Studies on *Anopheles dthali* Patton (Diptera: Culicidae) as a Malaria Vector in Jazan Region, Kingdom of Saudi Arabia (2014 -2017)

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Department of Medical Entomology and Vector Control
Blue Nile National Institute for Communicable Diseases

March, 2018
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DECLARATION

I hereby declare that with the exception of references to other people's work, which have been duly acknowledged, this thesis is the product of my own research efforts, under the supervision of Prof. Nabil H. H. Bashir, Prof. Samera Hamid Abdelrahman, Dr. Elsiddig M. N. Hassan and Dr. Adel A. H. Alsheikh and has not been presented elsewhere for the award of a degree or certificate.
DEDICATION

To the soul of my father Elsideeg
You will be in my heart forever.
ACKNOWLEDGEMENTS

Foremost, I would like to express my sincere gratitude to my supervisor Prof. Nabil Hamid Hassan Bashir, supervisor of the Department of Medical Entomology and Vector Control, BNNICD, U of G, and my co-supervisor Prof. Samira Hamid Abdelrahman Elamin, Dean, BNNICD, U of G, for their guidance and wisdom during this study.

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Studies on *Anopheles dthali* Patton (Diptera: Culicidae) as a Malaria Vector in Jazan Region, Kingdom of Saudi Arabia (2014-2017)

Waheed Eldeen Elsideeg Dafalla Mohammed

**Abstract**

Jazan region is known to be the most malarious region in Saudi Arabia accounting for an average of more than 50% of all locally malaria cases recorded in the country. *A.dthali* Patton (Diptera: Culicidae) is suspected to be a secondary vector of malaria in Jazan region. The aim of this study was to investigate the role of *Anopheles dthali* as a secondary malaria vector in the region and to study its ecology, distribution, behavior, vectorial capacity, as well as, to determine its insecticide resistance status in the region. A cross sectional study was conducted in the Harob and Eledabi malarious areas on October 2014 - September 2015. Selected breeding sites were monitored and all larvae sampled were reared to adults. Female *A.dthali* mosquitoes were used for insecticide susceptibility tests according to the WHO standard procedures. Adults *A.dthali* collected from study area were subjected to investigation of their vectorial capacity and other entomological parameters. Polymerase Chain Reaction (PCR) PCR technique was used to determine the sporozoite rate, blood meal source, whole genome sequencing and KDR mutation gene in the *A.dthali*. Results of ecological studies of the *A.dthali* in Jazan region indicated that the preferred breeding places were the edges of the valleys, animal footprints, shallow slow-current clear or turbid water, with or without vegetations, with rocky or sand substrate bottom and where the mean water temperature was 27°C, Total Dissolved Solid (TDS) ranged from 224 ppm to 1145ppm and pH ranged from 7.2 to 7.9. The adults were increased rapidly following the beginning of the rains in August with peak point in October. *A.dthali* was found to be the most predominant Anopheline species in Jazan region followed by *A.arabiensis* and *A.pretoriensis*. *A.multicolor* was collected only as larvae in the study areas. Laboratory analysis of adult *A.dthali* for other entomological parameters showed the following findings for Harob and Eledabi, respectively; the anthropophagic index was 11% and 15%, sporozoite rate 0.01 and 0.02, EIR 0.73 and 1.8, low vectorial capacity of 0.0001 and 0.0004. It is interesting to note that this the first time in the world to determine the vectorial capacity for *A.dthali*. The whole genome sequencing of *A.dthali* was established for the first time in the world and registered at the NCBI. Results of *A.dthali* insecticidal resistance status revealed its susceptibility to all pyrethroids tested, bendiocarb, malathion and DDT. However, it exhibited resistance to dieldrin and fenitrothion. On the other hand, the susceptibility of *A.dthali* larvae to various IGRs and temephos larvicides varied considerably; it was more susceptible to diflubenzuron than pyriproxyfen and methoprene, the latter had the lowest level of efficacy against *A.dthali*, while it was found to be resistant to temephos. Incriminating the *A.dthali* as a secondary vector of malaria in the Jazan region for the first time in the region and the kingdom of Saudi Arabia, along with its high density would create a burden to health authorities in the region and necessitate its periodical surveillance and prompted preventive and control measures.
دراسات عن انوفليس دتلاي باتون (ثنائية الأجنحة عائلة كيولسيدي) كناقل للملاريا في منطقة جازان بالمملكة العربية السعودية (2014-2017)
أحمد الصديق دف الله محمد

ملخص الدراسة
تعتبر منطقة جازان أكثر المناطق انتشاراً لمرض الملاريا بالمملكة العربية السعودية، حيث يتمثل أكثر من 50٪ من جمع حالات الملاريا المحلية المسجلة في البلاد. يشتبه في أن انوفليس دتلاي باتون (ثنائية الأجنحة عائلة كيولسيدي) ناقل ثانوي للملاريا في منطقة جازان. الهدف من هذه الدراسة هو تقصي دور انوفليس دتلاي كناقل ثانوي للملاريا في المجموعة، ودراسة بيئتها وتوزيعها وسلوكها وقدراتها الناقلة وكذلك لمعرفة مدى مقاومة هذا البعوضة للمبيدات الحشرية المستخدمة بالمنطقة.

لقد أجريت دراسة مقطعية في مناطق هروب وإلعيدابي في تشرين الأول / أكتوبر 2014 - أيلول / سبتمبر 2015. تم الاستكشاف الحشري لجمع اليرقات في مواقع التوالد المختارة حيث تم تربية اليرقات للحصول على الحشرة الكاملة (البالغة). تم استخدام الحشرات الكاملة لانوفليس دتلاي لإجراء اختبارات حساسيتها للمبيدات حشرية وفقا للإجراءات القياسية لمنظمة الصحة العالمية. تم استخدام مبيدات حشرية مختلفة من المنظمات النمو والثيموفوس (البيتا) وتم استخدام انوفليس دتلاي كناقل ثانوي للملاريا في المجموعة، بالإضافة إلى كثافتها العالية من شأنه أن يخلق عينا على السلطات الصحية في المنطقة ويستلزم مراقبتها الدورية والعمل على الوقاية منها.
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A. Anopheles
BC Biological Control
Bti B. thuringiensis var. israelensis
CDC Centers for Disease Control
CSP Circumsporozoite proteins
DDT Dichlorodiphenyltrichloroethane
DVs Disease Vectors
EIR Entomological Inoculation Rate
ELISA Enzyme-Linked Immunosorbent Assay
ENSO El Nino Southern Oscillation
FF Fresh Fed
GPIRM Global Plan for Insecticide Resistance Management
GR Gravid
HBR Human Biting Rate
HG Half Gravid
HLC Human Landing Catch
Hm Habitat modification
HM Habitat manipulation
Ib/p/year Infective bites per person per year
IGRs Insect Growth Regulators
IR Insecticide Resistance
IRS Indoor Residual Spraying
ITN Insecticide Treated Net
IVM Integrated Vector Management
Kdr Knockdown Resistance
KSA Kingdom of Saudi Arabia
LHM Larval Habitat Management
LLIN Long-Lasting Insecticidal Net
m Meters
m.a.s.l. Meters above sea level
MOH Ministry of Health
NCBI National Center for Biotechnology Information
OC organochlorine
OPs organophosphate
P Plasmodium
PCR Polymer chain reaction
PKD Pyrethroid Knockdown
PSC Pyrethrum Spray Collection
s.l. sensulato
s.s. sensustricto
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<td>SNNPR</td>
<td>Southern Nations, Nationalities and People’s Region</td>
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<td>spp.</td>
<td>species</td>
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<td>SR</td>
<td>Sporozoite Rate</td>
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<tr>
<td>TDS</td>
<td>Total Dissolved Solid</td>
</tr>
<tr>
<td>UF</td>
<td>Unfed</td>
</tr>
<tr>
<td>UL</td>
<td>Ultra Low Volume</td>
</tr>
<tr>
<td>USA</td>
<td>United State Of America</td>
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CHAPTER ONE

1. INTRODUCTION

1.1 General Introduction:

Throughout history descriptions of malaria appear in early medical texts of various civilizations. For the majority of this time, it was unknown as those mosquitoes were the carriers of this disease. Instead, for lack of knowledge it was blamed on "bad air". Hence it was named malaria by the Romans, which in Latin means bad air. The Greek physician Hippocrates, also remembered as the Father of medicine, wrote about the disease in the fifth century, dividing its associated fever in to different types. Malaria is suspected to have contributed to the decline of both the Greek and Roman empires, sapping the vitality of people and destroying their stamina (Sallares 2002 and Lalchhandama 2014).

