The oven temperature was held at 35 °C for 5 min, then programmed to increase at 10 °C/min to 280 °C and maintained at this temperature for 10 min. The volatile organic compounds were identified by comparing their mass spectra with library data (Adams2.L,

Chemecol.L and NIST05a.L) and with those of authentic standards where possible (see sources and purity under the chemical section below). The absolute areas of each constituent as calculated by the NIST05a.L software was used to estimate their amounts using an external calibration equation generated from known amounts of authentic compounds

3:7:0 Bacterial isolation from the infusion types

We prepared the Luria medium using premixed powder, and for each 950 mL of Distilled H_2O, we added 25 g of a pre-mixed formulation containing tryptone, NaCl, and yeast extract. Then, mix until the powder dissolved. We adjusted the pH using a sodium hydroxide (NaOH) solution to ~7.0. Then we adjusted the solution to a final volume of 1000 mL. Autoclaved using a liquid cycle, then dispensed into the culture plates and allowed to set before being stored at room temperature (Pioli, n.d.)

We used a 7-day-old infusion of each plant type selected. This was done by inoculating 100 µl of the respective plant leaf infusion into Luria Bertani media streaked using a sterile loop and incubated the plates at 36^0C to 37^0C for 12 - 18 hrs. The culture was done in four replicates of different batches of the 7-day-old plant leaf infusion. The culture plates were retrieved, and colony characteristics such as size, shape, texture, and pigmentation were examined to identify pure colonies. The pure colonies were sub-cultured in triplicate and used for DNA extraction for further identification of the bacteria.

3:7:1 DNA extraction from bacteria isolates

Bioline Isolate II Genomic DNA Kit was used to extract genomic DNA from the sub-cultured pure bacterial colonies. An inoculum from the single colony was picked using a sterile loop and placed in a clean microfuge tube, 200µl of Lysis Buffer G1 and 10 µl of Proteinase K were added. The samples were incubated at 72° C for 24 hr. The sample tubes were spun down at 5000g for 15 mins, 200 µl of Lysis Buffer G3 added, vigorously vortexed, and then incubated at 72^0C for 10 mins. The samples were then retrieved and vortexed, and then 200µl of 96%–100% ethanol was added and vortexed again. The samples were transferred and loaded into a DNA spin column and collection tubes, and then centrifuged at 11000 rpm for 3 minutes. The flow-through in the collection tubes

was discarded, and 400µl of Wash Buffer GW1 was added to the samples. The samples were centrifuged, flow-through discarded, 400µl of Wash Buffer GW2 added, and the samples centrifuged again. The DNA spin column was added to a new microfuge tube, and 100 µl of elution buffer G was added to the columns. The samples were incubated at room temperature for 5 minutes and then centrifuged. The DNA extracted from the process above was collected in the microfuge tube (Bioline, 2012).

3:7:2 Polymerase Chain Reaction Amplification

Pure colonies of each bacterial isolate were boiled in 50 µl of sterile distilled water for 10 min and immediately cooled on ice for 5 min. After centrifugation (1,960g, 30 sec), 2 µl of supernatant was used as DNA template in PCRs. The primer sets V_1-V_3 forward (5'CAGGCCTAACACATGCAAGTC-3') V_1- V_3 reverse (5'-GGTTACCTTGTTACGACTT-3') (Darwish *et al.*, 2021; Gichuhi *et al*., 2020; Marchesi *et al.*, 1998)were used to amplify the 16S rRNA gene. The PCR mixture contained 2 µl of template DNA, 0.2µmol concentrations of each primer, 5 µl of 10µl Pfx amplification buffer, 1.5 µl of 10 mM dNTP mixture, 1 µl of 50 mM MgSO4, and 1 unit of Platinum Pfx DNA Polymerase (Invitrogen), and sterile deionized water was added to achieve a final volume of 50 µl. After an initial step consisting of 3 min at 94°C, 30 cycles of amplification were performed; each amplification cycle consisted of 45 seconds at 94°C, 1 min at 55°C, and 1 min at 72°C(Marchesi *et al*., 1998). A final elongation step was carried out for 10 min at 72°C. PCR products were electrophoresed in a 1.5 % agarose gel, followed by ethidium bromide staining. Amplicon size and yield were determined by comparison to molecular mass standards (Low DNA Mass Ladder; GIBCO–BRL).

3:7:3 Sequencing and Identification of Bacterial Isolates.

The successfully amplified PCR products were purified using the QIAquik PCR purification kit (Qiagen) and for sequencing and identification of bacterial isolates, using the same primers above we outsourced laboratory services at Macrogen Inc. (Seoul, South Korea). The sequences were cleaned and edited using Bio-edit (Kaddumukasa *et al*., 2015; Tamura *et al*., 2013) and Codon code Aligner (Codon Code Aligner v.2.0.6, Codon Code Corporation) bioinformatics software to generate a consensus sequence using the forward and reverse strands. The individual sequences were then blasted using the Megablast application in the NCBI public database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences were aligned and the phylogenetic and molecular evolutionary genetic relationships were analyzed with construction of a phytogenic tree in MEGA v11 bioinformatics software (Lukenge *et al*., n.d.) Phylogenetic tree was computed with the Kimura 2-parameter model using the Maximum Likelihood method. The model was selected based on the Bayesian Information Criterion (BIC) values.(Kumar *et al.*, 2001; Tamura *et al.*, 2013) The bootstrap consensus tree was inferred from 1000 replicates, and less than 50% bootstrap replicates collapsed

3:8. Statistical data analysis.

We recorded for the entire study period the daily mosquito counts of the gravid *Ae. aegypti* retrieved from Gravid *Aedes* traps. To test the effects of the leaf infusion treatment on the catches, we subjected the recorded number of catches per treatment to a generalized linear model (GLM) with a negative binomial error structure. In addition, to compare chemical profiles of the volatile organic compounds, we subjected compounds based on quality (%) and peak rates The chemical analysis profile of the compounds identified from the different infusions was analyzed using the Non-metric multidimensional scaling analysis (NMDS) plot based on Bray-Curtis analysis of similarity ANOSIM(Best fit mode), sequential Bonferroni corrected. Statistical significance was considered for ($P < 0.05$). All graphs were drawn using the graph pad prism All GLMs were implemented in R v. 3.3.1 (Rdc, 2010) with the mass package at 95% significance level. Model validity was assessed by inspection of residuals.

CHAPTER 4:0. RESULTS

4:1. Comparison of mosquito catches from gravid *Aedes* traps baited with different infusions

A total number of 2151 mosquitoes were captured from the traps baited with different infusions the trapping was done for a period of 6 months with 256 replications of the trapping experiment. The traps baited with the prepared infusion attracted mosquitoes as follows: - Banana (N=565), Mango (N=1023), Cashew (N=383) and Neem (N=179) (Table 1.0). All the traps reported significantly distinct number of mosquitoes ($p<0.001$) (Figure 3.0). The recorded no. of the mosquito retrieved from the traps showed that mango baited traps had 2-7- fold increased captures than other infusions followed by the banana, cashew and neem infusion. The traps also captured other forms of mosquitoes in this case males and *culines / anophelines spp* (Table 1:0; Figure 3:0). Surprisingly, the trap baited with mango infusion similarly attracted the highest number of the aforementioned mosquitoes (Table: 1.0) compared to the traps baited with the other infusions. Gravid mosquitoes accounted for the highest number of trapped mosquitos' species irrespective of the trap (**n=1504**) compared to the males (**n=274**) and other species (**n=373**).

