Characterization of a local genetic sexing strain as well as a wild population of *Anopheles arabiensis* from KwaZulu Natal, South Africa.

Leonard Chikondi Dandalo

Thesis submitted to the Faculty of Health Science, University of the Witwatersrand, in fulfilment of the requirements for the degree of Doctor of Philosophy.

Johannesburg, 2017
DECLARATION

I declare that this thesis hereby submitted by me for the Degree of Doctor of Philosophy at the University of Witwatersrand, Johannesburg is my own independent work and has not been previously submitted for any degree or examination in any other University. I further more cede copyright of the thesis in favour of the University of Witwatersrand.

Signature:

On this 5th Day of November 2017
ABSTRACT

Malaria remains endemic in the north-eastern areas of KwaZulu-Natal (KZN), Mpumalanga and Limpopo provinces of South Africa (SA). *Anopheles arabiensis* is now implicated as the main malaria vector. This vector is not completely amenable to current vector control strategies which target indoor biting and resting mosquitoes. SA is moving towards malaria elimination and there is a need for additional vector control interventions to complement existing tools. The sterile insect technique (SIT) targeting *An. arabiensis* was selected as a potential intervention. In a mosquito SIT programme, only sterile males should be released because females are potential disease vectors. In order to achieve male releases only, a reliable sex separation strategy is needed. Additionally, it is imperative to gather entomological baseline information on the population density, species composition, and vectorial capacity of the targeted wild population. The aim of this study was to evaluate the use of a local genetic sexing strain for SIT and to determine the population dynamics of wild *An. arabiensis* in northern KZN. The following objectives were initiated in this study: development of a local genetic sexing strain (GSS), evaluation of the life history and reproductive effects of irradiation on *An. arabiensis*, and weekly mosquito surveillance was conducted over a period of 24 months.

A local GSS named GMK was established by introgressing a local wild-type population of *An. arabiensis* with an available GSS strain. The strain exhibited the following attributes: low egg hatch rates, fast developmental time, long adult survival and a high mating competitiveness. Dieldrin treatment of GMK eggs/larvae mainly produced males but this result remains controversial. The irradiation dose of 70 Gy induced male sterility without compromising their mating competitiveness and impacted negatively on female fitness, but not vectorial capacity. The perennial presence of *An. arabiensis*, the dominant anopheline species in Mamfene, was confirmed. Its population density fluctuated with season reaching a peak in summer. Clay pots were more productive than the other collection methods, collecting 16.3 mosquitoes per trap. This study recorded for the first time wild caught *An. arabiensis* and *An. vaneedeni* infected with *P. falciparum* in SA. *An arabiensis* sporozoite infection rates were 0.7% (2014) and 0.5% (2015). *Anopheles vaneedeni* has never been implicated as a malaria vector in nature. However, an infection rate of 1.96% was recorded (2014-2015), which implicate this species as a potential malaria vector.
These results highlight the importance of intensive mosquito surveillance to establish malaria vectors responsible for low level/residual malaria transmission. The data generated provides important baseline vector surveillance information and is valuable to stakeholders and researchers to make informed decisions regarding the use of SIT against vector mosquitoes in SA.
ACKNOWLEDGEMENTS

Special thanks should go to my supervisors; Prof. Lizette L. Koekemoer who conceived the project and played a significant role in guiding me through this work to its successful completion; Prof. Basil Brooke for his supervision and corrections of this thesis; and Dr. Givemore Munhenga for the wonderful supervision and guidance in laboratory techniques and field work experience. Sincere thanks goes to Prof. Maureen Coetzee for her valuable input and corrections of this thesis. A special acknowledgement goes to Dr. Maria Kaiser for her role in determining the genetic stability of the local genetic sexing strain established during this study, Ms. Ashley Burke for further analysis on species identification using nested PCR and sequencing and Dr. Annette Bennett for her guidance and support in conducting mosquito infections with *Plasmodium falciparum* and salivary gland diagnostics.

I would like to thank the people of Mamfene, KwaZulu-Natal, for letting us collect mosquitoes from this area and the Provincial malaria control team especially Mr. Sifiso Ngxongo and Mr. Jabulani Zikhali for the role they played in mosquito collections throughout the study period. My special gratitude goes to all Vector Control Research Laboratory staff and students for sharing their laboratory experience and vital support during laboratory experiments and field work. Finally, I thank all my friends in South Africa for moral support and encouragement. My stay in South Africa was wonderful and memorable because of you.

This project was funded by the International Atomic Energy Agency (Research Contract No. 17904; 19099 and SAF 5013/5014), the Industrial Development Corporation, and the South African Nuclear Energy Corporation (Necsa) through its Nuclear Technologies in Medicine and the Biosciences Initiative (NTeMBI) – a national platform funded by the Department of Science and Technology, the Global Diseases Detection/CDC grant (U19GH00622-01 MAL01) and the National Research Foundation (NRF) through Medical Faculty Research Endowment Fund (00140184612015121105RMVIRL015) and DST/NRF South African Research Chairs Initiative grant to Prof. Maureen Coetzee.
PUBLICATIONS


PRESENTATIONS


5) **Leonard C Dandalo**, Givemore Munhenga, Maria Kaiser and Lizette L Koekemoer. *Development of a Genetic Sexing Strain (GSS) of Anopheles arabiensis (Diptera: Culicidae) for KwaZulu Natal, South Africa.* 1st September, 2016. Faculty of Health Sciences Research Day, University of Witwatersrand, Johannesburg, South Africa. (Poster)

6) **Leonard C Dandalo**, Annette Bennett, Dewaldt Engelbrecht, Alan Kemp, Givemore Munhenga, Theresa Coetzee and Lizette L Koekemoer. *Effect of ionising (gamma) radiation on female Anopheles arabiensis: An investigative study towards the development of the Sterile*
Insect Technique to control malaria vectors in South Africa. 2\textsuperscript{nd} South African Malaria Research Conference, 31\textsuperscript{st} July – 2 August, 2016, Pretoria, South Africa. (Poster)

TABLE OF CONTENTS

Declaration
Abstract
Acknowledgements
Publications
Presentations
Table of contents
Appendices
List of Figures
List of Tables
Nomenclature

CHAPTER ONE - LITERATURE REVIEW
General Introduction
1.1 Malaria parasites 2
1.2 Malaria vectors 3
   1.2.1 Anopheles gambiae complex 4
1.3 Malaria transmission cycle 8
1.4 Malaria burden in sub-Saharan Africa 9
1.5 Malaria control in sub-Saharan Africa 10
1.6 Malaria in South Africa 11
1.7 Malaria vector control in South Africa 11
1.8 Other vector control strategies 13
1.9 Monitoring and evaluation of vector control strategies 14
1.10 Sterile Insect technique 15
   1.10.1 Advantages of the Sterile Insect Technique 15
   1.10.2 Disadvantages of the Sterile Insect Technique 16
   1.10.3 Reasons for failures of SIT programs from mid-1950s to the mid-1970s 17
   1.10.4 Some of the major requirements for successful implementation of SIT Programme 19
1.11 Sterile Insect Technique for major agricultural pests 24
1.12 Sterile Insect Technique for mosquitoes 28
1.13 Study rationale 31
1.14 Study aim and objectives 31
  1.14.1 Specific objectives 32

CHAPTER TWO - DEVELOPMENT OF A GENETIC SEXING STRAIN OF ANOPHELES ARABIENSIS FOR KWAZULU-NATAL, SOUTH AFRICA 33
2.1 Introduction 33
2.2 Materials and methods 33
2.3 Results 33
  2.3.1 Development of a genetic sexing strain of Anopheles arabiensis for KwaZulu-Natal, South Africa manuscript (Published) 34-35

CHAPTER THREE - MATING COMPETITIVENESS OF THE LOCAL STERILE GENETIC SEXING STRAIN MALES (GMK) OF ANOPHELES ARABIENSIS UNDER LABORATORY AND SEMI-FIELD CONDITIONS 36
3.1 Introduction 36
3.2 Materials and methods 37
  3.2.1 Study site 37
  3.2.2 Biological material 38
  3.2.3 Mosquito separation 39
  3.2.4 Mosquito irradiations 39
  3.2.5 Determining the optimal irradiation dose 41
  3.2.6 Mating competitiveness of the irradiated GMK₁ males 43
  3.2.7 Statistical analysis 46
3.3 Results 47
  3.3.1 Dose optimisation 47
  3.3.2 Mating competitiveness of the irradiated GMK₁ males 51
3.4 Discussion 58
  3.4.1 Dose optimisation 58
  3.4.2 Mating competitiveness 60
CHAPTER FOUR - EFFECT OF IONISING (GAMMA) RADIATION ON PHYSIOLOGICAL AND REPRODUCTIVE FITNESS OF FEMALE *ANOPHELES ARABIENSIS*