The Pontine Marshes southeast of Rome were notoriously known to travelers as the place of the bad air. It was more likely a large breeding ground for the Anopheles gambiae. Also, when the Europeans tried to settle on the west coast of Africa in the seventeenth century they suffered high mortality rates, so much so to name the whole region "The White Mans Grave" (Curtin, 1998).

Malaria is the most important and life-threatening parasitic disease of humans. Malaria remains a devastating global health problem. The disease is caused by protozoan parasites of the genus Plasmodium; (viz. Plasmodium falciparum, P. vivax, P. ovale and P. malariae) of which P. falciparum is responsible for severe morbidity and mortality followed by P. vivax. During the period from 1900 – 2002, the global population has grown from 1 to 6 billion, with the malaria risk population increasing from 0.9 to 3 billion (Hay et al., 2004). Worldwide, an estimated 300–500 million people with clinical episodes each year, resulting in one million or more deaths annually (Muentener et al., 1999; Sachs and Malaney, 2002; Trampuz et al., 2003, and Rasti et al., 2004).

An estimated 2.6 billion people in the tropical countries were at risk of P. falciparum malaria infection in 2010. Among these, 1.13 and 1.44 billion people were at
risk of unstable and stable infection, respectively. The highest level of *P. falciparum* transmission occurs in Africa, which contributes to 99% of the global- and 95% of the African *falciparum* malaria cases (Gething et al., 2011). In the same year, approx. 2.5 billion people were at risk of *P. vivax* infection. Among these, 1.5 and 1 billion were at risk of unstable and stable *P. vivax* malaria infection, respectively (Gething et al., 2012). The highest population at risk of *P. vivax* infection lived in Central Asia (2.05 billion), which was followed by Southeast Asia (215 million) and South and Central America (137.4 million). Africa has a population of 74.4 million at risk of *P. vivax* (Gething et al., 2012).

In the WHO Eastern Mediterranean Region, malaria is still endemic in several countries, including Saudi Arabia. The estimated incidence of malaria in this Region is about 10.5 million cases annually, more than 95% of which are estimated to occur in six countries: *viz.* Afghanistan, Pakistan, Djibouti, Somalia, Sudan and Yemen (WHO/EMRO, 2005).

Despite the intervention efforts, malaria continues to be a major public health problem (Noor, et al., 2014). In 2013, there were still 104 malaria-endemic countries and territories, where an estimated 3.4 billion people lived. Approx. 207 million cases and 627,000 deaths were documented in 2012. Most cases (80%) and deaths (90%) occurred in Africa, where children under the age of five years contributed to 77% of the total deaths (WHO, 2013a).

Malaria is one of the important endemic diseases in the South-western Saudi Arabia and is one of the country’s foremost health problems. Malaria used to have a wide distribution in the country in the past, but as a result of the extensive malaria control program, the disease has receded and is restricted now to the Tihama area, mainly in Jizan Region, where more than 50% of total cases are recorded. About 5% of the national population of Saudi Arabia is at risk (2.4 million). The average number of local cases per annum recorded in Saudi Arabia in the period from 1997 to 2008 was 5225, ranging from 61 in 2008 to 36,139 cases in 1998 (Alsheikh, 2011).
Jazan region is known to be the most malarious region in Saudi Arabia, accounting an average of more than 50% of all locally malaria cases recorded in Saudi Arabia (Al-sheikh, 2011).

Jazan region is divided into four zones of different endemicity; (Ageel, 1990)

- **Malaria free zone:** Malaria is absent in mountain areas at 1500 m above sea level (Fifa and al hashr areas) it is also absent in the Farasan Island.

- **Low endemicity zone:** Transmission in this zone is unstable and the area is subject to malaria epidemics. Periodic rains in the hills can lead to flooding in the valleys. This can leave water pockets suitable for breeding of mosquitoes on the coastal plain. In the past spleen rate was found to be less than 10%.

- **Moderate endemicity:** Malaria incidence in this zone also is rather unstable. Most of the inhabitants in this zone are exposed to malaria transmission during the period of up to six months every year, depending on pattern and amount of rain. This zone includes the interior plains and foothills (Al-Aridah, Harob, Samtah, AbuArish, Alraith, Sabya, and Aldarb) in these places the rain water collects in valleys and in natural pans, where most of the population reside, especially along the valleys. Spleen rate in this zone was 10-50%.

- **High endemicity:** Malaria in the Yemen border area is stable. Malaria cases reported in this zone constitute more than 50% of all cases reported in Jazan. Water suitable for mosquito breeding is available throughout the whole year. It is likely that malaria transmission continuously throughout seven months of the year.

Malaria transmission season in Jazan extends from October to April with a prominent peak in January. The major factor that influences the extent and intensity of transmission season is rain (Alsheikh, 2011).

**1.2 Rational:**

Routine entomological investigations showed the presence of high density of *A. dthali* in Jazan area and it has been found where *A. arabiensis* is present.

Previous studies showed that a number of *A. dthali* have been incriminated as secondary Malaria vector from the Southern part of Iran (Vatandoost et al., 2007).
Rishikesh (1961) stated that; from 14 \textit{A.dthali} collected from north of Somali one of these showed sporozoites in the salivary gland. Bruce \textit{et al.} (1966) revealed that; the precipitin tests in \textit{A.dthali} of specimens from Moracco and Saudi Arabia were 4-18.7% positive for human blood. Patton (1905), cited in Vatandoost \textit{et al.}(2007) mentioned that this species has been suspected as a vector of malaria in Saudi Arabia.

In spite of the above mentioned studies and field observations, no researches has been conducted to study the vectorial capacity, ecology and distribution of \textit{A.dthali} in the Jazan area-KSA. Therefore the purpose of this study was to assess the incrimination of \textit{A.dthali} as a potential malaria vector in Jazan region. It also covers the behavior, ecology of this vector, and any development of insecticide resistance among this species. These are being taken in order to provide evidence for the need to establish an entomological monitoring system in the area.

1.3 General objective:

The main objective of the study is to identify the spatio-temporal distribution, behavior and vectorial capacity of Anopheles dthali Patton (Diptera: Culicidae) as a Malaria vector in Jazan region-Saudi Arabia (Oct.2014-Sept.2015).

1.4 Specific objectives:

a) To identify the proportionate distribution of Anophelinae species in the Iazan region, Saudi Arabia.

b) To determine the distribution of \textit{A.dthali} in Jazan region.

c) To study the ecology and habitat of \textit{A.dthalit} with regard to breeding sites, vegetation, pH, shading, sunlight and salinity.

d) To describe the behavior of \textit{A.dthali}, its (resting behavior, feeding habits, seasonal variations, and the degree of man–vector contact and host preferences.

e) To assess the incrimination of \textit{A.dthali} as a malaria vector

f) To assess the susceptibility levels of \textit{A.dthali} to some adulticides (pyrethroid, DDT, malathion and bendiocarb) and larvicides used in the area,
víz the IGRs diflubenzron, methoprene, and pyriproxyfen and in addition temephos.
CHAPTER TWO

2. LITERATURE REVIEW

2.1 The Malaria Parasite

The discoveries of Pasteur and Koch in 1870s had precipitated a search for a bacterial cause for many diseases including malaria. The Italian Corrado Tommasi-Crudeli and the German, Theodor Albrecht Edwin Klebs, had claimed that they had isolated from the waters of the Pontine Marshes, where malaria was prevalent, a bacterium, \textit{Bacillus malariae}, which when isolated in culture and injected into rabbits caused febrile infections accompanied by enlarged spleens reminiscent of malaria (Klebs et al.,1879). It was against this background that Charles Louis Alphonse Laveran, a French army officer working in Algeria, began in 1880 to look for pigment in the fresh unstained blood of patients and observed it first in leucocytes and then in or on red blood cells (RBCs). He observed several different forms of erythrocytic organism including crescents, spherical motionless bodies with pigment, spherical moving bodies with pigment and bodies that extruded flagella-like structures all of which he thought were on the outside of the red cells (Larveran,1881). These observations are particularly interesting because Laveran not only used fresh blood but also a dry objective with a maximum magnification of $\times 400$ diameters. He also suggested a course of events that began with clear spots that grew, acquired pigment and filled the corpuscle which then burst coinciding with the fevers associated with malaria. Laveran meticulously examined the blood of 200 patients and in 148 observed the crescentic bodies in all cases of malaria but never in those without malaria. He also noted that quinine removed these stages from the blood. By 1884, Laveran had convinced the leading Italian malarialogists including Bignami, Golgi and Marchiafava that malaria was caused by a protozoan and not a bacterium (Laveran, 1884). As late as 1895 the American R. C. Newton wrote that ‘Aerial and aquatic transportation of malaria has been proved’ (Newton, 1895).
Thereafter began searches for other malaria parasites in reptiles, birds and mammals and this was facilitated by the accidental discovery of a methylene blueeosin stain by Dimitri Leonidovitch Romanowsky in 1891 (Garnham, 1966). Romanowsky’s stains became popular at the beginning of the 20th century and remain the basis of blood stains such as Leishman’s, Giemsa’s and Wright’s to the present day.