Table 1.0: Mosquito composition captured in GAT baited with different leaf infusions

Mosquito catches in gravid *Aedes* trap		
Type of infusion treatment	**Gravid** *Ae. aegypti*	**Males** *Ae. aegypti*	*Other spp_Culines/anophelines*
Banana	414	64	87
Cashew	256	43	84
Mango	730	158	136
Neem	104	09	66
TOTAL	**1504**	**274**	**373**

Analysis using generalized linear model (GLM) with the negative binomial fit structure (Fig: 3) the results based on the (*df*= 1.026; CI=95%, *p=0.001*). We evaluated the infusions competitively, the mean catches recorded, It depicts that mango had the highest mean (μ=2.86 +/- 0.17) followed by the banana (μ= 1.62 +/- 0.11), cashew (μ=1.0 +/-0.08) and neem (μ=0.41+/-0.04)
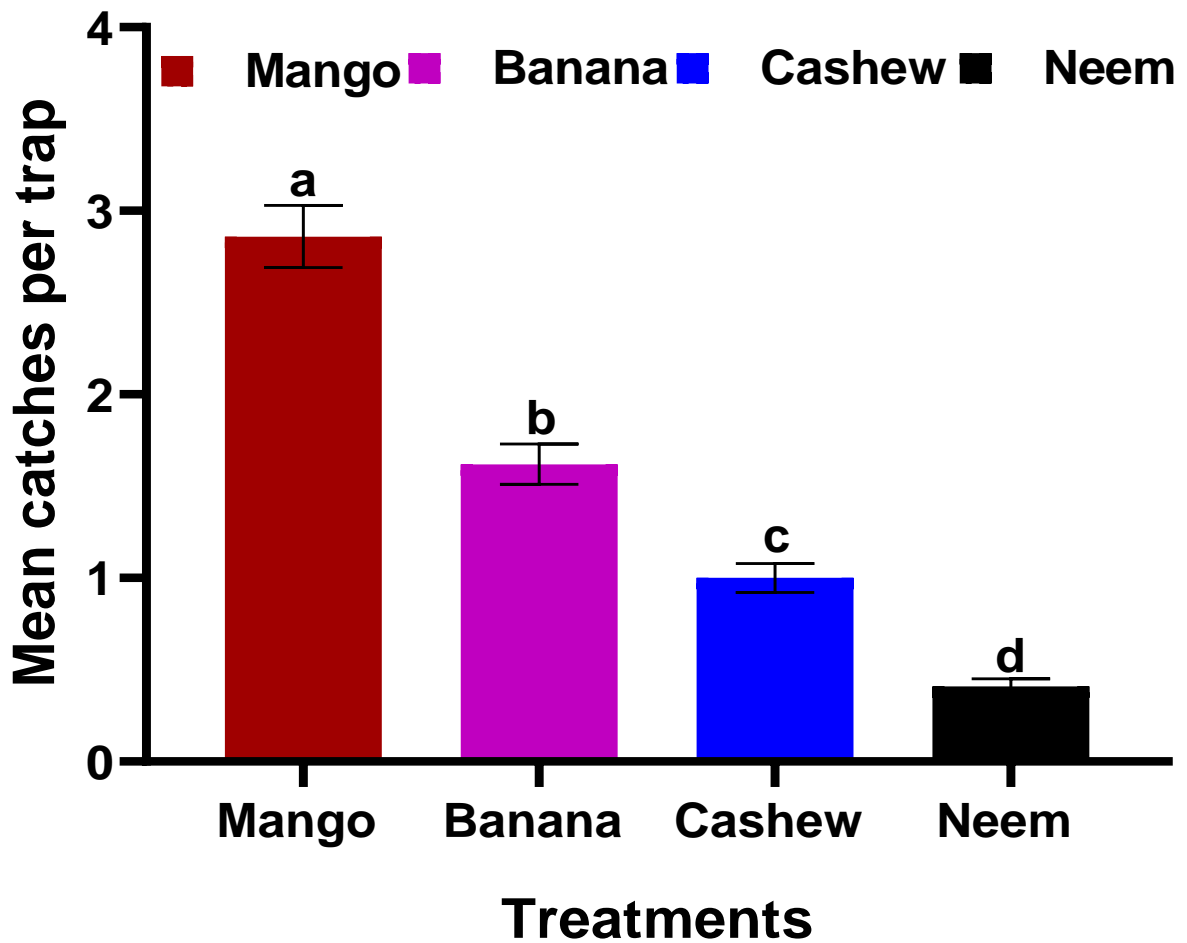
Figure: 4.0. Mean catches of Gravid *Aedes* mosquito per trap for the respective infusions a) Mango, b) Banana, c) Cashew and d) Neem

4:2. Chemical analysis of the volatile profile of the different plant leaf infusions

Different classes of compounds from volatile chemical were collected from the leaf infusions this includes ketones (38.4%), terpenes (26.2%), phenolic (24.7%), alcohols (7.4%), hydrocarbons (2.7%), esters (0.18%), indole (0.4%) and carboxylic acids (0.05%) (Fig 4.0) were identified by the gas chromatography mass spectrometry (GC-MS). A number of common compounds were was specifically associated with an infusion while some chemical compounds were common across the infusions. Mango had 19 chemical compounds which is the highest followed by cashew 14, banana 12 and neem 11.