4.1 Introduction  
4.2 Materials and methods  
4.3 Results  
   4.3.1 Effect of ionising (gamma) radiation on female *Anopheles arabiensis* Manuscript. (Published).  
   4.3.2 Susceptibility of irradiated *Anopheles arabiensis* to infection with *Plasmodium falciparum* manuscript (Under review)

CHAPTER FIVE - POPULATION DYNAMICS AND *PLASMODIUM FALCIPARUM* INFECTIVITY RATES FOR THE MALARIA VECTOR *ANOPHELES ARABIENSIS* AT MAMFENE, KWAZULU-NATAL, SOUTH AFRICA

5.1 Introduction  
5.2 Materials and methods  
5.3 Results  
   5.3.1 Population dynamics and *Plasmodium falciparum* (Haemosporida: Plasmodiidae) infectivity rates for the malaria vector *Anopheles arabiensis* (Diptera: Culicidae) at Mamfene, KwaZulu-Natal, South Africa manuscript (Published).  
   5.3.2 A new malaria vector mosquito in South Africa manuscript. (Published)

CHAPTER SIX – GENERAL DUSCUSSION AND CONCLUSIONS

REFERENCES
APPENDICES

Appendix 1A Genetic sex separation of *An. arabiensis* at egg/larval stages, the method described by Yamada *et al.*, 2012

Appendix 1B DNA extraction using prepGEM® Insect kit (*Bass et al.*, 2008)

Appendix 1C DNA extraction using Collins method (*Collins et al.*, 1987)

Appendix 1D Testing the presence or absence of Rdl mutation using the hydrolysis probe assay (*Bass et al.*, 2008; *Du et al.*, 2005)

Appendix 2A University of the Witwatersrand Human Research Ethics Committee (Medical) ethical clearance certificate

Appendix 2B KwaZulu-Natal Department of Health clearance certificate

Appendix 3 Dosimetry

Appendix 4 Detailed methodology on the effect of ionizing (gamma) radiation on physiological and reproductive fitness of female *Anopheles arabiensis*

Appendix 5 Additional information on population dynamics and *Plasmodium falciparum* infectivity rates for the malaria vector *anopheles arabiensis* at Mamfene, KwaZulu-Natal, South Africa methodology.

## CHAPTER THREE

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Experimental set-up for semi-field competitiveness assays A) semi-field</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>cages under tree canopies which provided shading during experiments; B)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>a white tray filled with water for additional humidity and a plastic jar</td>
<td></td>
</tr>
<tr>
<td></td>
<td>with sucrose solution soaked in cotton wool to provide energy to the</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mosquitoes; C and D) mosquito resting containers.</td>
<td></td>
</tr>
<tr>
<td>3.2</td>
<td>Gammacell 220 (MDS Nordion, Ottawa, Canada) used during irradiations</td>
<td>40</td>
</tr>
<tr>
<td>3.3</td>
<td>Doughnut shaped irradiation jig used during pupal irradiations</td>
<td>40</td>
</tr>
<tr>
<td>3.4</td>
<td>Experimental set up for the laboratory competitive assays</td>
<td>45</td>
</tr>
<tr>
<td>3.5</td>
<td>Mean % male adult emergence rate for GMK₁ pupae irradiated at different</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>doses and reared under standard insectary conditions until emergence and</td>
<td></td>
</tr>
<tr>
<td></td>
<td>corresponding controls. Control 1 (Baseline) consisted of wild type KWAG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>males mating with wild type KWAG females and Control 2 (Separation)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>consisted of unirradiated GMK₁ males mating with wild type KWAG females</td>
<td></td>
</tr>
<tr>
<td></td>
<td>treated the same way as irradiated pupae except that they remained</td>
<td></td>
</tr>
<tr>
<td></td>
<td>un-irradiated.</td>
<td></td>
</tr>
<tr>
<td>3.6</td>
<td>Kaplan – Meier survivorship curves for adult GMK₁ males reared under</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>standard insectary conditions using pupae drawn from cohorts irradiated at</td>
<td></td>
</tr>
<tr>
<td></td>
<td>varying doses and corresponding controls. Control 1 (Baseline) consisted</td>
<td></td>
</tr>
<tr>
<td></td>
<td>of wild type KWAG males mating with wild type KWAG females and Control 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Separation) consisted of unirradiated GMK₁ males mating with wild type</td>
<td></td>
</tr>
<tr>
<td></td>
<td>KWAG females treated the same way as irradiated pupae except that they</td>
<td></td>
</tr>
<tr>
<td></td>
<td>remained un-irradiated.</td>
<td></td>
</tr>
<tr>
<td>3.7</td>
<td>Mean percentage egg hatch rates of wild type KWAG females mated with GMK₁</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>males drawn from pupae irradiated at different dosages and their</td>
<td></td>
</tr>
<tr>
<td></td>
<td>corresponding controls. Control 1 (Baseline) consisted of wild type KWAG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>males mating with wild type KWAG females and Control 2 (Separation)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>consisted of unirradiated GMK₁ males mating with wild type KWAG females</td>
<td></td>
</tr>
<tr>
<td></td>
<td>treated the same way as irradiated pupae except that they remained</td>
<td></td>
</tr>
<tr>
<td></td>
<td>un-irradiated.</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.8 Mean temperature and relative humidity recorded during the mating period under natural environmental conditions (line graph represents humidity while bar graph shows temperature)

CHAPTER FOUR

Figure 4.1 Hemotek feeder: A) Power unit; B) Parafilm membrane disk with holder; C) Bottom surface of the parafilm membrane disk; D) Blood feeding mosquitoes in a cage

Figure 4.2 Midgut of uninfected mosquito (A and B) and infected mosquito with oocysts (C and D) under bright (A and C) and fluorescent fields (B and D).

Figure 4.3 Salivary gland with sporozoites highlighted in red circles

Figure 4.4 Mean percentage of irradiated and unirradiated *Anopheles arabiensis* females with oocysts post feeding *Plasmodium falciparum* gametocytes infected blood

Figure 4.5 Distribution of oocysts per mosquito in infected mid guts of irradiated and un-irradiated mosquitoes over 3 biological replicates

Figure 4.6 *An. arabiensis* sporozoites; A) The mean percentage of irradiated and unirradiated females with. B) Giemsa stained sporozoites. C) Salivary glands with sporozoites under phase contrast. D) Fluorescent sporozoites in salivary glands.
# List of Tables

## Chapter One

<table>
<thead>
<tr>
<th>Table 1.1</th>
<th>Some of the SIT programmes employed against major agricultural pests around the world</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1.2</td>
<td>Some of the SIT projects against different species of mosquitoes around the world</td>
<td>28</td>
</tr>
</tbody>
</table>

## Chapter Three

| Table 3.1 | Fecundity of females mated with males irradiated at different doses and corresponding controls | 49 |
| Table 3.2 | Percentage number of females recaptured after mating with males irradiated at different doses under laboratory and semi-field conditions | 52 |
| Table 3.3 | Mean percentage of inseminated females and fecundity of wild type KWAG females following mating competitiveness experiments under laboratory conditions | 54 |
| Table 3.4 | Mean percentage of inseminated females and fecundity of wild type KWAG females following mating competitiveness experiments under semi-field conditions | 55 |
| Table 3.5 | Experimental set up 1: Mating competitiveness values for *An. arabiensis* GMK₁ males irradiated at 70 Gy competing with wild type KWAG males for wild type KWAG females under laboratory | 56 |
| Table 3.6 | Experimental set up 2: Mating competitiveness values for *An. arabiensis* GMK₁ males irradiated at 70 Gy competing with wild type KWAG males for wild type KWAG females under laboratory conditions | 57 |
| Table 3.7 | Mating competitiveness values for *An. arabiensis* GMK₁ males irradiated at 70 Gy competing with wild type KWAG males for wild type KWAG females under semi-field conditions | 58 |
CHAPTER FOUR

Table 4.1 The feeding rate between irradiated and unirradiated females after standard membrane feeding

Table 4.2 Infection rates of laboratory colonized *An. arabiensis* infected with *P. falciparum*
NOMENCLATURE