By 1889 it had also become clear that the paroxysms characteristic of malaria coincided with the bursting of infected RBCs and the release of the products of multiplication something that Laveran, who had also realised that in the case of malignant tertian malaria the brain was involved, had proposed (Laveran, 1893). Thus by 1890 it was known that malaria was caused by a protozoan parasite that invaded and multiplied in red blood cells and, after a lot of confusion, that there were three species with specific periodicities and other characteristics responsible for benign tertian (Haemamoeba vivax), malignant tertian (Laverania malariae) and quartan (Haemamoeba malariae) malaria now respectively Plasmodium vivax, P. falciparum and P. malariae. In 1918, Stephens working in West Africa discovered a fourth species which resembled P. vivax which he described as P. ovale in 1922 (Stephens, 1922).

There are about 100 species of malaria parasites which infect a wide variety of hosts, including primates, reptiles, and birds. Of these, only four species are responsible for malaria in humans (WHO, 1999).

The malaria parasite that affects man and low vertebrates are species of the genus Plasmodium, family Plasmodiidae, order Heamosporica, and class Sporozoa (Belding, 1965). The life cycle of the malaria parasite consists of two phases: asexual phase or schizogony and a sexual phase or sporogony. The female anopheline mosquito is the insect vector and definite host of this parasite. The prevalence of the plasmodium (P) parasite in the insect vectors is an essential parameter in the description of the epidemiology of malaria (Githeko et al., 1996).

The four types of human malaria are P.vivax, P.malariae, and P.ovale and P. falciparum. P. falciparum and P. vivax are the most common, and P. falciparum the most deadly type of malaria infection. P. falciparum malaria is the most common in Africa south of Sahara, accounting in large part for the extremely high mortality in
this region (WHO, 2000). The malaria parasite enters the human host when an infected anopheline mosquito takes a blood meal. Inside the human host, the parasite undergoes a series of changes as part of its complex life-cycle. The various stages allow the *plasmodium* to invade the non-immune system, infect the liver and red blood cells, and finally develop into a form that is able to infect the mosquito again when it bites an infected person. Inside the mosquito, the parasite matures until it reaches the sexual stage where it can again infect the human host when the mosquito takes her next blood meal, 10 to 14 or more days later (Chwatt, 1980).

2.2 The Life-Cycle of the Malaria Parasite:

The malaria parasite exhibits a complex life-cycle involving an insect vector (mosquito) and a vertebrate host (human). The four *Plasmodium* species infect humans were previously mentioned exhibit a similar life-cycle with only minor variations Fig. (2.1).

The survival and development of the parasite within the invertebrate and vertebrate hosts, in intracellular and extracellular environments, is made possible by a toolkit of more than 5,000 genes and their specialized proteins that help the parasite to invade and grow within multiple cell types and to evade host immune responses (Greenwood *et al.*, 2008, and Florens *et al.*, 2002).

The parasite passes through several stages of development such as the sporozoites (the infectious form injected by the mosquito), merozoites (the stage invading the erythrocytes), trophozoites (nourishment; the form multiplying in erythrocytes), and gametocytes (sexual stages); all these stages have their own unique shapes, structures and protein complements. The surface proteins and metabolic pathways keep changing during these different stages that help the parasite to evade the immune clearance, while also creating problems for the development of drugs and vaccines (Florens *et al.*, 2002).

2.2.1 Sporogony cycle of *Plasmodium* within the Mosquitoes:

Mosquitoes are the definitive hosts for the malaria parasites, wherein the sexual phase of the parasite’s life cycle occurs. The sexual phase is called *sporogony* and results in the development of innumerable infecting forms of the parasite within the
mosquito that induce disease in the human host following their injection with the mosquito bite.

When the female *Anopheles* draws a blood meal from an individual infected with malaria, the male and female gametocytes of the parasite find their way into the gut of the mosquito. The male and female gametes fuse in the mosquito gut to form zygotes, which subsequently develop into actively moving ookinetes that burrow into the mosquito midgut wall to develop into oocysts. Growth and division of each oocyst produces thousands of active haploid forms called sporozoites. After the sporogonic phase of 8–15 days, the oocyst bursts and releases sporozoites into the body cavity of the mosquito, from where they travel to and invade the mosquito salivary glands. When the mosquito thus loaded with sporozoites takes another blood meal, the sporozoites get injected from its salivary glands into the human bloodstream, causing malaria infection in the human host. It has been found that the infected mosquito and the parasite mutually benefit each other and thereby promote transmission of the infection. The *Plasmodium*-infected mosquitoes have a better survival and show an increased rate of blood-feeding, particularly from an infected body (Barillas-Mury and Kumar, 2005, and Ferguson and Read, 2004).

### 2.2.2 Schizogony in the Human Host:

Man is the intermediate host for malaria, wherein the asexual phase of the life cycle occurs. The sporozoites inoculated by the infested mosquito initiate this phase of the cycle from the liver, and the latter part continues within the RBCs, which results in the various clinical manifestations of the disease.

#### 2.2.2.1 Pre-erythrocytic Phase – Schizogony in the Liver:

With the mosquito bite, tens to a few hundred invasive sporozoites are introduced into the skin. Following the intradermal deposition, some sporozoites are destroyed by the local macrophages, some enter the lymphatics, and some others find a blood vessel (Vaughan *et al.*, 2008 and Silvie *et al.*, 2008). The sporozoites that find a blood vessel reach the liver within a few hours. It has recently been shown that the sporozoites travel by a continuous sequence of stick-and-slip motility, using the thrombospondin-related anonymous protein (TRAP) family and an actin–myosin motor (Yamauchi *et al.*, 2007;
Munter, et al., 2009, and Baum et al., 2006). The sporozoites then negotiate through the liver sinusoids, and migrate into a few hepatocytes, and then multiply and grow within parasitophorous vacuoles. Each sporozoite develop into a schizont containing 10,000–30,000 merozoites (or more in case of P. falciparum) (Kebaier et al., 2009; Jones and Good, 2006). The growth and development of the parasite in the liver cells is facilitated by a favorable environment created by the the circumsporozoite protein of the parasite (Prudencio et al., 2006 and Singh et al., 2007). The entire pre-erythrocytic phase lasts about 5–16 days depending on the parasite species; an average 5-6 days for P. falciparum, 8 days for P. vivax, 9 days for P. ovale and 13 days for P. malariae. The pre-erythrocytic phase remains a “silent” phase, with little pathology and no symptoms, as only a few hepatocytes are affected (Vaughan et al., 2008). This phase is also a single cycle, unlike the next, erythrocytic stage, which occurs repeatedly. The merozoites that develop within the hepatocyte are contained inside host cell-derived vesicles called merosomes that exit the liver intact, thereby protecting the merozoites from phagocytosis by Kupffer cells. These merozoites are eventually released into the blood stream at the lung capillaries and initiate the blood stage of infection thereon (Silvie et al., 2008). In P. vivax and P. ovale malaria, some of the sporozoites may remain dormant for months within the liver. Termed as hypnozoites, these forms develop into schizonts after some latent period, usually of a few weeks to months. It has been suggested that these late developing hypnozoites are genotypically different from the sporozoites that cause acute infection soon after the inoculation by a mosquito bite, (Collins, 2007 and Cogswell, 1992) and in many patients cause relapses of the clinical infection after weeks to months.