Phenol was abundantly and uniquely found in all the 5 replicates for mango only. Other chemical volatiles were present in two or three infusions or all the infusions, for example styrene, isopentyl formate were present across the infusion, propanoic acid were present mango and neem infusions. Chemicals such as cyclohexanone, linalool oxide, hexanol and cedrol were present in mango, banana and cashew and absent in neem infusions (Fig 4.0)
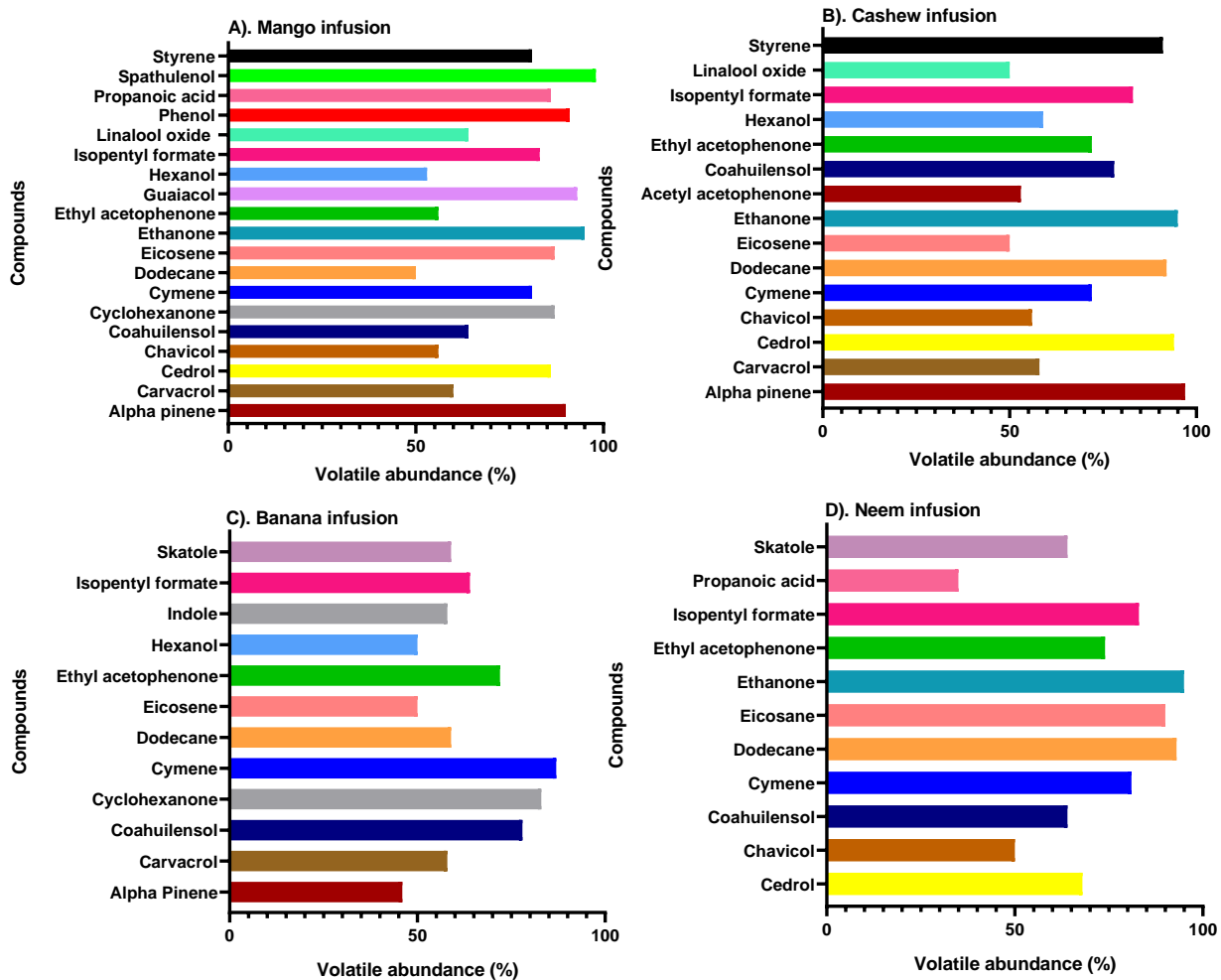
Figure: 5.0. Compounds identified from the different types of plant infusions used in the GAT by gas chromatography mass spectrometry A). Mango, B). Cashew B). Banana D). Neem infusion

Figure: 5.1. Non-metric multidimensional scaling analysis (NMDS) plot based on bray curtis showing comparison of chemical profiles of the leaf infusions for Mango, Cashew, and Banana and Neem

To analyze the (dis-) similarity of the volatile chemical profiles identified by the GC-MS a cross the infusions we subjected the profiles to Analysis of similarity R on bray Curtis (ANOSIM R: 0.33, *p*=0.0004) (Fig: 5.1). Here, the first two infusions, mango and banana made 82% of the total chemical profiles, while cashew and neem makes up 18% of the chemical volatiles.

4:3. Identification of bacterial isolates from the different infusions

We isolated a total number of 13 bacteria in 4 replicates of the different batches of the infusions, culturing the infusions using Luria Bertani media resulted in recovery of bacterial isolates which were identified by PCR and sequencing of the16S rRNA gene as belonging to families:- Bacillaceae (*Priesta filamentosa*, *Bacillus pumilus*, *B. methylotrophicus*, *B. halotolerans*, *B. velezensis*, *B. subtilis*, *B. exarquiensis*, *B. amyloliquefasciens*, *B. siamensis*) Pseudomonadaceae (e.g., *Pseudomonas mendocina*) Enterobacteriaceae (*Klebsiella pneumoniae*, *Citrobacter koseri*) Moraxellaceae (*Acinetobacter sp*.) (Fig 4.2)Mango associated with bacteria species in the family Bacillaceae (*Priesta filamentosa*, *Bacillus pumilus*, *B. methylotrophicus*, *B. halotolerans*, *B. velezensis*, *B. subtilis*, *B. exarquiensis*, *B. amyloliquefasciens*, *B. siamensis*), cashew by Bacillaceae (*B. subtilis*, *Bacillus pumilus*), Enterobacteriaceae (*Klebsiella pneumoniae*, *Citrobacteri koseri*)); banana: Bacillaceae (*B. exarquiensis*, *B. amyloliquefasciens*, *B. siamensis*), Moraxellaceae (*Acinetobacter sp*.), and neem by Pseudomonadaceae (e.g., *Pseudomonas mendocina*), Bacillaceae (*B. amyloliquefasciens*, *B. siamensis*) and Enterobacteriaceae (*Klebsiella pneumoniae*, *Citrobacteri koseri*) (Fig: 6.0, 6.1) some bacterial isolated for more than one infusion such as *Bacilllus pumilus* present in mango and cashew infusions, *Acinetobacter spp* in mango and banana, *B. amyloliquefasciens* in banana and neem. *Citrobacteri koseri* and *Acinetobacter spp* is common in more than two infusions (Fig 5.0)
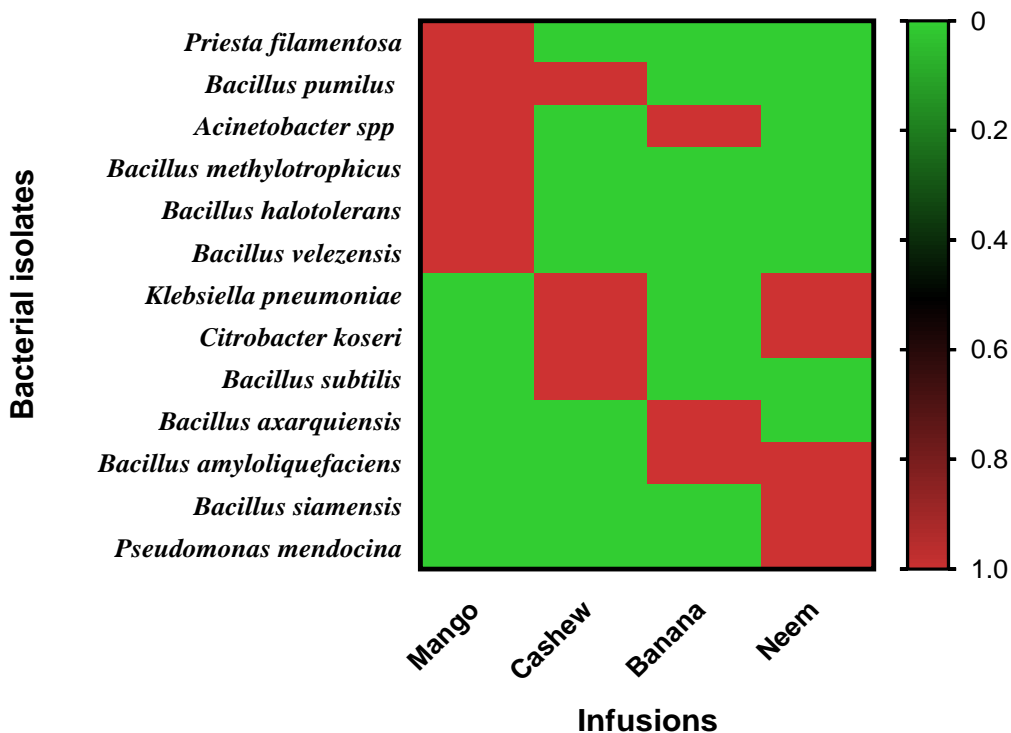
Figure: 6.0 Heat map showing different classes of bacteria identified from the different plant infusions a). Mango, b). Cashew c). Banana and d). Neem; color intensity of bands ranges [0-low (Green), 1.0- high (Red) indicates the number of bacterial species.