1:1:1  Ratio of 1 to 1 to 1
1:1:3  Ratio of 1 to 1 to 3
1:1:5  Ratio of 1 to 1 to 5
%  Percentage
µg  Micrograms
µl  Microliters
µM  Micromole
1st use  First use
2nd use  Second use
3rd use  Third use
AB  Antibody
ABTS  2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
ACTs  Artemisinin-based combination therapies
ANO IPCL  Anopheles arabiensis genetic sexing strain developed at the Food and Agriculture Organization/International Atomic Energy Agency Insect Pest Control Laboratory (IPCL, Seibersdorf, Austria)
ANOVA  Analysis of variance
ArwPIT  Aedes albopictus strain originating from Italy
AW-IPM  Area-wide integrated pest management
AW-IVM  Area wide integrated vector management
BB  Blocking buffer
bp  Basepairs
C  Competitive Index
CI  Confidence Interval
cm  Centimeters
CO₂  Carbon dioxide
CS  Circumsporozoite
DDT  Diethyl diphenyl trichloroethane
DFID  Department for International Development
DNA  Deoxyribonucleic acid
dNTP  Deoxynucleotide triphosphate
EDTA  Ethylenediaminetetraacetic acid
ELISA  Enzyme linked Immunosorbent assay
et al  and others
Etc   et cetera
F     Filial
GAMA  *Anopheles arabiensis* strain derived from the genetic sexing strain, ANO IPCL strain (Austria) and *An. arabiensis* from Kruger National Park (named AMAL), South Africa
Gamma γ (penetrating electromagnetic radiation)
GMK   *Anopheles arabiensis* strain derived from GAMA strain (South Africa) and wild type KWAG strain from Mambene, KwaZulu-Natal, South Africa.
GSS   genetic sexing strain
Gy    Gray
IAEA  International Atomic Energy Agency
IPTp  Intermittent preventive treatment in pregnancy
IRS   Indoor residual spraying
IS    Induced Sterility
ITNs  Insecticide treated nets
ITS2  Internal transcribed spacer 2
IVM   Integrated vector management
KAc   Pottassium Acetate
kb    Kilobase
KGB   *Anopheles arabiensis* strain originating from Kanyemba, Zimbabwe
KWAG  *Anopheles arabiensis* strain from KwaZulu-Natal
KZN   KwaZulu-Natal
L     Litre
L1    First instar larvae
M     Molar
Max   Maximum
mg    Milligrams
MgCl₂  Magnesium chloride
Min   Minimum
ml    Millilitre
mM    Millimole
mm    Millimetre
MMR   Mark-release-recapture
N     Total number
NaCl  Sodium chloride
NF54-GFP A transgenic *P. falciparum* NF54 strain that expresses green fluorescent protein (gfp-luciferase)
NICD  National Institute for Communicable Diseases
nM    Nanomole
°C    Degrees celcius
P     Probability level
PABA  Para-aminobenzoic acid
PBS   Phosphate buffered saline
PBS-TW Phosphate buffered saline – tween
PCR   Polymerase chain reaction
Pf    *Plasmodium falciparum*
Pg    Pictogram
pH    Potential of hydrogen
Pmol  Per mole
Ppm   Parts per million
Rdl   Resistance to dieldrin
RdlF  Resistance to dieldrin forwad primer
Rdlr  Resistance to dieldrin reverse primer
rDNA  Ribosomal deoxyribonucleic acid
rFAL  *Plasmodium falciparum* specific primer
rPLU  *Plasmodium* (genus) primer
Rpm   Revolutions per minute
$R^2$ Square of the sample correlation coefficient in regression analysis
s.l. Sensu lato
s.s. Sensu stricto
SA South Africa
SD Standard deviation
SDS Sodium dodecyl sulfate
SEM Standard error of mean
Ser Serine
SIT Sterile insect technique
SMFA Standard membrane feeding assay
Spp Species
SPSS Statistical Package for the Social Sciences
SSA Sub-Saharan Africa
ssRNA Single-stranded ribonucleic acid
TAE Tris(hydroxymethyl) aminomethane-acetate
Taq Thermus aquaticus polymerase
TE Tris(hydroxymethyl) aminomethane ethylenediaminetetraacetic acid
Tris-Cl Tris(hydroxymethyl) aminomethane chloride
Tsl Temperature sensitive lethal
V Voltage
VCRL Vector Control Reference laboratory
WHO World Health Organization
WRIM Wits Research Institute for Malaria
WT2 Wild Type 2
$X^2$ Chi-square
CHAPTER 1
GENERAL INTRODUCTION
Malaria remains a major public health concern in South Africa (SA). Significant efforts to control malaria have successfully reduced the burden of malaria in SA and the transmission of the disease is now limited to the low-altitude northern and north-eastern regions of Limpopo, Mpumalanga and KwaZulu-Natal provinces (Morris et al., 2013). In 2015, 11 000 cases of malaria were officially recorded. Of these, 36% were locally acquired (Department of Health, unpublished records). Such transmission levels represent a malaria case incidence of <1/1000 population at risk which meets the World Health Organization (WHO) criteria for pre-elimination (Maharaj et al., 2013). The South African government is committed to eliminate malaria transmission by 2020 (Malaria Elimination Group, 2009; WHO, 2016a).

The success of reducing malaria transmission to pre-elimination levels in SA is largely attributed to sustained vector control efforts. These efforts primarily depend on indoor residual spraying (IRS) of households with either DDT (in traditional mud-walled houses) or synthetic pyrethroids (in modern cement-brick houses) (Brooke et al., 2013). However, as SA shifts towards malaria elimination it is unlikely that current malaria control efforts can successfully eliminate Anopheles arabiensis due to its variable feeding and resting behavior. This necessitated research into additional vector control tools such as the Sterile Insect Technique (SIT) to complement existing strategies.

The SIT entails artificial mass-rearing of the target vector species, sterilizing the males and releasing them in large numbers. The aim is to inundate the target population with sterilized males to a point where the vast majority of matings do not produce progeny, leading to target population suppression and a reduction in disease transmission (Vreysen, 2001). The successful implementation of the SIT as an insect intervention strategy is dependent on a number of factors. These include prior knowledge on the bionomics of the targeted species as well as a thorough understanding of the species diversity in the area targeted for releases. Another essential requirement for applying the SIT is the development of a sexing system to exclusively obtain males for sterilization and releases.
Female mosquitoes, even when sterilized with ionizing radiation, cannot be released, because of their capacity as disease vectors. It is against this background that this study was conceived.

This research consisted of four parts: establishing a local genetic sexing strain (GSS) that can be used to exclusively obtain males only for sterilization, characterizing fitness parameters of the strain, assessing the effect of irradiation on the physiological and reproductive fitness of GSS females, and understanding the population dynamics of *An. arabiensis*, its vector status and mosquito species diversity in an area targeted for pilot SIT releases.

1.1 Malaria parasites

Malaria is an infectious disease caused by parasitic protozoan in the genus *Plasmodium*. There are approximately 156 *Plasmodium* species that infect various vertebrate species (Berger and Marr, 2006), but only six of these cause human malaria. However, the five species that exclusively utilize human host as intermediates and are considered true parasites of humans are: *P. ovale curtisi*, *P. ovale wallikeri*, *P. malariae*, *P. vivax* and *P. falciparum*. The sixth species, *P. knowlesi* has been detected in humans and has an intermediate host; macaque monkeys (van Hellemond et al., 2009). No information is available to ascertain whether transmission from human to human via the mosquitoes occurs and it is therefore still regarded as zoonotic malaria (Singh and Daneshvar, 2013). Malaria symptoms include cyclical fever and shivering, joint pains, vomiting, headache and general body pain while severe cases include convulsions and/or kidney failure resulting in severe complications of acute anaemia, transient or permanent neurological effects, and death (Schumacher and Spinelli, 2012).

*Plasmodium ovale curtisi* and *P. ovale wallikeri* occurs in sub-Saharan Africa and some western Pacific islands. These two species differ in their biology with *P. ovale wallikeri* having a shorter latent period than *P. ovale curtisi*. These parasites cause milder illness with few fatalities in comparison with *P. vivax* and *P. falciparum* (Nevill, 1990).
*Plasmodium malariae* has a wide distribution, found in sub-Saharan Africa, South America, Asia, Indonesia and on islands of the western Pacific, but is less frequent than *P. falciparum* in terms of infection (Collins and Jeffery, 2007). Its asexual stages multiply every 3 days in blood and never cause severe infections (van Hellemont *et al.*, 2009).