2.2.2.2 Erythrocytic Schizogony–Centre Stage in Red Cells

Red blood cells are the ‘centre stage’ for the asexual development of the malaria parasite. Within the red cells, repeated cycles of parasitic development occur with precise periodicity, and at the end of each cycle, hundreds of fresh daughter parasites are released that invade more number of red cells. The merozoites released from the liver recognize, attach, and enter the (RBCs) by multiple receptor–ligand interactions in as
little as 60 sec. This quick disappearance from the circulation into the red cells minimises the exposure of the antigens on the surface of the parasite, thereby protecting these parasite forms from the host immune response (Greenwood et al., 2008; Silvie et al., 2008, and Cowman and Crabb, 2006). Within the red cells, the parasite numbers expand rapidly with a sustained cycling of the parasite population (Silvie et al., 2008). The erythrocytic cycle occurs every 24 hr in case of *P. knowlesi*, 48 hr in cases of *P. falciparum*, *P. vivax* and *P. ovale* and 72 hr in case of *P. malariae*. During each cycle, each merozoite grows and divides within the vacuole into 8–32 (average 10) fresh merozoites, through the stages of ring, trophozoite, and schizont. At the end of the cycle, the infected red cells rupture, releasing the new merozoites that in turn infect more RBCs. With sunbridled growth, the parasite numbers can rise rapidly to levels as high as $10^{13}$ per host (Greenwood et al., 2008). A small proportion of asexual parasites do not undergo schizogony but differentiate into the sexual stage gametocytes. These male or female gametocytes are extracellular and nonpathogenic and help in transmission of the infection to others through the female anopheline mosquitoes, wherein they continue the sexual phase of the parasite’s life-cycle. Gametocytes of *P. vivax* develop soon after the release of merozoites from the liver, whereas in case of *P. falciparum*, the gametocytes develop much later with peak densities of the sexual stages typically occurring 1 wk after peak asexual stage densities (Pukrittayakamee et al., 2008, and Miller et al., 2002).
**Fig.(2.1) Life Cycle of Malaria Parasite**

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2.3 The Population of the Malaria Vectors

Of the various arthropods of medical significance, the family Culicidae or mosquitoes are, without a doubt, the most important. The genera that are of major medical importance are the Anopheles, Aedes and Culex (Herms and James, 1961). Knowledge of the biology and ecology of the mosquitoes is vital because of the global resurgence of mosquito-borne diseases. Lack of proper understanding of the various aspects of mosquito-bionomics has frequently hampered vector control (VC) programme (WHO, 1963a). Out of the 2,500 species of mosquitoes over 300 named species, the anophelines are known to be the most important as malaria vectors, but the females of over 60 species are recognized as vectors of human malaria (WHO, 1963b). The Anopheles belongs to the order Diptera, sub-order Nematocera, family Culicidae and tribe Anophelini in the zoological classification. With tribe Anophelini, the genus Anopheles has several sub–genera (Chawttes, 1980). Most of the species within these genera are not sufficiently closely associated with Man to be important as malaria vectors. The geographical, seasonal, or ecological distribution of the Anopheles is therefore not necessarily related to the presence of malaria, and Anophelism without malaria is not only possible but may represent the final outcome of malaria eradication, in which the elimination of the vector is not primarily intended (WHO, 1963b).

2.3.1 The Distribution of Anopheline mosquitoes

Anophelines are found worldwide except Antarctica. Malaria is transmitted by different Anopheles species, depending on the region and the environment. Anophelines that can transmit malaria are found not only in malaria-endemic areas, but also in areas where malaria has been eliminated. The latter areas are thus constantly at risk of re-introduction of the disease.
2.3.1.1 Geographical distribution

In the ordinarily sense, this is describable in term of latitudes and longitudes. Elevation and climatic zones are, of course, interrelated, in as much as mountain tops at the equator may reach atmospheric conditions comparable to these at sea level in arctic regions.

2.3.1.2 Seasonal distribution

The factors that determine the distribution and severity of malaria are diverse and complex, but climate can be considered the major determinant. Temperature and rainfall limit malaria to the warm, humid regions of Africa, where the mosquitoes and parasite can breed and develop, and transmission occurs. In general terms, seasonal changes in the *Anopheles gambiae* complex population tend to follow the seasonal patterns of rainfall, in particular *Anopheles arabiensis* (MARA/ARMA, 1998).

2.3.1.3 Microclimatic distribution

This is especially important in survey work as each species has its characteristic ecology for both larval and adult stages, and some thrive under conditions of temperature and humidity, salinity or sunlight that others do not tolerate. The anopheline mosquitoes are somewhat more abundant in the tropics than elsewhere. It tends to be restricted by oceans barriers, mountain ranges, adverse winds, and long stretches of deserts. The spread of the species, which might well adapt themselves to distances, could cross these barriers. Other species, however, seem to have their range limited by less tangible physiological or ecological factors (WHO, 1975a).

2.3.1.4 Epidemiological zones

Biologists distinguish between six zoogeographical regions. MacDonald (1957) in his work on the epidemiology and control of malaria, recognized 12 zones of malaria epidemiology outside of which very few anophelines are known to exist.

2.4 Malaria Vectors of the WHO Eastern Mediterranean Region:

The WHO Eastern Mediterranean region comprises the following countries: Afghanistan, Bahrain, Cyprus, Egypt, Ethiopia, Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Libya, Oman, Pakistan, Qatar, Saudi Arabia (KSA), Somalia, Sudan, Syria,
Tunisia, United Arab Emirates (UAE), and Yemen. Zahar (1974) reviewed the malaria vector in this region as follows:

- *A. gambiae* Giles found to be as an important malaria vector of the Ethiopian zoogeographical region (Yemen, Saudi Arabia, Sudan, Ethiopia and Somalia Map 2.1).
- *A. funestus* Giles is the second most important malaria vector in the Ethiopian zoogeographical region it occurs in Sudan, Ethiopia and Somalia.
- *A. pharoensis* Theobald is widely distributed in Ethiopia, Sudan, Egypt and Somalia.
- *A. sergenti* Theobald is an important vector of malaria in Egypt (Oases and Fayoum) and Libya. In the eastern part of the Region, it is recorded from Iran, Iraq, Jordan, Saudi Arabia, and some pockets in the Syria and Tunisia.
- The role of *A. multicolor* Cambouliu, where it exists with *A. sergenti* or alone in these oases, remains unknown. *A. multicolor* Cambouliu has not been incriminated in nature, but is suspected to be a vector on epidemiological grounds as it has been found alone in some oases where malaria is transmitted.
- *A. labranchiae* Falleroni has been reported from north-western part of Libya and in the northern governorates of Tunisia.
- *A. claviger* Meigen is widely distributed in the Middle East. In 1970, is found to be responsible of malaria outbreak in Syria.
- *A. pulcherrimus* Theobald occurs in the north-eastern part of the Eastern Mediterranean Region, including eastern Saudi Arabia, Syria, Iraq, and Afghanistan.
- *A. superpictus* Grassi has been recorded in Jordan, Saudi Arabia, Syria, Iraq and Pakistan.
- *A. fluviatilis* James has a wide distribution in the region where it exists in Saudi Arabia, Iraq, Iran and Pakistan.
- *A. dthali* Patton is widespread in semi-arid parts of the Region. It is found in north Ethiopia and Somalia, Socotra, north Africa to north west of Pakistan, Southwest of Saudi Arabia, around the Red sea and Adan Gulf (Christophers,
1933; De Meillon, 1947; Stone et al., 1959, and Gillies and Meillon, 1968), in Iran (Manouchehri and Rohani, 1975) and Eritrea (Shililu et al., 2003).

In Somalia, Rishikesh (1961) found one specimen of *A. dthali* Patton with sporozoite-positive glands among 14 dissected females. In Iran gland infection in *A. dthali* was reported by Manoochehri et al. (1972) and Manouchehri and Rohani (1975); this species was repeatedly found infected during 1965-1967. The sporozoite rate was low; 1%, 2.1% and 7.7% in three different areas. Also it was reported by Shililu, et al. (2003) as predominant species in Anseba area of Eritria.

*A. sacharovi* Favre is a major vector in Syria Iraq and Iran. *Anopheles stephensi* Liston (the oriental malaria vector) is present over a fairly large area in the northern and eastern parts of the Region. *A. stephensi mysorensis* Liston has been identified in the Eastern Province of Saudi Arabia (Gillies and Coetzee, 1987), Iraq and in southern Iran.