To evaluate the relationship between the bacteria isolated from leaf infusions in this study to other bacterial sequences stored genebank we constructed a phylogenetic tree for *Bacillaceae, Moraxellaceae, Pseudomanaceae, and Enterobacteriaceae* members using MEGA v11 Sequences were aligned using Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Majority of the bacteria identified belongs Bacillacea family with clustered relationships with other families *Moraxellaceae, ,* and *Enterobacteriaceae* which forms the least number of the bacteria with *Klebsiella pneumoniae* and *Pseudomonas mendocina* distinctively different from other bacteria (Fig:6.1)
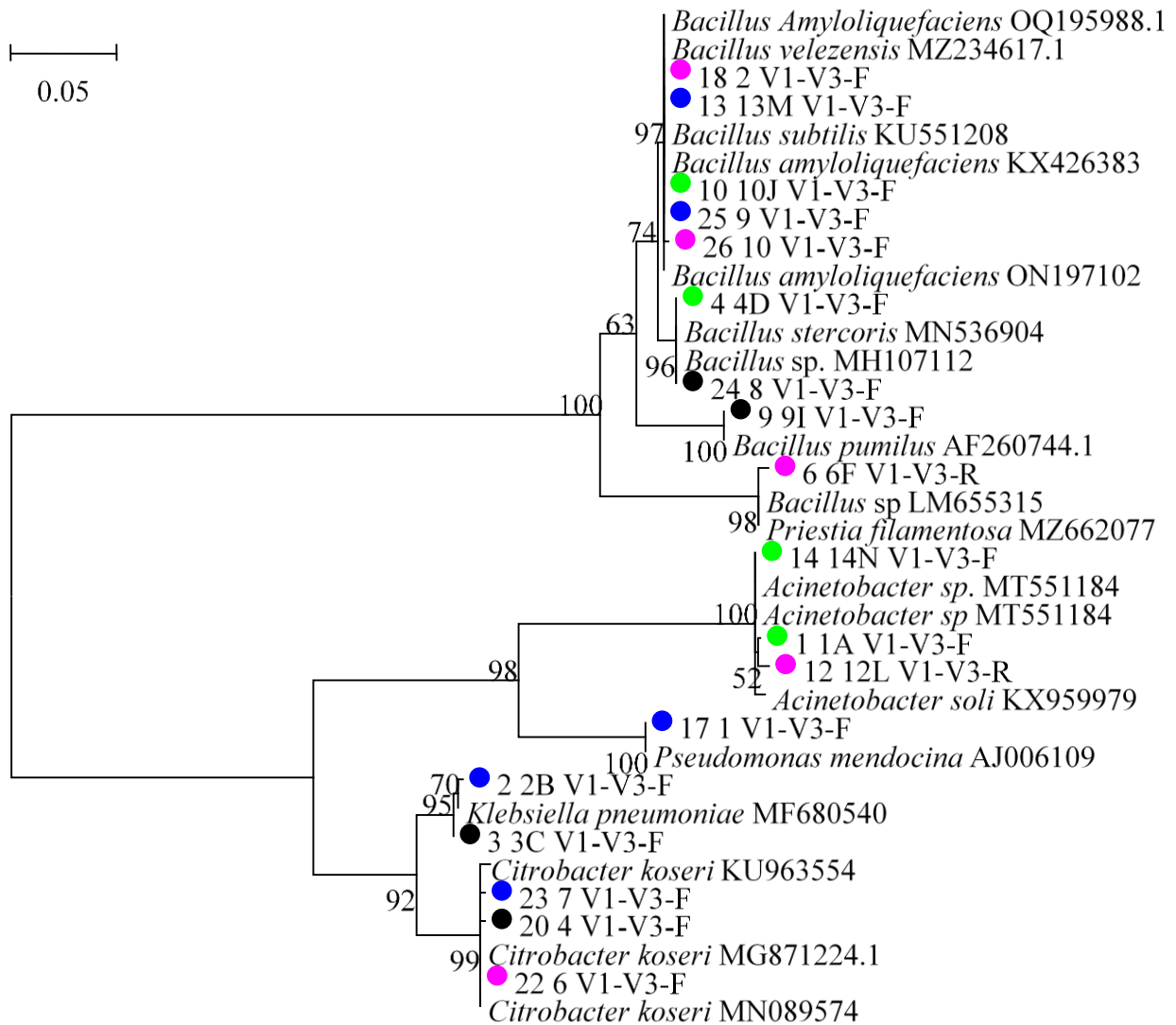
Fig: 6.1. Molecular Phylogenetic analysis tree by Maximum Likelihood method for *Bacillaceae, Moraxellaceae, Pseudomanaceae, and Enterobacteriaceae* isolated from the leaf infusion. The model selected based on the Bayesian Information Criterion (BIC) values. The bootstrap consensus tree was inferred from 1000 replicates, and less than 50% bootstrap replicates collapsed

CHAPTER 5:0 DISCUSSION, CONCLUSION AND RECOMENDATIONS

5:1 Discussion

Mosquito surveillance is an important component of disease epidemiologic risk assessments. This is mostly achieved through the use of effective trap tools to maximize detection of viral pathogens (Tchouassi *et al.,* 2013). Gravid *Aedes* trap (GAT) is among the universally employed tool to monitor blood fed adult mosquito populations. Findings from this study demonstrated that baiting GAT with plant infusions from mango increased the number of mosquito catches compared to the use of infusions from cashew, banana and neem ((p<0.001) (Figure 4.0). Infusions have previously been shown to be effective attractants for both larval and adult mosquito populations. For instance, hay grass infusion *Megathyrsus maximus* and, *Azadirachta indica* (Neem) extracts as well as *Bacillus thuringiensis israelensis* formulations are effective attractants for both *Aedes* and *Culicidae* larvae and adults respectively(Alouani *et al.*, 2009; da Silva *et al.,* 2022). The disparity in number of mosquito catches among the different plant infusions could be attributed to the difference in composition and potency. Irrespective of the type of infusion baited in the trap, GAT attracted male *Aedes* mosquitoes as well as mosquitoes from the other species. The number of trapped gravid mosquitoes were however higher demonstrating the efficiency of this trap which has previously been used in surveillance of dengue vectors (Ritchie *et al*., 2013).