*Plasmodium knowlesi* occurs in long-tailed and pig-tailed macaque monkeys inhabiting forested areas in south-east Asia (van Hellemont *et al.*, 2009). It was known to cause malaria among these monkeys but in recent years has also been discovered to cause malaria in humans (Nevill, 1990; WHO, 2003a). Asexual stages of *P. knowlesi* resemble those of *P. malariae* but differ in that they multiply daily and high parasitemia can cause death in humans (van Hellemont *et al.*, 2009).

*Plasmodium vivax* is the most frequent and widely distributed species causing recurring malaria but is less virulent than *P. falciparum*. It is mainly found in South America, Asia and some parts of Africa with the exception of sub-Saharan Africa (Gething *et al.*, 2012). This parasite is carried by at least 71 mosquito species most of which live in temperate climates. The hypnozoite form of the parasite can become dormant in the liver for days to years, causing no symptoms, and being undetectable in blood tests. The sexual stages can multiply in the bloodstream before the patient shows symptoms. The liver stages allow relapses up to 14 months after elimination from the bloodstream (Adak *et al.*, 2001).

*Plasmodium falciparum* is the deadliest species, causing severe illness (Nevill, 2009). It is less widespread in comparison with *P. vivax*. It is found in many tropical and subtropical regions of the world and accounts for almost all malaria deaths that occur in sub-Saharan Africa (Snow and Omumbo, 2006). In 2015, estimated 429,000 malaria deaths cause by this species were reported worldwide and 92% of these occurred in Africa (WHO, 2017)

### 1.2 Malaria vectors

Mosquitoes in the genus *Anopheles* act as malaria vectors. In this genus, members of the *Anopheles gambiae* complex and *An. funestus* group are responsible for the bulk of malaria transmission in the Afro-tropical region (Gillies and De Meillon, 1968; White, 1974; Gillies and Coetzee, 1987). *Anopheles gambiae* s.s., *An. coluzzii* and *An. arabiensis* are the major malaria vectors from the *An. gambiae* complex (Coetzee *et al.*, 2013), whereas *An. funestus* s.s. and *An. rivulorum* are the only
main malaria vectors from the *An. funestus* group (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987; Coetzee and Koekemoer, 2013). The other members from the *An. gambiae* complex (*An. merus*, *An. melas* and *An. bwambae An. quadriannulatus* and *An. amharicus*) and (*An. paresis*, *An. vanee deni*, *An. leesonii*) from the *An. funestus* group are either non-malaria vectors or play a very limited role (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987; Wilkes *et al.*, 1996; Kweka *et al*., 2013; Coetzee *et al*., 2013). This study focused on *An. arabiensis*, implicated as a likely major malaria vector in South Africa. The following literature review on malaria vectors is restricted to the *An. gambiae* complex.

1.2.1 *Anopheles gambiae* complex


(i) *Anopheles gambiae sensu stricto* Giles

Populations of *An. gambiae* are thought to be undergoing speciation and have historically been defined in two ways: chromosomal forms and molecular forms (Lanzaro and Lee, 2013). There are five chromosomal forms that carry different strain specific combinations of inversions and differ in their vectorial capacity, namely, Bissam, Forest, Mopti, Savanna and Bamako. The forms occur sympatrically but are segregated environmentally (Coluzzi *et al*., 1979; Favia *et al*., 1994; Bayoh *et al*., 2001; Sogoba *et al*., 2008). Apart from chromosomal forms, this species has also been divided into two genetically distinct molecular forms, “M” and “S” (Lehmann and Diabate, 2008). The M form has been renamed *An. coluzzii* Coetzee and Wilkerson, sp.n [Described in 1.2.1(ii)] whereas the S form retains the nominotypical name *An. gambiae* s.s Giles described further in this section (Coetzee *et al*., 2013).

*Anopheles gambiae* s.s. has the broadest distribution occurring throughout sub-Saharan Africa and is regarded as one of the most efficient malaria vectors (Lanzaro and Lee, 2013). This species is often localized in areas with closed canopy and breeds in a great variety of shallow open sunlit
pools such as borrow-pits, drains, brick-pits, ruts, car-tracks, hoof prints, round ponds and water holes (Gillies and De Meillon, 1968). Although *An. gambiae* thrives under rather cool conditions, it is tolerant of relatively high temperatures (Gillies and De Meillon, 1968). In general terms, seasonal changes in *An. gambiae s.s* populations tend to follow the seasonal pattern of rainfall. Thus, in savanna zones with a single rainy season per year, numbers start to rise explosively soon after the first main rainfall, reaching a peak in the middle of the rainy season and declining steadily thereafter as the levels of water become stabilized and vegetation and predators become established (Gillies and De Meillon, 1968). Female *An. gambiae s.s* are highly anthropophilic, feeding preferentially on humans (White, 1974; Coluzzi et al., 1979). The endophilic and anthropophagous behaviours of *An. gambiae s.s* create a very close association between the human reservoir and the insect vectors of malaria.

(ii) *Anopheles (Cellia) coluzzii* Coetzee & Wilkerson, sp.n.

This species was historically known as *An. gambiae* molecular m form (della Torre et al., 2001). Its distribution extends from northern Senegal in the west to east central Africa and south to coastal Angola. This species is found in the Zambezi valley in Zimbabwe and is also regarded as one of the most efficient vectors of *P. falciparum* (della Torre et al., 2001, Coetzee et al., 2013). Larval breeding sites originate from longer lasting human activities. In savannah areas these tend to be irrigated sites (e.g., man-made rice fields, reservoirs and drainage ditches) whereas in forest areas these tend to be urban pools (Favia et al., 1994; Sika et al., 2010; Coetzee et al., 2013).

(iii) *Anopheles arabiensis* Patton

*Anopheles arabiensis* is widely distributed in Africa ranging from Madagascar to Senegal (Coetzee et al., 2000). Its range and relative abundance tend to be influenced by climatic factors, especially total annual precipitation. It prefers to breed in fresh temporary sunlit or rain water pools (Lindsay et al., 1998). This species is more tolerant to higher temperatures and is able to survive in drier conditions (Gillies and Coetzee, 1987; Petrarca et al., 2000). It has been observed that adult females persist in arid conditions by laying their eggs on damp surfaces, rather than water, with hatching being delayed in a proportion of eggs (Lindsay et al., 1998) and aestivate during periods of prolonged dryness (Omer and Cloudsley-Thomson, 1970). *Anopheles arabiensis* has a more opportunistic feeding behaviour, it can be entirely zoophilic, as recent studies from Madagascar
have shown (Duchemin et al., 2001). This species also tends to be more exophagic and exophilic (Gillies and Coetzee, 1987). The variable behaviour of An. arabiensis females, being anthropophilic and zoophilic as well as endophilic and exophilic makes them incompletely vulnerable to house-spraying (White, 1974).

The seasonal abundance of An. arabiensis with peaks following the onset of rains makes it largely responsible for malaria transmission in southern African countries including South Africa (Gillies and Coetzee, 1987; Hargreaves et al., 2003; Maharaj et al., 2013).

(iv) **Anopheles quadriannulatus** Theobald

*Anopheles quadriannulatus* species have a limited distribution associated perhaps with more subtropical climates than the other members of the complex. They are freshwater species and are adapted to lower developmental temperatures (Gillies and De Meillon, 1968). They are not known to transmit malaria parasites (Coetzee, 1989). The species that retained its nominotypical name *An. quadriannulatus* s.s. Theobad was previously called *An. quadriannulatus* s.s. species A and is widespread in Southern Africa (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987). This species feeds on cattle and rests outside houses (Gillies and De Meillon, 1968).

(v) **Anopheles amharicus** Hunt, Wilkerson & Coetzee, sp.n.

This species was previously called *An. quadriannulatus* species B (Hunt et al., 1998) and has been named *An. amharicus* based on chromosomal, cross-mating and molecular evidence (Coetzee et al., 2013). Its distribution is limited to Ethiopia (Fettene et al., 2002; Coetzee et al., 2013) covering Bahir Dar, Bako, Bedele, Dejen and Jimma areas, and along Omo River. This species is associated with cattle and is found abundantly in animal shelters or mixed human/animal dwellings but no information is available for the larval habitats (Coetzee et al., 2013).