### 2.5 Malaria vectors of Saudi Arabia:

Glick (1992) reviewed the following species of *Anopheles* mosquitoes as primary malaria vectors in the region; *A. gambiae* s.l., *A. culicifacies* sensu lato, *A. fluviatilis* James, *A. sergentii* Theobald, *A. steohensi* Liston and *A. superpictus* Grassi. Although many of these are important in malaria transmission in other areas of the region, only *A. gambiae* s.l (Southern part), *A. sergentii* (Western region), *A. stephensi* (Eastern region) and *A. superpictus* (Northern region) are of major importance in malaria transmission in Saudi Arabia (Sebai, 1988). In addition to the four mentioned species, the following *Anopheles* have been reported in the country: *A. cinereus* Illiger (Abha, Asir region), *A. dthali* (Western and Madinah region), *A. fluviatilis* James (Eastern region), *A. multicolor* Cambouliu (Eastern, western and Medinah region), *A. pharoensis* Theobald (Tabuk region), *A. pretoriensis* Theobald (jazan), *A. pulcherrimus* Theobald (Eastern), *A. rhodesiensis rupicola* Lewis (Yanbu in Medinah region), *A. turkhuudi* Theobald (Western and Medinah region), *A. coustani* Laveran and *A. tenebrosus* Donitz (Eastern region; Mattinggly and Knight 1956; Zahar, 1985), and Glick, 1992). None of these species have yet been incriminated in malaria transmission in Saudi Arabia (Zahar, 1985).
2.6 Anophelinae Vector in Jazan Region:

Very few studies on malaria vectors have been made in Jazan region. Zahar (1985) reported only two Anopheline mosquitoes, viz. *A.gambiae s.l* and *A.pretoriensis* Theobald. Abdullah and Merdan (1995) reported one more species, *i.e.* *A.tenebrosus* Donitz, recorded as larval stage only. Previous studies only stated the importance of *A. gambiae s.l.* as a potential malaria vector in the Tihama (Mattingly and Knight, 1956; Zahar, 1974, 1985 and Sebai, 1988). Al-sheikh (2011) revealed that *A. arabiensis* is the most important malaria vector in this region.

2.7 Malaria control in KSA:

Over the last 50 yr, systematic efforts to control malaria in the KSA have successfully shrunk the extent of *P. falciparum* and *P. vivax* risks. Starting with the oil rich areas in the Eastern region, the use of annual indoor residual house spraying (IRS) with dichlorodiphenyltrichloroethane (DDT) and dieldrin, between 1948 and 1957, led to a dramatic decline in malaria case incidence (Daggy, 1959) and local transmission was interrupted by 1975 (Sebai, 1988).

From 1956, significant progress was made in reducing the malaria risks maintained by *A. superpictus* through the application of DDT IRS and larvicides in the northern borders with Jordan and Iraq. Active transmission of malaria in the northern regions was interrupted in the 1970s (Snow *et al*., 2013, and Sebai, 1988).

The hardest areas to control were located along the Red Sea, where *A. sergentii* and *A.arabiensis* sustained transmission (Sebai, 1988, and Farid, 1956). The pilgrimage routes used by those on the Hajj were protected through Abate® larviciding and DDT IRS in rural households through the 1970s. Small residual foci remained in the lower reaches of the Hijaz Mountains and persistent *A. arabiensis* foci in the foothills of Mecca (Sebai, 1988; Magzoub, 1980). Malaria control activities in the south-western regions of the Kingdom did not start until 1972.

Epidemics were reported during the mid-late 1990s in the south-western regions of the Kingdom and malaria case incidence began to rise (Snow *et al*., 2013; Coleman *et al*., 2014; Al-Zanbagi, 2014). By 2003, >70 % of the total national case burden occurred in this region, mainly Jazan and Asir Provinces. Renewed efforts to achieve malaria pre-
elimination were launched in 2004. The last 10 yr have witnessed significant declines in malaria in the south-western provinces. Malaria transmission was eventually constrained to Qunfudha and Al Lith sectors of Mecca Province, Asir and Jazan Province. In 2009, only 61 autochthonous cases were reported and all came from foci in Jazan and Asir (Al Zahrani, 2010).

2.8 Malaria control in Jazan Region:

Two principal malaria vectors exits in Jazan, *A.sargentii*, located more frequently on border with Asir Province in the north, and *A. arabiensis* more commonly associated with transmission closer to Yemeni border. Other Anopheline species identified in the province but never implicated in malaria transmission include *A.dthali*, *A.rupicola*, *A.multicolor*, *A.turkhudi*, *A.pretoriensis* and *A.tenebrosus* (Mattingly and Knight, 1956; Al-Sheik, 2011, and Bakr et al., 2014).

Although there are some reports of larviciding in the town of Jazan in 1973, vector control did not start until the early 1980s (Lin, 1986). From 1983, the malaria control programme in Jazan focussed on attacking the adult vectors using annual indoor residual spray (IRS) rounds of DDT in pre-defined areas of endemic risk accompanied by Abate® (temphos 50EC) larviciding in persistent foci covering circa 800 villages (Anon., 1986). After 1 year of aggressive VC, and reductions in slide positivity, the regional control programme abandoned IRS in favour of only larviciding. Ultra-low volume (ULV) spaying was undertaken using the pyrethroid Reslin® (Permethrin 20%) in some selected persistent foci in the mid-1980s (Anon., 1986). Investment in malaria control financing and staff were reduced during the late 1980s. Epidemics were recorded between 1986–1988, 1992, 1995–1996 and the severe epidemic of 1997–1998, lead to renewed efforts at VC accompanied by large increases in funding made available to the Jazan malaria programme. DDT resistance was detected in 1987, resulting in a malaria outbreak in 1988. DDT was replaced the same year with the organophosphates (Ops) fenitrothion and Malathion for IRS with two annual rounds aimed at blanket spraying. In 1995, the preferred insecticide for IRS was switched to the pyrethroid lambdacyhalothrin 10 CS.

Abate was used as a larvicide for many years in Jazan. Insecticide susceptibility tests conducted in 2002 showed reduced sensitivity to *Culex tritaeniorhynchus* (one of
vectors of Rift valley fever; RVF) and larviciding was switched to biological control using *Bacillus thuringiensis israelensis* (Bti), insect growth regulators (IGRs) diflubenzuron (25 %) and pyriproxyfen (0.5 %), and to a lesser extent chemical control using pyridafenthion. Larviciding is routinely undertaken in 103 wadis (valleys) and 534 tributaries carrying water all the year round with total length of 3469 km. Additional larviciding activities are undertaken at 13,800 breeding sites inside focal transmission villages. Lambdacyhalothrin 10 CS was used for IRS between 1995 until 2003 when a switch was made to deltamethrin WG 250, within the pyrethroid insecticide class.

Between 2011 and 2014, lambdacyhalothrin 10 CS was re-introduced. Since 2000, spraying occurs three times every 12 months in September, December and March. Communities identified for IRS are those where known or emerging foci have been described, covering approx. 1200 villages. Over the period 2004–2014, between 51,000 and 63,000 households have been protected by IRS, amounting to 83–93 % of target households and protecting circa 550,000 people. Bioassay data for deltamethrin and lambdacyhalothrin have shown consistently high 24-h mortality rates and have been fully susceptible between 2004 and 2014. ITN have been available in the Kingdom for malaria control, provided free of charge, since the late 1980s (Jamjoom *et al*., 1994).

Between 2000 and 2006 circa 500,000 ITN were distributed to communities in Jazan. Approx. 50,000 LLITNs have been distributed, each year, between 2007 and 2012; these have been targeted to active foci of transmission and all communities along the border with Yemen. 51,000 and 63,000 households have been protected by IRS, amounting to 83–93 % of target households and protecting circa 550,000 people. Bioassay data for deltamethrin and lambdacyhalothrin have shown consistently high 24-h mortality rates and have been fully susceptible between 2004 and 2014. ITN have been available in the Kingdom for malaria control, provided free of charge, since the late 1980s (Jamjoom *et al*., 1994).

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Map (2.1) Location of Saudi Arabia in the Tropical Zoon
2.9 Ecology and biology of Anopheles Mosquitoes:

2.9.1 Breeding sites:

The availability of the appropriate types of surface water may be affected by many different factors, depending on the types of surface. There is often a close general agreement between the topography (T), vector distribution (VD), breeding potentialities (BP) and malaria endemicity (ME) (WHO, 1975b). The equilibrium between malaria and the community is often disturbed by changes in environmental conditions that enhance agricultural activities. Unusual combinations of tide and rainfall, development of new roads and other such activities that are favourable for vector breeding (VB) have over the years caused malaria cases to appear in large numbers. The suitability of the available surface water for breeding of different species may further be affected by light and shade, emerging vegetation, temperature, salinity and other physiochemical characteristics, competitors, predators and parasites (Wernsdorfer and Gerger, 1988).

A. arabiensis breed mostly in small collections of stagnant and muddy seepages and rainwater. Vegetation can be present or absent with partial or full sunlight. The species avoids polluted water (http://www.malaria.org.zw/vectors_control.html welcome to the Southern Africa Malaria Control website).