Further chemical analysis of the chemical profiles of the plant infusions identified different classes of compounds including Esters, Ketones, Phenolic, Hydrocarbons and Carboxylic acids. The plant infusions reported different types and quantities of metabolites(Meza *et al.,* 2020). Mango infusion with evidence of attracting a significantly higher number of mosquitoes (Fig 4.0) reported phenol, spathulenol and guaiacol as the dominant compounds, which might be attributed to the higher number of mosquitoes, observed in the traps. Other compounds identified include linalool oxide,

which have been implicated in attraction of *Ae. aegypti* in field trails (Nyasembe *et al*., 2015) .Phenol is a strong attractant for both *Ae. aegypti* and *C. tarsalis* (Wooding *et al*., 2020).while on the contrast spathulenol has been shown to be a deterrent to *Ae. aegypti* mosquito (Cantrell *et al*., 2018)

Mosquito breeding habitats are rich in organic matter and microorganisms, which have been hypothesized to have a role in mosquito breeding sites previously but their actual role has yet to be determined and substantiated. Additionally, the specific species of bacteria, which influence a range of mosquito behaviors, such as the preference for egg-laying site(Coon *et al*., 2016; Ranasinghe & Amarasinghe, 2020). For instance, (Xia *et al*., 2021) demonstrated a 20 times increase in the number of eggs laid by *Ae. aegypti* in bamboo infusions from *Bambusa spp* than distilled water. Similarly, *Aedes* females were shown to lay more eggs when challenged with infusions from bamboo species *Arundinaria gigantea* or leaf infusion from white oak than distilled water (Ponnusamy *et al*., 2008a). Here, both studies attributed the mosquito oviposition behavior to the compounds released from the microbes. In our study, the infusions released a number of compounds and diverse microbial community including *Bacillus, Acinetobacter, Citrobacter* and *Klebsiella.* The *Acinetobacter spp* bacteria has been associated with nutritional benefits to the mosquito larva as well aid in digestion by metabolizing amino acids such as valeric acid and glycine(Mosquera *et al.*, 2021) (Coon *et al.*, 2016). Directly affecting the mosquito preference for an oviposition site. Mango infusion reported the highest number of microorganisms including Bacillus, *Acinetobacter spp* and *Priesta spp* (Figure 6:0). *Bacillus thuringensis* has majorly been implicated in the stimulation of *Ae. aegypti* to the oviposition sites while acting as a deterrent to *Ae. albopictus* and *Culex quinquefasciatus* and with no reported response to *An. Gambiae* (Girard *et al.*, 2021).The bacillus species identified in mango infusion together with *Priesta filamentosa* are gram positive bacteria

33

mostly found in marine environments known to be suitable breeding sites for *Ae. aegypti.* However, the role of this microbes and their respective compounds in oviposition is yet to be elucidated on whether they act independently or together. Apart from *Bacillus* species, cashew infusion reported *Citrobacter koseri* as one of the possible microbe influencing oviposition behavior. Although little is known about *C koseri, C freundii* has been shown to stimulate *Ae. aegypti* and *Ae. albopictus* oviposition behavior (Ponnusam*y et al*., 2008b) .Despite the low numbers observed in the GAT traps baited with neem infusion, microbial community of this infusion reported *Pseudomonas mendocina, Klebsiella pneumoniae, Citrobacter koseri* and *Bacillus siamensis.* The bacterial classes have been attributed with effect on oviposition behavior hence the repellency could be volatiles emitted from the neem infusion(Sharma *et al*., 1995)

5:2 Conclusion

In conclusion, our study demonstrates that plants emit natural odor signatures, which can be utilized as lures in GAT for surveillance. The lures that are tested in the field experiment will improve, making the surveillance reliable, particularly in the arbovirus endemic frontiers in Kenya and beyond. In this study, mango infusion specifically attracted a significant number of mosquitoes owing to the abundance of volatiles and signature cues such as phenol as well as the diverse microbial population, which can be attributed to the potential preference of the gravid mosquitoes to visit the trap. Neem infusion, a naturally repellant for mosquitoes, attracted relatively low number of mosquitoes owing to the effect of volatiles emitted from the infusion. The microbial community identified from the infusions together with their respective compounds lays a framework for the development of potent ovipositional attractants which can be commercialized, which will contribute significantly in the improvement of arbovirus surveillance tools and subsequently control of *Aedes*-borne viral diseases.

5:3. Recommendations

This study presents a significant step in elucidating important plant volatile organic compounds (VOCs) mediating mosquito-plant interactions as well as their interaction with the microbial organisms in mosquito breeding sites. Development of potential lures using the identified compounds could be envisaged. we recommend further laboratory bioassays such as Coupled gas Chromatography Electroantennographic Detection (GC-EAD) of the individual volatile compounds in order to understand the mosquito antennal response to the distinct chemicals in this identified by GC-MS.

REFERENCES

A Polson, K., Curtis, C., Moh Seng, C., G Olson, J., Chantha, N., & C Rawlins, S. (2002). *The use of ovitraps baited with hay infusion as a surveillance tool for Aedes aegypti mosquitoes in Cambodia.*

Agha, S. B., Alvarez, M., Becker, M., Fèvre, E. M., Junglen, S., & Borgemeister, C. (2021). Invasive Alien Plants in Africa and the Potential Emergence of Mosquito-Borne Arboviral Diseases—A Review and Research Outlook. *Viruses*, *13*(1), Article 1. https://doi.org/10.3390/v13010032

Alouani, A., Rehimi, N., & Soltani, N. (2009). *Larvicidal Activity of a Neem Tree Extract (Azadirachtin) Against Mosquito Larvae in the Republic of Algeria*. *2*(1).

Arslan, A., Rathor, H., Mukhtar, M., Mushtaq, S., Bhatti, A., Asif, M., Arshad, I., & Ahmed, F. (2016). Spatial distribution and insecticide susceptibility status of Aedes aegypti and Aedes albopictus in dengue affected urban areas of Rawalpindi, Pakistan. *Journal of Vector Borne Diseases*, *53*, 136–143.

Barrera, R. (2022). New tools for Aedes control: Mass trapping. *Current Opinion in Insect Science*, *52*, 100942. https://doi.org/10.1016/j.cois.2022.100942

Bhatt, S., Gething, P. W., Brady, O. J., Messina, J. P., Farlow, A. W., Moyes, C. L., Drake, J. M., Brownstein, J. S., Hoen, A. G., Sankoh, O., Myers, M. F., George, D. B., Jaenisch, T., Wint, G. R. W., Simmons, C. P., Scott, T. W., Farrar, J. J., & Hay, S. I. (2013a). The global distribution and burden of dengue. *Nature*, *496*(7446), Article 7446. https://doi.org/10.1038/nature12060

Bhatt, S., Gething, P. W., Brady, O. J., Messina, J. P., Farlow, A. W., Moyes, C. L., Drake, J. M., Brownstein, J. S., Hoen, A. G., Sankoh, O., Myers, M. F., George, D. B., Jaenisch, T., Wint,

G. R. W., Simmons, C. P., Scott, T. W., Farrar, J. J., & Hay, S. I. (2013b). The global distribution and burden of dengue. *Nature*, *496*(7446), Article 7446. https://doi.org/10.1038/nature12060

Bonizzoni, M., Dunn, W. A., Campbell, C. L., Olson, K. E., Marinotti, O., & James, A. A. (2012). Complex Modulation of the Aedes aegypti Transcriptome in Response to Dengue Virus Infection. *PLOS ONE*, *7*(11), e50512. https://doi.org/10.1371/journal.pone.0050512

Brady, O. J., & Hay, S. I. (2020). The Global Expansion of Dengue: How Aedes aegypti Mosquitoes Enabled the First Pandemic Arbovirus. *Annual Review of Entomology*, *65*(1), 191–208. https://doi.org/10.1146/annurev-ento-011019-024918