(vi) **Anopheles merus** Dönitz

*Anopheles merus* is a saltwater breeding member of the *An. gambiae* complex involved in low rate malaria transmission (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987; Temu et al., 1998; Cuamba and Mendis, 2009). This species has a limited distribution occurring in the East coast of
Africa, as well as the adjacent inland areas and coastal islands (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987; Sharp, 1983). This species also occurs inland in some malaria endemic areas in South Africa (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987; Obala, 1995; Mouatcho et al., 2009; Munhenga et al., 2014). Larvae of An. merus are found in coastal Avicennia mangrove swamps, salt pans, brackish ponds (Gillies and De Meillon, 1968) or mineral springs (Coetzee and Le Sueur, 1988).

In the absence of domestic animals, An. merus bites man readily both indoors and outdoors. It has also been observed that in the presence of other hosts An. merus is more attracted to cattle (Gillies and De Meillon, 1968).

(vii) Anopheles melas Theobald

Anopheles melas is the West Coast salt-water member of the An. gambiae complex. It can utilise saline environments yet it does not require brackish water for its larval development (Sinka et al., 2010). It occurs in patches of salt grass in tidal swamps and in pools, ponds or lagoons flooded by spring tides, and also in Avicennia mangrove swamps (Gillies and De Meillon, 1968). Anopheles melas is generally restricted to coastal areas (Coetzee et al., 2000), with the exception of Gambia, where it occurs far inland along the Gambia River (Bogh et al., 2003).

This species shows opportunistic biting behaviour being highly anthropophilic and zoophilic (Sinka et al., 2010). It feeds on humans and feeds as readily indoors as outdoors even in the presence of domestic animals (Gillies and De Meillon, 1968). This species generally rests outdoors after feeding. Anopheles melas is an important vector of malaria at many points along the West African coast but is considered to be a vector of lesser importance where it occurs in sympathy with An. gambiae s.s or An. arabiensis (Sinka et al., 2010). It is also capable of transmitting Brancoftian filariasis (Gillies and De Meillon, 1968).

(viii) Anopheles bwambaæ White

The distribution of An. bwambaæ is very limited being defined by its breeding sites in the Buranga brackish water hot mineral springs in the Semliki forest in Bwamba County, Toro district, Uganda (White, 1985; Sinka et al., 2010). The natural hosts of this species in the Semliki forest are
unknown but the adults readily bite humans from the nearby villages. *Anopheles bwambae* is regarded as a potential malaria vector due to its susceptibility to *P. falciparum*. *Plasmodium falciparum* infection rates of 0.7% were recorded in indoor collections from Bwamba, Uganda (White, 1985). This species is also regarded as a vector of filariasis (White, 1985).

### 1.3 Malaria transmission cycle

Malaria parasite life cycle involves two hosts: humans and female *Anopheles* mosquitoes. During a blood meal, a malaria infected female *Anopheles* mosquitoes inoculates sporozoites into the human host. The sporozoites infect liver cells and mature into schizonts, which rupture releasing merozoites into the blood stream. The merozoites infect red blood cells and are responsible for the clinical manifestation of the disease (usually fever, headache, chills and vomiting) (Aly *et al.*, 2009). Female *Anopheles* mosquitoes ingest male and female’s gametocytes during a blood meal. Inside the mosquito’s stomach, the gametocytes generate zygotes, which develop into oocysts. The oocysts grow, rupture and release sporozoites which migrate to the mosquito’s salivary glands (Huff, 1947). The inoculation of the sporozoites into a new human host perpetuates the malaria life cycle.

The susceptibility of anopheline mosquito species to *Plasmodium* infection varies depending on species. Some species are more permissive to infection than others (Hume *et al.*, 2007). *Plasmodium* refractory lines of *Anopheles* have previously been selected in laboratories (Hurd *et al.*, 2005; Voordouw *et al.*, 2008; Eldering *et al.*, 2016). Refractory mosquitoes are poor malaria vectors as they impede parasite development in their midguts (Hurd *et al.*, 1995). Several parasite – inhibiting mechanisms have been reported and these include: 1) Failure of the parasite to exflagellate or to undergo syngamy in the midgut of mosquitoes (Nijhout, 1979), 2) Failure in activation of parasite chitinase for penetration of the peritrophic matrix (Shahabuddin *et al.*, 1993), 3) Melanotic encapsulation of the developing oocyst that occurs between the basolateral epithelial cell membrane and the basement membrane (Collins *et al.*, 1986), 4) Salivary gland barriers that prevent sporozoite penetration or reduce sporozoite survivourship (Roseberg, 1985), 5) The incompatibility between the mosquito and the parasite also referred to as a refractory condition. Loss of parasites without trace might be attributed to lysis in the midgut epithelial cells (Vernick *et al.*, 1995; Yan *et al.*, 1997; Hurd *et al.*, 2005).
There is fitness cost associated with refractoriness in female mosquitoes. Previous laboratory and field studies have shown that malaria infected mosquitoes are less fit than uninfected ones. There is a reduction in the reproductive success (fecundity and fertility) for infected mosquitoes (Hogg and Hurd, 1995, 1997; Hurd et al., 2005) and also a reduction in survival rate (longevity) (Anderson et al., 2000; Ferguson and Read, 2002). However, Hurd et al., 2005 reported that parasite infection has no effect on survivorship of mosquitoes whereas Ve’zilier et al., 2012 reported that parasite infection increases longevity of mosquitoes. The increase in longevity arises through a trade-off between reproduction and survival. The elevated expression of oxidoreductive genes in refractory strains is responsible for causing fitness costs following infection. Hence refractoriness to parasites is as costly as tolerance (Hurd et al., 2005). The refractory population has a significantly shorter egg to adult developmental time and small body size. The reduction in body size allows the females to take smaller blood meal and subsequently lay fewer eggs than their susceptible counterparts (Yan et al., 1997).

The immune system of many insects decreases with an increase in age leading to the prediction that older females are more prone to infection than the young ones. In contrast to this theory, older female mosquitoes have a lower probability of becoming infected with the parasite. However, the age effect is entirely reversed when old mosquitoes have taken one previous non-infected blood meal (Pigeault et al., 2015).

1.4 Malaria burden in sub-Saharan Africa
Malaria is among the most dangerous human diseases in the world. An estimated 3.2 billion people are at risk of malaria, with populations living in sub-Saharan Africa (SSA) having the highest risk of acquiring malaria (WHO, 2016b). Approximately 80% of cases and 78% of deaths occur in sub-Saharan Africa (SSA), with children under five years of age and pregnant women most severely affected (WHO, 2016b). Malaria causes death to young children in three ways. Firstly, an acute infection of cerebral malaria may kill the child directly and quickly. Secondly, repeated infections cause severe anaemia which increases the risk of death. Thirdly, malaria infection in pregnant women results in children born with low birth weight thereby increasing the risk of death (Steketee et al., 2001).
In addition, children who survive severe malaria may suffer long-term consequences of the infection such as reduced appetite, and restricted playing and social interaction, which may eventually affect their educational opportunities (Steketee et al., 2001). An estimated 2% of children who survive cerebral malaria suffer from learning impairments and disabilities due to brain damage (Murphy and Breman, 2001).

Despite causing mortality and morbidity, malaria is also a burden to African health systems expenditure. In some countries with heavy malaria burden, malaria may account for 40% of public expenditure, up to 25-35% of all outpatient clinic visits and 20-45% of all inpatient admissions (WHO, 2016b; DFID, 2010). Malaria also has direct costs to individuals in highly endemic areas. Individuals or families spend their income on insecticide treated nets (ITNs), antimalarial drugs, transport to health facilities, etc. The direct and indirect costs associated with malaria pose a substantial burden on poor households in SSA because people living in poorer households are more at risk of contracting malaria. This is most likely due to them living in dwellings that offer little protection against mosquitoes. They are also less likely to afford effective malaria treatment/transport to health facilities or insecticide treated nets (Chima et al., 2003; Worrall et al., 2005).

1.5 Malaria control in sub-Saharan Africa

One approach used to reduce malaria transmission is through vector control. The most effective forms of vector control in SSA are the distribution of insecticide treated nets (ITNs) and indoor residual sprays (IRS). In this region, in 2014, 56% of the population received ITNs and 6% of the population at risk of malaria lived in households protected by IRS (WHO, 2015). Administration of intermittent preventive treatment in pregnancy (IPTp) for malaria is another method of control used in SSA. However, the acceptance of ITNs and IPTp depends on cultural, behavioural and demographic factors (Govern et al., 2000).