Thus, while mosquitoes as groups are found breeding in an almost infinite variety of sizes and types of water bodies, each species is generally associated with certain types of breeding places. In some species, however, breeding is restricted to a narrow-range of habitats while others breed readily in a wide-range of water types (WHO, 1982). The anophelines tend to breed in sluggishly moving streams or in stagnant pools, especially where there is a luxurious growth of weeds or grass. They are not commonly found in rapidly flowing streams (Richard and Cook, 1959).
2.9.2 The life-cycle and morphology of *Anopheles* mosquitoes:

After mating and blood feeding, a female *Anopheles* mosquito lays 30-300 small brown or blackish boat-shaped eggs per oviposition, singly onto a water surface. The eggs hatch a free swimming larva, which passes through four larval instars (Wernsdorfer and Gregon, 1988). Anopheline larvae are easily recognized by their appearance as they float horizontally on the surface of the water and feed by means of their mouth brushes, which sweep the floating particles towards the mouth. The larva moves by sharp jerks, and if disturbed, sinks below the surface (Chwattes, 1980).

The body of a larva is divided into three regions: the head, the thorax and the abdomen. The head bears mouth brushes, a pair of antennae and a pair of compound eyes. The thorax is roundish and has hairs, which are usually long and conspicuous. Its segmented abdomen has hairs (either unbranched or branched). The last abdominal segment has two paired groups of long hairs that form the caudal setae, and a larger fan-like group comprising the ventral brush. The last abdominal segment ends in two pairs of transparent, sausage-shaped anal papillae, which undertake osmoregulation. Most of the abdominal segments have a pair of palmate hairs, which aid the larva in keeping parallel to the water surface. *Anopheles* larva must come to the surface to breathe, and take in atmospheric air through a pair of dorsally situated spiracles. It feeds on yeast, bacteria, protozoa and numerous other micro-organisms, as well as on decaying plant and animal materials found on the water’s surface (Service 2012).

The fourth instar larva (L4) moults into comma-shaped pupa. The head and thorax of the pupa are combined to form cephalothorax, which dorsally has a pair of respiratory trumpets. It comes to the surface frequently to breathe through its trumpets, but does not feed. After a few days, depending on the local temperature, the dorsal surface of the cephalothorax splits and the adult mosquito emerges (Service 2012).

After emerging from the pupa, the female mates with male and finds a blood meal for its eggs to mature. An adult *Anopheles* has a slender body divided into a head, thorax and abdomen. The head contains the eyes and a pair of long many-segmented antennae, which help to detect the host odour and breeding site. The head also has an elongated forward-projecting proboscis used for feeding and two sensory palps. The
thorax is specialized for locomotion, and is the place where three pairs of legs and a pair of wings are attached. The abdomen is specialized for food digestion and egg development, and expands considerably during the blood meal. The blood is digested over time, and serves as a source of protein for egg production (Service, 2008, and WHO, 2013b).

2.9.3 Habitats of larvae:

Naturally, rainfall is the primary source for the formation of larval habitats, which include the landward edges of floodplains, footprints, ponds, rain pools, puddles, tire tracks and hoof prints (Minakawa et al., 1999). In some cases, however, rainfall can negatively affect mosquitoes by flushing their eggs and larvae, and also by killing them directly (Paaijmans et al., 2007). Not all water collections serve for mosquito breeding, as a great majority of them could be transient and live a short period of time before the maturation of larvae into pupae and adults. Anopheline mosquito breeding generally occurs in different types of water and a wide range of habitats which may be natural or man-made, temporary or permanent, shaded or sunny (Machault, et al., 2009). Certain environmental parameters are particularly influential in determining larval habitat suitability for the different anopheline vectors, including size and permanence of the water body, water salinity and turbidity, amount of sunlight, and presence of emergent or floating vegetation (Service, 1989). Each anopheline species has its preferred breeding site for oviposition, depending on factors such as weather conditions, physical geography and human activity (Liu et al., 2012). The physico-chemical parameters of the water probably determine the selection of larval habitats. Various physico-chemical properties of the larval habitat such as pH, optimum temperature and concentration of ammonia, nitrate and sulphate have been found to affect larval development and survival (Mutero, et al., 2004). The breeding habitat is crucial for mosquito population dynamics, since it is the location where many important life-cycle processes, e.g. oviposition, larval development and emergence take place (Overgaard et al., 2002).

Anopheles mosquitoes inhabit diverse larval habitats, including water overflows, irrigation ditches, borrow pits, wheel ruts, hoof prints, foot prints, rice field puddles, small streams, dams, riverbed pools, seepage springs, shallow wells, ponds, irrigation
channels, the edges of lakes, lake lagoons, slow-flowing rivers, natural depressions in the ground, swamps, pools in drying stream beds, disused goldmines, plant hollows and cavities, epiphytic arboreal and terrestrial bromeliads, rock holes in stream beds, tree holes, water-filled bamboo stump, pitcher plants, leaf axils in a banana tree, pineapples and other plants, water-filled split coconut husks and snail shells. Larvae also occur in “man-made container-habitats”, such as wells, clay pots, water-storage jars, tin cans, discarded kitchen utensils and motor vehicle tires. A few mosquitoes breed almost exclusively in brackish or salt water, while some species are less specific in their requirements and can inhabit a wide range of breeding habitats including lakes (Abeiku et al., 2003; Minakawa, et al., 2012, and WHO, 1975b).

Members of the *A. gambiae* s.l. breed more abundantly in the aquatic habitats of pasture land than in farmland, indicating their preference for sunlight and higher temperatures. Larval habitats in pasture lands are exposed to sunlight for a long time, which helps to provide suitable habitats for larval growth and oviposition by gravid female *Anopheles* mosquitoes (Mutuku et al., 2006, and Minakawa et al., 2006). They occur in small, open disused goldmines, hoof prints and in cultivated swamps. Grass covered habitats cause a decrease in the abundance of *An. gambiae* s.l. and an increase in *A. funestus* and other *Anopheles* larvae (Kweka et al., 2012).

In Arabia areas, Patton (1905) found the larvae of *A. dthali* in springs and wells. At Muscat, Oman, Gill (1916) found them in pools, and especially in holes of volcanic rock fed by underground water. In Hormozgan Province of Iran this species breeds in pebbly margins of rivers, springs, pits around the springs with or without vegetation, pools in dried-up river beds and palm irrigation canal (Vatandoost et al., 2007). In Bandar Abbas county of Iran larvae were also found in mineral water. They are also found in water with high salinity (2.7 parts per 1000). The temperature was between 13 °C and 28 °C with a pH of 6.9-8.0. Larvae were more abundant during September and October, which is the end of the hot season in southern Iran (Manouchehri and Rohani, 1975). Amani et al. (2014) stated that *A. dthali* preferred mostly larval nests without plants, more adapted with running water, habitats with sandy bottom, and among the artificial larval nests *A. dthali* select mostly plots of rice cultivations.
Manouchehri et al., (1992) caught A. dthali along with A. stephensi and A. fluviatilis in mountainous areas of Hormozgan Province, Iran. Larval nests of A. dthali were found on sides of rivers, holes of river bed, grasslands, streams, and the plots of the rice cultivations. (Azari-Hamidian, 2011). The breeding places for A. dthali are clay or sandy beds. The chemical analysis of water samples of breeding sites showed a pH of 7.14 – 8.9. Water salinity was 180–392 mg/l.

In Saudi Arabia, the larvae of A. arabiensis were predominant in stream edges and stream bed pools (Shililu et al., 2007 and AlSheikh, 2011) collected A. dthali larvae from valleys in Jazan region from the same habitat of A. arabiensis.

In the dry seasons of western Kenya, burrow pits and pools in stream beds have contributed to a significant increase in A. Arabiensis pupal productivity (Mutuku et al., 2006). In Eritrea, mosquito breeding persists year round in stream bed pools, but significantly decreases with an increase in rainfall (Shililu et al., 2007).

2.9.4 Dispersal

Dispersal refers to a goal-oriented flight of Anopheles mosquitoes from one place to another. In normal atmospheric circumstances, most individuals of the tropical Anopheles mosquitoes apparently fly within a range of 1-3 km, although there are records of a few species or occasional individual mosquitoes flying much further (WHO, 1975b).