Brown, J. E., Scholte, E.-J., Dik, M., Den Hartog, W., Beeuwkes, J., & Powell, J. R. (2011). Aedes aegypti Mosquitoes Imported into the Netherlands, 2010. *Emerging Infectious Diseases*, *17*(12), 2335–2337. https://doi.org/10.3201/eid1712.110992

Cantrell, C. L., Ali, A., & Jones, A. M. P. (2018). Isolation and identification of mosquito biting deterrents from the North American mosquito repelling folk remedy plant, Matricaria discoidea DC. *PLOS ONE*, *13*(10), e0206594. https://doi.org/10.1371/journal.pone.0206594

Charrel, R. N., De Lamballerie, X., & Raoult, D. (2007). Chikungunya Outbreaks—The Globalization of Vectorborne Diseases. *New England Journal of Medicine*, *356*(8), 769–771. https://doi.org/10.1056/NEJMp078013

Cianci, D., Hartemink, N., & Ibáñez-Justicia, A. (2015). Modelling the potential spatial distribution of mosquito species using three different techniques. *International Journal of Health Geographics*, *14*(1), 10. https://doi.org/10.1186/s12942-015-0001-0

Coon, K. L., Brown, M. R., & Strand, M. R. (2016). Mosquitoes host communities of bacteria that are essential for development but vary greatly between local habitats. *Molecular Ecology*, *25*(22), 5806–5826. https://doi.org/10.1111/mec.13877

Crepeau, T. N., Healy, S. P., Bartlett-Healy, K., Unlu, I., & Farajollahi, A. (2013). *Effects of Biogents Sentinel Trap Field Placement on Capture Rates of Adult*.

da Silva, H., Oliveira, T. M. P., & Sallum, M. A. M. (2022). Bacterial Community Diversity and Bacterial Interaction Network in Eight Mosquito Species. *Genes*, *13*(11), Article 11. https://doi.org/10.3390/genes13112052

Darwish, N., Shao, J., Schreier, L. L., & Proszkowiec-Weglarz, M. (2021). Choice of 16S ribosomal RNA primers affects the microbiome analysis in chicken ceca. *Scientific Reports*, *11*(1), Article 1. https://doi.org/10.1038/s41598-021-91387-w

Demanou, M., Pouillot, R., Grandadam, M., Boisier, P., Kamgang, B., Hervé, J. P., Rogier, C., Rousset, D., & Paupy, C. (2014). Evidence of Dengue Virus Transmission and Factors Associated with the Presence of Anti-Dengue Virus Antibodies in Humans in Three Major Towns in Cameroon. *PLOS Neglected Tropical Diseases*, *8*(7), e2950. https://doi.org/10.1371/journal.pntd.0002950

Eiras, A. E., Buhagiar, T. S., & Ritchie, S. A. (2014). Development of the Gravid Aedes Trap for the Capture of Adult Female Container–Exploiting Mosquitoes (Diptera: Culicidae). *Journal of Medical Entomology*, *51*(1), 200–209. https://doi.org/10.1603/ME13104

Farlow, R., Russell, T. L., & Burkot, T. R. (2020). Nextgen Vector Surveillance Tools: Sensitive, specific, cost-effective and epidemiologically relevant. *Malaria Journal*, *19*(1), 432. https://doi.org/10.1186/s12936-020-03494-0

Gichuhi, J., Khamis, F., Berg, J. V. D., Mohamed, S., Ekesi, S., & Herren, J. (2020). *Influence of Bactrocera Dorsalis Larval Gut Bacteria on its Susceptibility to the Entomopathogenic Fungus, Metarhizium Anisopliae* [Preprint]. In Review. https://doi.org/10.21203/rs.3.rs-55527/v1

Girard, M., Martin, E., Vallon, L., Raquin, V., Bellet, C., Rozier, Y., Desouhant, E., Hay, A.-E., Luis, P., Valiente Moro, C., & Minard, G. (2021). Microorganisms Associated with Mosquito Oviposition Sites: Implications for Habitat Selection and Insect Life Histories. *Microorganisms*, *9*(8), Article 8. https://doi.org/10.3390/microorganisms9081589

Gubler, D. J. (2002). The Global Emergence/Resurgence of Arboviral Diseases As Public Health Problems. *Archives of Medical Research*, *33*(4), 330–342. https://doi.org/10.1016/S0188-4409(02)00378-8

Harwood, J. F., Rama, V., Hash, J. M., & Gordon, S. W. (2018). The Attractiveness of the Gravid Aedes Trap to Dengue Vectors in Fiji. *Journal of Medical Entomology*, *55*(2), 481–484. https://doi.org/10.1093/jme/tjx221

Huang, Y.-M., & Rueda, L. M. (2016). <p>A pictorial key to the sections, groups, and species of the Aedes (Diceromyia) in the Afrotropical Region (Diptera: Culicidae)</p>. *Zootaxa*, *4079*(2), Article 2. https://doi.org/10.11646/zootaxa.4079.2.9

Kaddumukasa, M. A., Kayondo, J. K., Masiga, D., Akol, A. M., Lutwama, J. J., & Masembe, C. (2015). High proportion of mosquito vectors in Zika forest, Uganda, feeding on humans has implications for the spread of new arbovirus pathogens. *African Journal of Biotechnology*, *14*(16), Article 16. https://doi.org/10.4314/ajb.v14i16

Khormi, H. M., & Kumar, L. (2014). Climate change and the potential global distribution of Aedes aegypti: Spatial modelling using geographical information system and CLIMEX. *Geospatial Health*, *8*(2), Article 2. https://doi.org/10.4081/gh.2014.29

Konongoi, S. L., Nyunja, A., Ofula, V., Owaka, S., Koka, H., Koskei, E., Eyase, F., Langat, D., Mancuso, J., Lutomiah, J., & Sang, R. (2018). Human and entomologic investigations of chikungunya outbreak in Mandera, Northeastern Kenya, 2016. *PLOS ONE*, *13*(10), e0205058. https://doi.org/10.1371/journal.pone.0205058

Kramer, L. D., & Ciota, A. T. (2015). Dissecting vectorial capacity for mosquito-borne viruses. *Current Opinion in Virology*, *15*, 112–118. https://doi.org/10.1016/j.coviro.2015.10.003

Kumar, S., Tamura, K., Jakobsen, I. B., & Nei, M. (2001). MEGA2: Molecular evolutionary genetics analysis software. *Bioinformatics*, *17*(12), 1244–1245. https://doi.org/10.1093/bioinformatics/17.12.1244

Lehmann, I., & Kioko, E. (2005). LEPIDOPTERA DIVERSITY, FLORISTIC COMPOSITION AND STRUCTURE OF THREE KAYA FORESTS ON THE SOUTH COAST OF KENYA. *Journal of East African Natural History*, *94*(1), 121–163. https://doi.org/10.2982/0012-8317(2005)94[121:LDFCAS]2.0.CO;2

Logan, J. G., Stanczyk, N. M., Hassanali, A., Kemei, J., Santana, A. E., Ribeiro, K. A., Pickett, J. A., & Mordue (Luntz), A. J. (2010). Arm-in-cage testing of natural human-derived mosquito repellents. *Malaria Journal*, *9*(1), 239. https://doi.org/10.1186/1475-2875-9-239

Lukenge, M., Birungi, J., Kayondo, J., & Mukwaya, L. G. (n.d.). *Isolation and molecular characterization of Gram positive entomopathogenic bacteria against the major malaria vector Anopheles gambiae in Uganda*.