There are several reasons why ITNs are not used in certain communities and these include: 1) Unpleasant smell produced by the nets (Sangare et al., 2012), 2) Chemicals used to treat ITNs are associated with family planning (Alaii et al, 2003), 3) Discomfort due to heat and humidity (Adam et al., 2008), 4) Educational status – low ITN usage is associated with low level education and the vice versa, 5) People from low density areas are more likely to own a bed net compared to those
from high density areas, 6) People from monogamous families are more likely to sleep under bed net than their counterparts from polygamous families (Aluko and Oluwatosin, 2012).

The number of women receiving IPTp in SSA remains below national targets (WHO, 2015). IPTp usage is limited mainly due to lack of knowledge regarding this method among pregnant women (Adam et al., 2008). The effective drugs being administered in SSA against P. falciparum are the artemisinin-based combination therapies (ACTs). In 2014, the African region accounted for 98% of ACT manufacturer deliveries (WHO, 2015). However, the progress in malaria control is being hampered by rapid development and spread of drug and insecticide resistance.

1.6 Malaria in South Africa

Despite great strides in malaria control, malaria remains a public health concern in SA. The disease is still endemic in the north-eastern areas of KwaZulu-Natal (KZN), Mpumalanga and Limpopo provinces. Members of the An. gambiae complex and An. funestus group are predominant malaria vectors in SA. Anopheles gambiae s.s, An. arabiensis, An. merus and An. funestus s.s. have been implicated in malaria transmission in this country (Maharaj et al., 2013). Anopheles funestus s.s was eliminated in the 1960s but resurfaced in 1999 and was re-eliminated after 2000. Anopheles merus only played a minor role in malaria transmission up to the present time (Coetzee et al., 2013). Currently, malaria transmission is presumed to be maintained predominantly by An. arabiensis, and approximately 90% of malaria cases in SA are attributed to P. falciparum infections (Maharaj et al., 2013).

1.7 Malaria vector control in South Africa

Malaria vector control constitutes the back-bone of successful malaria control in SA with insecticide residual spraying (IRS) of dwellings being at the center of this success. IRS has been in place for the past 70 years and various insecticides have been used (Coetzee et al., 2013).

Currently, the South African malaria control programme uses synthetic pyrethroids to spray cement-brick structures and DDT is sprayed in traditional mud-walled structures, while in some instances carbamates are used (Brooke et al., 2013). Like any insecticide based vector control strategy the current approach has several shortfalls. The greatest challenge for vector control using
insecticides is the development of insecticide resistance in target vector populations because of the limited number of insecticides recommended for vector control. There are only four classes of insecticides (DDT, pyrethroids, organophosphates and carbamates) which share 2 modes of action approved by WHO for use in insecticide residual spray and insecticide treated bed nets (Constant et al., 2012). It has been known that insects, including mosquitoes, could become resistant behaviourally by avoiding exposure to lethal dose or physiologically by finding ways to survive a normally lethal dose to insecticides (Mullin and Scott, 1992). Behavioural mechanisms for resistance have been less studied than physiological (Sparks et al., 1989). Physiological resistance is divided into 3 mechanisms; reduced cuticular penetration, altered target size, increased metabolic detoxication or sequestration (Oppe noorth, 1985; Scott, 1991). Increased excretion is also a possible resistance mechanism though it has not convincingly demonstrated (Mullin and Scott, 1992).

However, multiple insecticide resistance may occur in the same mosquito population. Multiple insecticide resistance has been detected among major malaria vectors in Africa (WHO, 2012; Djoura ka et al., 2016; Riveron et al., 2016). Multiple insecticide resistance mechanisms have been expressed in mosquitoes where a single mutation at a target site can result in mosquito resistance to DDT and pyrethroids or to organophosphates and carbamates (Perera et al., 2008). In An. gambiae sl mosquitoes, mutations in the DDT/pyrethroid target site [Knockdown resistance (kdr) alleles] have been found in conjunction with resistance acetylcholinesterase gene alleles (Ace-1R), the target site of organophosphates and carbamates (Yewhalaw et al., 2011). This multiple resistance poses a big challenge in malaria control programmes. Hence, for effective vector control, there is need for application of intergrated vector control approach that can include SIT and other biological methods which are not insecticide based.

In addition, the adverse effects of these synthetic pesticides, such as high toxicity from residues in food, and contamination of water and the environment poses a health hazard to humans (Bhattacharyya and Mukhopadhyay, 2013). This approach is also proving to be challenging when applied in low malaria transmission situations where An. arabiensis is the main vector.
Anopheles arabiensis females feed on both cattle and humans, and rest both inside human habitations and outdoors (White, 1974) making it difficult to control them using IRS (Gillies and Coetzee, 1987). These concerns and the mandate by the South African government to eliminate malaria by 2020 necessitated the search for additional vector control strategies to complement the existing tools. One such vector control method is the Sterile Insect Technique (SIT) (Howell and Knols, 2009).

1.8 Other vector control strategies
Genetic control methods have been explored to achieve population suppression/elimination e.g. Sterile insect technique (SIT) and to modify population achieved by replacement or alteration of the hereditary material with the aim of reducing the reproductive potential of insects e.g. Gene drives (Curtis and Graves, 1988). Advances in new genetic tools, such as gene drives and SIT have been explored for mosquito control. Gene drives are genetic systems that greatly increase the chance of inherited gene or set of genes to be passed on to offspring. The genes are subsequently spread to all members of the population (Macias et al., 2017). Gene drives can be achieved by editing desirable site-specific genes with CRISPR/Cas9 systems (Macias et al., 2017). Gene drive has been applied to prevent the spread of insects that carry pathogens such as mosquitoes that transmit malaria, dengue, and zika pathogens (Yen and Barr, 1973; Stouthamer et al., 1999). The first successful synthetic gene drive was demonstrated in the flour beetle (Tribolium castaneum) (Chen et al., 2007). In mosquitoes, the most successful gene drive was the development of “Flightless female” Ae. aegypti. This strain contains a genetic element that encodes a toxin to destroy the wing muscles of females.

Since the wings fail to function normally, these females are unable to mate, to reach food or to search places for oviposition (Fu et al., 2010). The gene set that encodes these phenotypes is spread into the population by the males who are unaffected by the transgene.

These males are being released in Brazil and Florida as a control strategy to suppress Ae. aegypti population (Harris et al., 2012; Carvalho et al., 2015). Gene drive has also been successful in Ae. albopictus and An. stephensi, but have not yet been utilized to control wild population (Labbé et al., 2012; Marinotti et al., 2013).
Genetic control of mosquitoes using gene drives has an advantage over SIT in that gene drives can be applied to multiple mosquito species while as SIT can only target a specific species (Macias et al., 2017). However, gene drives might not be adopted under local settings due to technical complexity and challenges in public acceptability of releasing genetically modified mosquitoes coupled with tight regulatory approvals associated with them.

1.9 Monitoring and evaluation of vector control strategies

Knowledge of the local vector species and their susceptibility to insecticides as well as vector and human behaviours that may reduce contact is the key to effective malaria vector control (WHO, 2015). There is a need to periodically collect such data to evaluate the impact of vector control strategies on malaria transmission. It is also important to monitor the coverage, usage, quality and durability of vector control interventions following their deployment (WHO, 2015). Performance of vector control strategies varies greatly when employed in different settings and this has a bearing on the overall impact on malaria transmission. Therefore, evaluation of the impact of intervention methods on malaria outcomes is very important. There are a number of entomological parameters that can be assessed to evaluate the performance of vector control strategies in an area.

The entomological parameters (human biting rates (HBR), sporozoite rates (SR) and the entomological inoculation rates (EIR) determines intensity of malaria parasite transmission. The HBR is calculated as the total number of freshly fed mosquitoes of a particular species divided by the total number of human occupants in houses used for collection (Aju-Ameh et al., 2016). The SR is calculated as the number of sporozoites positive mosquitoes divided by the number of mosquitoes dissected (Omalu et al., 2015). The EIR is the average number of infectious bites, per person, per year in a given area; defined as the product of HBR and SR (Aju-Ameh et al., 2016).

In experimental laboratory studies, the oocyst rate (OR) (calculated as total number of oocysts in female mosquito’s mid gut divided by the total number of females examined) is used to determine mosquito infectivity (Ndiathi et al., 2011). The high rates of entomological parameters indicate high malaria parasite intensity and the vice versa.