Knowledge regarding the dispersal of adult vectors from their breeding sites helps to identify areas where control methods such as LLINs and IRS are better applicable. The movement of mosquitoes is governed by a number of factors, including temperature, humidity, host attractiveness and the attractiveness of breeding sites depending on their physiological conditions. The flight of gravid female Anopheles mosquitoes to breeding places is stimulated by fully developed ovaries and the characteristics of the breeding site, as they disperse in the direction of post emergence or oviposition, resting, feeding, daytime resting and breeding sites (Service, 1997).

The typical active flight of most Anopheles mosquitoes is short and under their control, but some such as A. pharoensis can actively fly long distances (Pener et al.,
The population size of *Anopheles* mosquitoes decreases with an increasing distance from their source of breeding places or release points (Service, 1997) and is non-random, but related primarily to their distribution, number of resting sites and blood meal sources.

In Sri Lanka, a marked number of *A. culicifacies* were caught at 498 m from a release point within a day after marking (Curtis and Rawlings, 1980). Another study showed that the proportion of dispersing *A.gambiae* s.l. declined exponentially with an increasing distance starting from a larval habitat along The River Gambia, and that 90% of their movements were within 1.7 km (Thomas *et al.*, 2013).

Flight distance differs among the *Anopheles* species. In Senegal, (Trape, *et al.*, 1992) observed a significant decrease in the density of indoor-occurring *A. arabiensis* with an increase in distance up to 910 m from a permanent marshy area. Inhabitants close to the marsh experienced a maximum risk of malaria infection than those further away. In Burkina Faso, the mean distance moved by individual *A. arabiensis* and *A. gambiae* s.s. mosquitoes ranged from 350-650 m/day (Costantini *et al.*, 1996) under a condition survival of the mosquitoes was where the daily estimated to be 80–88%. In Korea, 85% of the released *A.sinensis* were recaptured within 6 km from the release point (Cho *et al.*, 2002). Highly localized dispersion activities provide *Anopheles* mosquitoes with a better opportunity for breeding (Costantini *et al.*, 1996).

When a female Anopheles mosquito feeds on humans to nourish its eggs, it may acquire *Plasmodium* gametocytes from a carrier. After several feeding cycles, the mosquito becomes infectious, and on biting a second human host it transmits the parasites. During a single rainy season, proximity to mosquito breeding sites predicted human malaria infection when homesteads were upwind of larval sites, but not when they were downwind of larval sites. This indicates that following oviposition, female Anopheles mosquitoes fly upwind searching for human hosts, and hence increasing the risk of malaria transmission. Because of this, malaria transmission could be disrupted by targeting vector larval sites in close proximity to human dwellings and downwind of malaria hotspots (Midega *et al.*, 2012). In addition, the adult *Anopheles* mosquito's
prevention tools, e.g. LLINs and IRS, can better be applied in the upwind direction in order to minimize the risk of malaria transmission.

Host availability, proximity to breeding site, sugar source, resting site, preferred flight direction and season could affect dispersal of vectors (Midega et al., 2012). In Mali, large population size and migration was observed during the wet season, but with very low numbers and no sign of migration during the dry-season. The study suggested that VC measures could be more efficient in the region and other seasonal riparian habitats by targeting the disruption of mosquito populations by the river during the dry-season. This would decrease the size of an already small population, and would likely delay an explosive growth in vector abundance in inland villages as rainfall increases (Baber et al., 2010).

After emerging from pupa, the female Anopheles mosquito rests for some hours in the vicinity of the breeding site and undertakes mating. It then flies to areas where hosts are available, orientated by the hosts’ stimuli. Males generally tend to be more concentrated in the area of their breeding site and to remain in outdoor shelters, although a good number of males of endophilic species accompany females to their resting places (WHO, 1975b; and Service, 1997).

Passive dispersion occurs when a mosquito is transported by external factors, including cattle, air currents, ships, airplanes, trains and vehicles. The movement of cattle from a breeding place in the evening to remote villages led mosquitoes to longer distances by accompanying cattle (Cho et al., 2002). Wind also causes dispersion over a wide range. For example, *A. pharoensis* in Egypt was found at distances of 56 km and 29 km from the nearest possible breeding places. In contrast, under a condition with a very low wind speed, mosquitoes can detect air-carried, host-specific odours from a distance and orient themselves to the host by flying upwind (WHO, 1975b).
2.9.5 Feeding and resting behavior:

Flight, host-seeking and the feeding activities of *Anopheles* mosquitoes can take place if the relative humidity and temperature are not limiting (Chwatt, 1980). Many female *Anopheles* mosquitoes bite humans to obtain a blood meal, and a few feed on humans in preference to animals (Chwatt, 1980). Mosquitoes are attracted to hosts by various stimuli emanating from their breath or sweat, such as carbon dioxide, lactic acid, octenol, body odors and warmth. Some species feed more or less indiscriminately at any time of the day or night (Service, 1997).

After having their blood meal, mosquitoes seek resting places in which to shelter until their meal is digested and their ovaries are matured. Adults of *A. gambiae* s.l. are primarily indoor-feeding (endophagic) and indoor-resting (endophilic), as opposed to outdoor-feeding (exophagic) and outdoor-resting (exophilic) mosquitoes. Few mosquitoes entirely feed on humans (anthropophagic) or animals (zoophagic), or possibly zoo-anthropophagic feeding on both depending on availability. Feeding occurs between dusk and dawn in species associated with open terrain or sunlit habitat (WHO, 2013b). Even so, the feeding behavior of a species may change over time (Service, 2008, and WHO, 1975b).

The biting behavior of female *Anopheles* mosquitoes is important in the epidemiology of malaria. During the hot and dry seasons, a substantial number of people may sleep outdoors and as a result, be bitten more frequently by exophagic mosquitoes. Some mosquitoes bite predominantly in forests or wooded areas, so people will only get bitten when they visit these places. Thus, the behavior of both people and mosquitoes is relevant in malaria transmission (Service, 2008).

The human blood-feeding activity of female *Anopheles* mosquitoes is responsible for malaria transmission. This activity is part of their intrinsic behaviour, as blood proteins are essential nutrients for egg production, metabolic energy and reproductive fitness (Service, 2008). Blood quality, and hence host type, affects reproductive output, which suggests the host-preference is likely to be more common given the evolutionary association between insect vector and pathogen. According to Takken and Verhulst
(2013), host-preference is defined as the trait to preferentially select certain host species above others. This selective behavior has a great influence on disease transmission. Host-preference according to the same authors, resulting from selective behavior exists not only between different species, but also between populations of the same species, and even within a given population, due to several extrinsic and intrinsic factors (Takken and Verhulst, 2013).

External factors, *e.g.* such as an absence of the preferred host and a reduced response threshold for host-selection owing to low metabolic energy or adverse weather, prevent mosquitoes from venturing far from their local habitat. This may force them to change their feeding and resting preference (Williams, *et al.*, 2010).

The extrinsic determinants of host-preference include odorants (and their production by skin bacteria), carbon dioxide, blood quality/host species, color, body heat, RH, body mass, gender, age, defensive behavior, parasites and climate (Takken and Verhulst, 2013, Smallegange *et al.*, 2010) and the potential suitability of a host. Skin emanations contain host-specific cues that play a role in host-preference. For example, (s)-lactic acid is an excretory product of humans and an important cue in the host-selection process of *A. gambiae* s. s. (Smallegange *et al.*, 2011).

The body mass of a host may affect preference, presumably because a larger host would exude a higher quantity of olfactory cues. A well-known example of this is the production of metabolic CO$_2$, which is positively associated with body size (Torr,*et al.*,2006). Young children are bitten less often by mosquitoes than their parents are, with mosquitoes expressing different degrees of preferences for humans. These preferences are supposed to be associated with differences in odor profiles, which differ between men and women, as well as between people of the same sex (Havlicek, *et al.*, 2005). Lindsay *et al.* (2000) demonstrated that *An. gambiae* s.s. were more attracted to pregnant women than to women who were not pregnant.

The intrinsic factors that determine the host-preference of mosquitoes include physiology, genetics and plasticity (learning, divergence after the implementation of ITNs and IRS and host abundance; Takken and Verhulst, 2013). Soon after emergence from the pupal stage, male and female mosquitoes express a strong behavioral response
to nectar that serves them as a source of the metabolic energy needed for flight and an emotactic behaviors (Foster and Takken, 2004). Following mating, female mosquitoes search for blood. Choice experiments showed a preference of *A. quadriannulatus* and *An. arabiensis* for a cow’s odor, while *A. gambiae* s.s. preferred a human’s volatiles (Pates *et al.*, 2001). Nonetheless, the nutritional state of the insects may overrule the inherent host-preference, because the principal strategy of the insect is to safeguard reproduction, for which animal blood is required. Under such circumstances, the mosquitoes lower their threshold for host-preference, and may feed on a non-preferred host. The age of the mosquito does not affect host-preference, though adaptive learning through a memorized host encounter was shown to affect the choice for a specific host species (Chilaka *et al.*, 2012).