Lutomiah, J., Barrera, R., Makio, A., Mutisya, J., Koka, H., Owaka, S., Koskei, E., Nyunja, A., Eyase, F., Coldren, R., & Sang, R. (2016). Dengue Outbreak in Mombasa City, Kenya, 2013–2014: Entomologic Investigations. *PLOS Neglected Tropical Diseases*, *10*(10), e0004981. https://doi.org/10.1371/journal.pntd.0004981

Marchesi, J., Sato, T., Weightman, A., Martin, T., Fry, J., Hiom, S., Dymock, D., & Wade, W. (1998). Design and Evaluation of Useful Bacterium-Specific PCR Primers That Amplify Genes Coding for Bacterial 16S rRNA. *Applied and Environmental Microbiology*, *64*, 2333. https://doi.org/10.1128/AEM.64.2.795-799.1998

Melo, N., Wolff, G. H., Costa-da-Silva, A. L., Arribas, R., Triana, M. F., Gugger, M., Riffell, J. A., DeGennaro, M., & Stensmyr, M. C. (2020). Geosmin Attracts Aedes aegypti Mosquitoes to Oviposition Sites. *Current Biology*, *30*(1), 127-134.e5. https://doi.org/10.1016/j.cub.2019.11.002

Mesquita, R. D., Vionette-Amaral, R. J., Lowenberger, C., Rivera-Pomar, R., Monteiro, F. A., Minx, P., Spieth, J., Carvalho, A. B., Panzera, F., Lawson, D., Torres, A. Q., Ribeiro, J. M. C., Sorgine, M. H. F., Waterhouse, R. M., Montague, M. J., Abad-Franch, F., Alves-Bezerra, M., Amaral, L. R., Araujo, H. M., … Oliveira, P. L. (2015). Genome of Rhodnius prolixus, an insect vector of Chagas disease, reveals unique adaptations to hematophagy and parasite infection. *Proceedings of the National Academy of Sciences*, *112*(48), 14936–14941. https://doi.org/10.1073/pnas.1506226112

Meza, F. C., Roberts, J. M., Sobhy, I. S., Okumu, F. O., Tripet, F., & Bruce, T. J. A. (2020). Behavioural and Electrophysiological Responses of Female Anopheles gambiae Mosquitoes to Volatiles from a Mango Bait. *Journal of Chemical Ecology*, *46*(4), 387–396. https://doi.org/10.1007/s10886-020-01172-8

Milugo, T. K., Tchouassi, D. P., Kavishe, R. A., Dinglasan, R. R., & Torto, B. (2021a). Root exudate chemical cues of an invasive plant modulate oviposition behavior and survivorship of a malaria mosquito vector. *Scientific Reports*, *11*(1), Article 1. https://doi.org/10.1038/s41598-021-94043-5

Milugo, T. K., Tchouassi, D. P., Kavishe, R. A., Dinglasan, R. R., & Torto, B. (2021b). Root exudate chemical cues of an invasive plant modulate oviposition behavior and survivorship of a malaria mosquito vector. *Scientific Reports*, *11*(1), 14785. https://doi.org/10.1038/s41598-021-94043-5

Mosquera, K. D., Martinez Villegas, L. E., Pidot, S. J., Sharif, C., Klimpel, S., Stinear, T. P., Moreira, L. A., Tobias, N. J., & Lorenzo, M. G. (2021). Multi-Omic Analysis of Symbiotic Bacteria Associated With Aedes aegypti Breeding Sites. *Frontiers in Microbiology*, *12*, 703711. https://doi.org/10.3389/fmicb.2021.703711

Mulatier, M., Boullis, A., Dollin, C., Cebrián-Torrejón, G., & Vega-Rúa, A. (2023). Chikungunya Virus Infection and Gonotrophic Cycle Shape Aedes aegypti Oviposition Behavior and Preferences. *Viruses*, *15*(5), Article 5. https://doi.org/10.3390/v15051043

Ndenga, B. A., Mutuku, F. M., Ngugi, H. N., Mbakaya, J. O., Aswani, P., Musunzaji, P. S., Vulule, J., Mukoko, D., Kitron, U., & LaBeaud, A. D. (2017a). Characteristics of Aedes aegypti adult mosquitoes in rural and urban areas of western and coastal Kenya. *PLOS ONE*, *12*(12), e0189971. https://doi.org/10.1371/journal.pone.0189971

Ndenga, B. A., Mutuku, F. M., Ngugi, H. N., Mbakaya, J. O., Aswani, P., Musunzaji, P. S., Vulule, J., Mukoko, D., Kitron, U., & LaBeaud, A. D. (2017b). Characteristics of Aedes aegypti adult mosquitoes in rural and urban areas of western and coastal Kenya. *PLOS ONE*, *12*(12), e0189971. https://doi.org/10.1371/journal.pone.0189971

Nyasembe, V. O., Tchouassi, D. P., Mbogo, C. M., Sole, C. L., Pirk, C., & Torto, B. (2015). Linalool oxide: Generalist plant based lure for mosquito disease vectors. *Parasites & Vectors*, *8*(1), 581. https://doi.org/10.1186/s13071-015-1184-8

Obonyo, M., Fidhow, A., & Ofula, V. (2018). Investigation of laboratory confirmed Dengue outbreak in North-eastern Kenya, 2011. *PLOS ONE*, *13*(6), e0198556. https://doi.org/10.1371/journal.pone.0198556

Ochieng, C., Ahenda, P., Vittor, A. Y., Nyoka, R., Gikunju, S., Wachira, C., Waiboci, L., Umuro, M., Kim, A. A., Nderitu, L., Juma, B., Montgomery, J. M., Breiman, R. F., & Fields, B. (2015). Seroprevalence of Infections with Dengue, Rift Valley Fever and Chikungunya Viruses in Kenya, 2007. *PLOS ONE*, *10*(7), e0132645. https://doi.org/10.1371/journal.pone.0132645

Omondi, W. P., Owino, E. A., Odongo, D., Mwangangi, J. M., Torto, B., & Tchouassi, D. P. (2019). Differential response to plant- and human-derived odorants in field surveillance of the dengue vector, Aedes aegypti. *Acta Tropica*, *200*, 105163. https://doi.org/10.1016/j.actatropica.2019.105163

Owino, E. A. (2018). Aedes spp mosquitoes and emerging neglected diseases of Kenya. *Int. J. Mosq. Res*, *5*, 1–11.

Owino, E. A., Sang, R., Sole, C. L., Pirk, C., Mbogo, C., & Torto, B. (2015). An improved odor bait for monitoring populations of Aedes aegypti-vectors of dengue and chikungunya viruses in Kenya. *Parasites & Vectors*, *8*(1), 253. https://doi.org/10.1186/s13071-015-0866-6

Pioli, P. D. (n.d.). *Protocol: Luria-Bertani (LB) Media/Broth Recipe*.