In Africa, the EIR is variable and ranges from <1 to >1000 infection bites per person per year (Aju-Ameh et al., 2016). There are several factors that affect malaria transmission such as: (i) Human
factor- infectiousness of human carriers having gametocyte reservoir (Coosemans et al., 1992) (ii) Entomological factors – influenced by the natural environment and also its modification by man. Malaria transmission is enhanced by the increase in vector density, associated with high humidity, high temperatures (Omar et al., 2015) and availability of water pools due to irrigation or rainfall (Coosemans et al., 1992). (iii) Man-vector contact - the frequency of contact between man and vector is another important factor. High contact may lead to high malaria incidences (iv) Longevity of the vector also plays a significant role. Malaria transmission will only be possible if the vector lives long enough to complete sporogony (Coosemans et al., 1992). In Mamfene, malaria transmission is associated with irrigation schemes on the Makhathini flats. Stagnant water bodies in irrigation schemes act as mosquito breeding sites (Obala, 1995).

1.10 Sterile Insect technique
SIT relies on the mass production of target insects in mass-rearing facilities, sterilization, and mass release of the sterile insects into the natural habitat. The sterile insects are released in sustained numbers to outnumber the wild pest population (Vreysen, 2001). The released sterile males compete with wild males to mate with wild females. The sterile males inseminate wild females with sterile sperm, which results in the production of non-viable offspring leading to overall reduction of the target population. This technique was developed by Edwin F. Knipling as early as 1937 (Bartlett and Staten, 1996).

1.10.1 Advantages of the Sterile Insect Technique
As a vector control strategy, the SIT has several advantages as outlined below:
1) It is species-specific and the released sterile males can only mate with females of the same species. This enhances its efficacy in terms of reducing or eliminating the targeted insect/pest (Vreysen, 2001).
2) It is an environmentally friendly, non-polluting method. The chances of targeting other species beside the intended target species are minimal when employing this method and no toxic chemicals are released into the environment as is the case of using traditional insecticides (Alphey et al., 2010).
3) Released sterile males cannot establish in the released area since they provide no viable offspring. This is unlike other releases in biological control programmes where introduced insects as control agents become established causing damage to crops (Benedict and Robson, 2003).

4) SIT can easily be integrated with other vector control methods already in place such as integrated vector management (IVM) programs using several approaches simultaneously (Dyck et al., 2005).

5) SIT can be cost effective since the sterile males released in targeted areas disperse themselves and can even invade protected areas or inaccessible areas, unlike chemicals where human intervention is required (Alphey et al., 2010; Bloem et al., 2005).

1.10.2 Disadvantages of the Sterile Insect Technique

Like any other vector control method, SIT also has limitations that need to be considered before project implementation. These are outlined below.

1) This technique is suitable in areas with a single dominant vector for a particular disease. Due to its species-specificity it might be very difficult to deploy in areas where several vector species need to be controlled simultaneously. Two vectors might be manageable but beyond that the technique might not be effective (Alphey et al., 2010).

2) Released sterile males can disperse anywhere in the area of release and dispersal cannot be controlled.

Insects with high dispersal range can migrate outside of the control area thereby reducing efficiency of the program while insects that do not disperse readily need to be released at fine spatial scale, which might increase program cost (Alphey et al., 2010).

3) SIT programmes do not show immediate effects on vector numbers. Reduction of vector population is observed in subsequent generations and hence impact is slow (Bloem et al., 2005).

4) Depending on the stage of the released insect, there might be density dependent effects. For example, when larval stages are released, they tend to compete for food and space thereby reducing their survival rate which may lead to reduction in efficiency of the SIT programme (Rogers and Randolph, 1984).
5) SIT requires cheap and alternative techniques that can effectively suppress populations to low densities on an area-wide basis. This also applies to areas where there is no human population or where there is very difficult terrain. The technique is less effective when the vector population is of very high density since this might require release of higher numbers of sterilized males (Rogers and Randolph, 1984; Vreysen, 2001).

1.10.3 Reasons for failures of SIT programs from mid-1950s to the mid-1970s
The reasons for the disappointing outcomes for SIT programmes that were conducted between mid-1950s to mid-1970s were commonly believed to be due to poor male competitiveness (as a result of the mass rearing and/or the effect of sterilization process), political interference and immigration of already mated males from nearby untreated areas (Becker et al., 2010). Some of the reasons are discussed below.

1) Low competitiveness of released sterile males
A field trial was conducted in 1969 on an island (5km²) in Lake Kariba, Zimbabwe to control of Glossina morsitans Westwood. The adult flies that were sterilized after emergence and held in captivity suffered an 80% loss in field competitiveness (Klassen and Curtis, 2005). Another study was conducted in Pensacola, Florida where irradiated (110-180 Gy) Aedes aegypti were released for two seasons (1960-1961) by the Centers for Disease Control. The released mosquitoes were not effective due to reduced competitiveness caused by irradiation in pupae stage (Dame et al., 2009).

2) Reduced longevity of released sterile males due to irradiation
In 1991, a large field trial to eradicate introduced Sea lampreys Petromyzon marinus from the Great Lakes (USA) was initiated and lasted for several years. Radiation sterilization was considered but yielded unsatisfactory results regarding male survival and competitiveness (Helinski et al., 2009).

3) Use of chemosterilants
Release of sterile male lampreys (*Petromyzon marinus*) in the Great Lakes (USA) using chemosterilant bisazir was also initiated. Chemosterilizing agents being mutagenic, present a potential hazard to humans during the treatment process, their use was abandoned (Helinski *et al.*, 2006a). Concerns were raised about the effects of the chemicals on the environment and non-target organisms, particularly when large numbers of treated insect were released. Spiders that fed on a diet of only chemosterilized mosquitoes subsequently became sterile (Helinski *et al.*, 2006a; 2009).

4) Change in mating behaviour of released sterile males

The release of sterile *Anopheles quadrimaculatus* males by the United States Department of Agriculture (USDA) in South Florida in 1959-1960 led to reduced incidence of mating with wild females due to a change in mating behavior influenced by colonization and this resulted in programme failure (Dame *et al.*, 2009). Another study that showed assortative mating involved the release of *Culex tarsalis* in 1981. The released sterile males showed a significant inability to seek out and mate with wild females (Reisen *et al.*, 1982).

5) Political interference/civil war

Large scale mosquito field trials were conducted in El Salvador and India in the mid-1970s. In El Salvador, the target was the malaria vector *Anopheles albimanus*. Unfortunately, the study was interrupted by eruption of civil war.

Similarly, in India, the study targeting *Aedes aegypti* never materialized due to the political problem encountered. There were false accusations that the project was intended to collect data on biological warfare (Klassen, 2009).

6) Influx of already mated females from outside the targeted release areas

The initial releases of sterile male *Culex quinquefasciatus* in India (mid-1970s) in targeted villages achieved only limited levels of sterility in eggs laid by wild females because of the influx of already- mated females from outside targeted release area (Robinson *et al.*, 2009; Klassen, 2009).
1.10.4 Some of the major requirements for successful implementation of SIT programme

Successful implementation of a SIT programme depends on several factors and these need thorough investigation prior to project implementation. Some of these requirements are discussed below:

1) A comprehensive knowledge of the ecology and behaviour of the target population is required. Before SIT programme initiation, it is very important to know the ecology and behaviour of the targeted species. Mating barriers may exist between species complexes or cryptic species. In cases where strong pre-mating barriers exit between two or more sibling species of the target species, rearing and releasing only one type may be of limited benefit (Alphay et al., 2010). For example, in areas where *An. gambiae s.s.* and *An. arabiensis* are both major vectors and occur in sympatry, mating barriers between different chromosomal forms or types of these species may occur. However, SIT programs against agricultural pests have typically not found significant mating barriers between widely separated populations despite genetic differences (Cayol et al., 2002). Similarly, Girod et al., 2001 found no mating incompatibility between genetically distinguishable populations of *An. arabiensis*. It is also very important to know detailed information on population dynamics (spatial and temporal), mating behaviour, breeding sites and flight distance of the target population (Helinski et al., 2006b).

2) Colonization

The insect pest targeted for SIT should be amendable to colonization. The colony or strain characterization like fertility, fecundity etc. should be known. The cost of colonization and mass production of target insects should be reasonable in order to meet the required numbers for release (Helinski et al., 2006b). It is very important to have an estimate of the relative cost of the SIT programme before implementation. The cost will be determined by the area to be controlled and the number of sterile insects to be mass-reared and released. One way of reducing the cost is to release the sterile males when the targeted wild population is at its lowest because relatively low numbers of sterile males may be released to suppress the wild population (Alphay et al., 2010).