Ponnusamy, L., Xu, N., Böröczky, K., Wesson, D. M., Abu Ayyash, L., Schal, C., & Apperson, C. S. (2010a). Oviposition Responses of the Mosquitoes Aedes aegypti and Aedes albopictus to Experimental Plant Infusions in Laboratory Bioassays. *Journal of Chemical Ecology*, *36*(7), 709–719. https://doi.org/10.1007/s10886-010-9806-2

Ponnusamy, L., Xu, N., Böröczky, K., Wesson, D. M., Abu Ayyash, L., Schal, C., & Apperson, C. S. (2010b). Oviposition Responses of the Mosquitoes Aedes aegypti and Aedes albopictus to Experimental Plant Infusions in Laboratory Bioassays. *Journal of Chemical Ecology*, *36*(7), 709–719. https://doi.org/10.1007/s10886-010-9806-2

Ponnusamy, L., Xu, N., Nojima, S., Wesson, D. M., Schal, C., & Apperson, C. S. (2008a). Identification of bacteria and bacteria-associated chemical cues that mediate oviposition site preferences by Aedes aegypti. *Proceedings of the National Academy of Sciences*, *105*(27), 9262–9267. https://doi.org/10.1073/pnas.0802505105

Ponnusamy, L., Xu, N., Nojima, S., Wesson, D. M., Schal, C., & Apperson, C. S. (2008b). Identification of bacteria and bacteria-associated chemical cues that mediate oviposition site preferences by Aedes aegypti. *Proceedings of the National Academy of Sciences*, *105*(27), 9262–9267. https://doi.org/10.1073/pnas.0802505105

Ranasinghe, H. a. K., & Amarasinghe, L. D. (2020). Naturally Occurring Microbiota Associated with Mosquito Breeding Habitats and Their Effects on Mosquito Larvae. *BioMed Research International*, *2020*, e4065315. https://doi.org/10.1155/2020/4065315

Rdc, T. (2010). R: A language and environment for statistical computing. *(No Title)*. https://cir.nii.ac.jp/crid/1370294721063650048

Ritchie, S. A., Cortis, G., Paton, C., Townsend, M., Shroyer, D., Zborowski, P., Hall-Mendelin, S., & Van Den Hurk, A. F. (2013). A Simple Non-Powered Passive Trap for the Collection of

Mosquitoes for Arbovirus Surveillance. *Journal of Medical Entomology*, *50*(1), 185–194. https://doi.org/10.1603/ME12112

Sang, R., Lutomiah, J., Chepkorir, E., & Tchouassi, D. P. (2022a). Evolving dynamics of Aedes-borne diseases in Africa: A cause for concern. *Current Opinion in Insect Science*, *53*, 100958. https://doi.org/10.1016/j.cois.2022.100958

Sang, R., Lutomiah, J., Chepkorir, E., & Tchouassi, D. P. (2022b). Evolving dynamics of Aedes-borne diseases in Africa: A cause for concern. *Current Opinion in Insect Science*, *53*, 100958. https://doi.org/10.1016/j.cois.2022.100958

Sant'ana, A. L., Roque, R. A., & Eiras, A. E. (2006). Characteristics of Grass Infusions as Oviposition Attractants to Aedes (Stegomyia) (Diptera: Culicidae). *Journal of Medical Entomology*, *43*(2), 214–220. https://doi.org/10.1093/jmedent/43.2.214

Sergon, K., Njuguna, C., Kalani, R., Ofula, V., Onyango, C., Konongoi, L. S., Bedno, S., Burke, H., Dumilla, A. M., & Konde, J. (2008). Seroprevalence of chikungunya virus (CHIKV) infection on Lamu Island, Kenya, October 2004. *The American Journal of Tropical Medicine and Hygiene*, *78*(2), 333–337.

Sharma, S. K., Dua, V. K., & Sharma, V. P. (1995). Field studies on the mosquito repellent action of neem oil. *The Southeast Asian Journal of Tropical Medicine and Public Health*, *26*(1), 180–182.

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), Article 1. https://doi.org/10.3390/ijerph10010249

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*, *30*(12), 2725–2729. https://doi.org/10.1093/molbev/mst197

Tatem, A. J., Hay, S. I., & Rogers, D. J. (2006). Global traffic and disease vector dispersal. *Proceedings of the National Academy of Sciences*, *103*(16), 6242–6247. https://doi.org/10.1073/pnas.0508391103

Tchouassi, D. P., Agha, S. B., Villinger, J., Sang, R., & Torto, B. (2022). The distinctive bionomics of Aedes aegypti populations in Africa. *Current Opinion in Insect Science*, *54*, 100986. https://doi.org/10.1016/j.cois.2022.100986

Tchouassi, D. P., Sang, R., Sole, C. L., Bastos, A. D. S., Teal, P. E. A., Borgemeister, C., & Torto, B. (2013). Common Host-Derived Chemicals Increase Catches of Disease-Transmitting Mosquitoes and Can Improve Early Warning Systems for Rift Valley Fever Virus. *PLOS Neglected Tropical Diseases*, *7*(1), e2007. https://doi.org/10.1371/journal.pntd.0002007

Tchouassi, D. P., Torto, B., Sang, R., Riginos, C., & Ezenwa, V. O. (2021). Large herbivore loss has complex effects on mosquito ecology and vector-borne disease risk. *Transboundary and Emerging Diseases*, *68*(4), 2503–2513. https://doi.org/10.1111/tbed.13918

Tongaonkar, S. S., & Ghosh, S. N. (1973). Production of Interferon by Arboviruses in Suckling Mouse Brains. *Current Science*, *42*(19), 680–682. https://www.jstor.org/stable/24076028

Trexler, J. D., Apperson, C. S., & Schal, C. (1998). Laboratory and Field Evaluations of Oviposition Responses of Aedes albopictus and Aedes triseriatus (Diptera: Culicidae) to Oak Leaf Infusions. *Journal of Medical Entomology*, *35*(6), 967–976. https://doi.org/10.1093/jmedent/35.6.967

Trexler, J. D., Apperson, C. S., Zurek, L., Gemeno, C., Schal, C., Kaufman, M., Walker, E., Watson, D. W., & Wallace, L. (2003). Role of Bacteria in Mediating the Oviposition Responses of <I>Aedes albopictus</I> (Diptera: Culicidae). *Journal of Medical Entomology*, *40*(6), 841–848. https://doi.org/10.1603/0022-2585-40.6.841

Wang, X., Liu, T., Wu, Y., Zhong, D., Zhou, G., Su, X., Xu, J., Sotero, C. F., Sadruddin, A. A., Wu, K., Chen, X.-G., & Yan, G. (2018). Bacterial microbiota assemblage in Aedes albopictus mosquitoes and its impacts on larval development. *Molecular Ecology*, *27*(14), 2972–2985. https://doi.org/10.1111/mec.14732

WHO, 2016. (2009). *Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control*. World Health Organization.

Wooding, M., Naudé, Y., Rohwer, E., & Bouwer, M. (2020). Controlling mosquitoes with semiochemicals: A review. *Parasites & Vectors*, *13*(1), 80. https://doi.org/10.1186/s13071-020-3960-3

Xia, S., Dweck, H. K. M., Lutomiah, J., Sang, R., McBride, C. S., Rose, N. H., Ayala, D., & Powell, J. R. (2021). Larval sites of the mosquito Aedes aegypti formosus in forest and domestic habitats in Africa and the potential association with oviposition evolution. *Ecology and Evolution*, *11*(22), 16327–16343. https://doi.org/10.1002/ece3.8332