3) Sex separation system

It is preferable that insects released in an SIT programme shouldn’t have unintended consequences (Klassen and Curtis, 2005). In a mosquito SIT programme, the release of sterile females is not
acceptable due to the risk of disease transmission (Robinson et al., 2009). It is therefore a prerequisite that all females be removed from the released population. A number of separating methods have been used in SIT programmes:

(i) Morphological separation – In most insects, the external morphology in pupal or adult stages has been used to separate males from females (Klassen and Curtis, 2005). Sexual dimorphism in size can be used to separate the sexes in some mosquitoes such as Aedes mosquitoes, Culex quinquefasciatus (Papathanos et al., 2009) and in Lepidoptera but the consideration overlap in the size renders the sorting inefficient (Parker, 2005).

(ii) Classical genetics – This approach confers a selectable trait to males with the aim of eliminating females. In mosquitoes, a selectable trait such as insecticide resistance is translocated to the male determining factor such as the Y-chromosome (Curtis et al., 1976). A Genetic Sexing Strain (GSS) has been successfully established in An. gambaie (Curtis et al., 1976), An. albimanus (Kaiser et al., 1978) and An. arabiensis (ANO IPCL) based on dieldrin resistance (Oliva et al., 2012; Yamada et al., 2012).

An effective sexing strain has been developed for Mediterranean fruit fly based on a temperature sensitive lethal mutation where a white pupae (wp) mutant is linked to the temperature sensitive lethal (tsl) mutation and male determining chromosome. Male pupae are brown and female pupae are white and thus provide a visual indicator for the GSS stability (Franz, 2005). Various GSS have been developed for 20 insect species (Robinson, 2002) but very few strains have been developed to the point of being mass reared and released in area wide integrated vector management (AW-IVM) (Papathanos et al., 2009). Elimination of females based on exposure of eggs to insecticide or high temperature can reduce rearing costs during mass production and gives flexibility for the insectlife stage to be released (e.g. pupae or adult; Papathanos et al., 2009).

(iii) Transgenic methods – Several new methods have been proposed such as sex-linked expression of a fluorescence-protein, combined with fluorescence-based sorting. This has been achieved in a Mediterranean fruit fly GSS where separation was achieved by scoring newly hatched larvae for the fluorescent trait (Condon et al., 2007; Papathanos et al., 2009). Sex limited expression of transgenes has also been achieved in An. stephensi where a sperm specific promoter from the An.
gambiae β2-tubulin gene was used to express the fluorescent protein and conditional femalespecific lethality where females die during larval development (Catterucia et al., 2000; Fu et al., 2007).

4) Low population density
There is an advantage to having very low population density of the target insect in the control area before program initiation. This can be achieved by employing economically feasible suppression techniques such as biological control methods or insecticide sprays before sterile insect release (Vreysen, 2001). The releases may also be timed to coincide with natural seasonal declines in the numbers of target pests, which is mostly observed in the winter months of the year (Becker et al., 2010).

5) Sterile male competitiveness
An SIT programme can only be successfully applied if the released sterile males can successfully compete for wild females against wild males. Therefore, there is a need to thoroughly evaluate the competitiveness of the sterile male insects that are released. Competitiveness of sterile male insects is compromised by various factors such as colonization, sterilization (Helinski et al., 2006a), handling, distribution and genetic manipulations. These factors may reduce quality of released males such as longevity, dispersal as well as the ability to find and successfully court females (Kaiser et al., 1979; Benedict et al., 2009; Alphey et al., 2010). For example, colonized males are homogeneous entities due to bottlenecks which select specific traits required to survive under artificial conditions and therefore not as genetically diverse as wild populations and this might alter mating ability (Norris et al., 2001). The modified insectary environment has been shown to lead in fixation of alleles or behaviour that can result in assortative mating between the released colonized sterile males and wild females (McDonald, 1979). This was observed in colonized Cx. tarsalis in which released sterile colony males swarmed in different arenas compared to wild vectors (Reinsen et al., 1982). This was also reported in An. culicifacies (Reinsen et al., 1981) and An. albittarsis (Lima et al., 2004) which had been under colonisation for several generations. Genetic manipulation during development of genetic sexing strains particularly mutations and chromosome rearrangements of the GSS strains have been shown to significantly reduce physiological fitness (Alphey et al., 2010).
However, despite these adverse effects of colonization, the mating competitiveness of colonized males can be enhanced in different ways which include:

(i) Creating an ideal rearing environmental conditions that can increase the mating competitiveness during laboratory rearing regimes (Ng’habi et al., 2005) i.e. increasing the mating rank. This can be done by reducing larval crowding to reduce competition of food. It has been shown that larval crowding has a strong effect on the mating competitiveness of male mosquitoes (Ng’habi et al., 2005).

(ii) Increasing adult male size – Low larval crowding results in emergence of large males (Bargielowski, 2010). It has been shown that body size has a strong effect on the mating competitiveness of male mosquitoes. Studies conducted by Ng’habi et al., (2005) and Bargielowski, (2010) on *An. gambiae* and *Ae. aegypti* mosquitoes respectively, showed that:

- Larger males were more likely to acquire mates than smaller individuals.
- Larger males were more likely to acquire the first mating. Large males of *An. gambiae* won females by approximately 11 times more often than the smaller males.
- The proportion of total matings between larger males and smaller ones did not differ.

However, the females showed no propensity to re-mate over several gonotrophic cycles.

(iii) Increasing teneral reserves – Males with high teneral energy reserves have a competitive advantage over males with low teneral energy reserves (Kaspi and Yuval, 2000). In *An. gambiae* s.s., males with sugar reserves showed high insemination capacity and high survival rate at temperatures, 23°C and 27°C than males without sugar reserves (Gary Jr. et al., 2009). Females are more likely to oviposit when mated with young males (2-3 days) than when mated with older males (Chambers and Klowden, 2001). High teneral energy reserves in colonized males can be achieved by lowering larval density. However, some species like *An. gambiae* s.s. inherently build a very small teneral reserves (Brigiel, 1990). Males that emerge from low teneral reserves are more vulnerable to starvation if they are unable to acquire sugar soon after emergence (Magnarelli, 1986; Foster and Takken, 2004).

(iv) Heterosis (Hybrid vigour) - Enhanced hybrid males can be produced in colonized insects to increase their mating ability. Heterosis has been achieved in *An. coluzzii* by crossing two colonized
strains, > 35 and 8 years old. Heterotic males were more fertile, increased female fecundity and showed increased longevity (Ekechukwu et al., 2015). Hybridization of two colonized *An. gambiae* strains evaluated for 20 generations led to increased performance in fecundity, body size and adult longevity (Menge et al., 2005). Heterosis can also be achieved by refreshing laboratory strain with newly wild caught material (Baeshen et al., 2014).

6) Capability of released males to disperse.
The males earmarked for release males should be able to disperse for effective SIT programme. The dispersal ability of males is crucial in determining appropriate release strategies before programme implementation. Male dispersal rates can be investigated using mark-release-recapture (MMR) techniques (Verhulst, 2013).

However, there is also a need to investigate environmental conditions of the area targeted for release because dispersal is affected by environmental factors such as wind/wind direction (Midega et al., 2012), rainfall (Hausermann, 1971), vegetation, humidity (Lacroix et al., 2012), topography (Thomas et al., 2013) etc. In mosquitoes, dispersal rates are greatly affected by human settlement (Trpis and Hausermann, 1986). Both male and female mosquitoes travel longer distance in uninhabited areas than in inhabited areas (Harrington et al., 2005). Even though male mosquitoes do not blood feed, they are readily recaptured in households due to their mating behaviour (Hartberg, 1971; Harrington et al., 2005). Due to the effects of colonization, the mean dispersal rates of both male and female mosquitoes are lower than their wild counterparts (Lacroix et al., 2012; (Hausermann, 1971).

7) Isolated population
The SIT is very effective when applied to the total target population or part of the population that can be isolated by natural or artificial barriers such as mountain ranges, lakes/oceans etc. (Helsinki et al., 2006b). The immigration of fertile insects from non-targeted breeding sites reduces programme efficiency hence the artificial or natural barriers play an important role in preventing immigration of fertile insects from neighbouring sites outside the area of intervention.