Host-choice depends not only on the innate host-preference of the mosquito species, but also on the tendency of the mosquito to feed indoors or outdoors and the time of feeding. These behavioral characteristics may be driven by selection, and therefore have a genetic background. Studies have confirmed the existence of genetic control for the behavioral differences between the strains, although none of the behavioural preferences was strongly fixed in the population. The anthropophilic behavior of *A. gambiae* s. s. is found to be strongly fixed in a population, but not complete (Takken and Verhulst, 2013). Intervention strategies should not only consider the feeding preferences of vectors but also their peak biting time, which varies between species, populations of the same species and the age of individual mosquitoes. Nulliparous female *A. gambiae* s.l. in Sierra Leone and *A. punctulatus* in Papua New Guinea showed a tendency to bite earlier than the parous ones (Bockarie, *et al.*, 1996). Additionally, *Anopheles* mosquitoes infected with *P. vivax* were observed to bite earlier than those infected with *P. falciparum*. On average, mosquitoes containing *P. vivax* sporozoites are expected to be younger than those infected with *P. falciparum* sporozoites. This is because the duration of *P. vivax* sporogony (7 days) is shorter than that of *P. falciparum* (9 days) at 30 ºC. The early biting tendency of younger parous females than older ones may help
explain the early biting habit of mosquitoes infected with \textit{P. vivax} in comparison to mosquitoes with \textit{P. falciparum} (Bockarie \textit{et al.}, 1996).

IRS is mostly targeted against the indoor-resting malaria vectors. However, these mosquitoes may avoid the impact of IRS by changing their behavior to outdoor feeding and outdoor resting (Russell \textit{et al.}, 2013). \textit{A.sundiacus} Rodenwald1 and \textit{A.albimanus} Wiedemann modified their indoor-biting and indoor-resting behaviours in response to residual house spraying with DDT (Sundararaman, 1958). On Bioko Island in Equatorial Guinea, \textit{A.gambiae} s.s., which was primarily an indoor-feeding and indoor-resting vector, was observed to seek hosts outdoors at least as much as it did indoors (Reddy \textit{et al.}, 2011). In the Temotu Province of the Solomon Islands, \textit{A.farauti} Laveran (1902) showed the tendency of early and outdoor biting following intensive IRS (DDT and lambda–cyhalothrin spray) and LLIN use (Bugoro \textit{et al.}, 2011). In southern Zambia, a doubling in the amount of rainfall in the 2005 – 2006 rainy-season resulted in a 10-fold increase in the number of \textit{A. arabiensis} resting inside human sleeping quarters each night (Kent \textit{et al.}, 2007).

The introduction of ITNs brought behavioral changes such as shifts toward outdoor and/or earlier biting. Like other aspects of its behavior, the nightly biting activity of \textit{A.arabiensis} varies dramatically across Africa, as peak biting after midnight has been observed in Senegal, Chad and Kenya (Lemasson \textit{et al.}, 1997, and Kerah-Hinzoumbe \textit{et al.}, 2009). However, in Mozambique, Tanzania and Ethiopia, biting was observed as early as 9 pm (Geissbuhler \textit{et al.}, 2007, and Yohannes and Boele, 2012). In southern Zambia, \textit{A.arabiensis} biting was observed throughout the night, with peak activity starting before midnight at approx. 10 pm. Although most persons have gone to bed by this hour, roughly 14% of the \textit{A.arabiensis} biting occurred prior to this time when residents were finishing dinner and preparing for bed and were not protected by ITNs. In the area, despite ITN use \textit{A.arabiensis} remained highly anthropophilic, and also appeared to be relatively exophagic, biting outdoors immediately after sunset and before sunrise, thereby circumventing the protective effect of ITNs (Fornadel \textit{et al.}, 2010).
A. dthali found mainly outdoors in animal houses, as well as human dwelling. Manouchehri and Rohani (1975) found that this species is more prevalent in animal shelters, tent and human dwellings. This species rests in water reservoir, warehouse, septum of well, cave and natural shelters e.g. holes in riverbanks. The adults are very sensitive to light and often disturbed by torchlight. Gillies and De Meillon (1968) reported that in southern Arabia, Mauritania and Somalia the adults are not uncommon indoors. Precipitin tests of this species showed that 12.5% were positive for human blood (Edrissian et al., 1985). Another precipitin tests on females caught in houses from the north Bandar Abbas (Iran) showed 20.8% positive for human blood. The results of precipitin tests from this species caught in houses from mountainous area of Bandar Abbas showed that 20% were positive for human blood (Manouchehri et al., 1972). Precipitin tests on specimens from Morocco and KSA showed that 4 - 18.7% were positive for human blood (Bruce-Chwatt et al., 1966).

2.10 Entomological Parameters:

2.10.1 Entomological inoculation rate (EIR):

Humans become infected with malaria as a result of their exposure to blood-feeding infective Anopheles mosquitoes. Sporozoite-stage parasites inoculated by even a single infectious mosquito can cause human malaria infection and life-threatening disease. To estimate the intensity of malaria parasite transmission under field conditions, it is standard practice to determine the EIR, the product of the mosquito biting-rate times the proportion of mosquitoes carrying sporozoites in their salivary glands (Macdonald, 1957).

The EIRs are expressed in terms of average numbers of infective bites/person/unit time. Unlike most other parts of the world where it is difficult to determine EIRs because of exceedingly low sporozoite rates, many valuable studies of transmission have been conducted in Africa where sporozoite rates generally range from 1 to 20% (Wirtz and Burko, 1991). The EIRs in endemic areas of Africa range from 1 to 1,000 infective bites/year (Trape and Rogier1996).

Based on the analysis, the relationship between EIR and malaria prevalence in Africa is such that any detectable EIR is associated with prevalence rates of
*P.falciparum* malaria large enough to seriously impact public health. It is obvious that high prevalence rates can even be seen with extremely low or non-detectable EIRs. For example, on the Kenyan coast, one site with an EIR of 0.001 and another where no infected mosquitoes could be found had *P.falciparum* prevalence rates of 44.7% and 49.3%, respectively (Kabiru, 1994, and Mbogo *et al.*, 1995).

Effective VC measures decrease the incidence of malaria infections because there is a linear relationship between EIRs and malaria incidence. In fact, studies in Saradidi in western Kenya showed that 74% of the variation in *P.falciparum* incidence is explained by EIRs (Beier *et al.*, 1994). Many studies documented the basic relationship between EIRs and malaria prevalence in Africa. One of the most important points is that unacceptably high levels of *P.falciparum* prevalence exist at very low levels of transmission by local vector populations. Even at the lowest EIRs, there are high rates of malaria prevalence and associated severe diseases (Trape and Rogier, 1996; Mbogo *et al.*, 1993, 1995 and Trape., 1987).

The substantial reductions in malaria prevalence are only likely to be achieved when EIRs are reduced to levels <1 infective bite/person/yr (Beier *et al.*, 1999). The EIR measures the intensity of malaria transmission in a particular area (Kelly-Hope and McKenzie, 2009). Estimation of the EIR is important for quantifying the potential level of human exposure to infected mosquitoes and for assessing the impact of interventions on malaria transmission in a given area (Shaukat *et al.*, 2010, and Ulrich *et al.*, 2013).

Many studies have reported huge variations in malaria transmission intensity in Africa (Hay *et al.*, 2000), not only between urban and rural settings but even within the same locality (Omumbo *et al.*, 2005). It has been reported that the EIR varies between 0.01 and 1000 infectious bites/person/yr (ib/p/year) in malaria-endemic countries in Africa (Trape and Rogier, 1996).

The human landing catch (HLC) has been the most widely accepted method for estimating the human-biting rate (WHO, 1975b) because it measures actual human vector contact, but it has obvious ethical and technical problems (Lines *et al.*, 1991). Other methods such as the Centers for Disease Control (CDC) light trap have been
evaluated to replace the HLC by calibrating and determining an index equivalent to the human biting rate (HBR).

It is believed that CDC light traps when set near sleeping persons protected by nets capture the anthropophilic mosquito species and thus can provide an indirect estimate of the HBR (Lines, et al., 1991).

Some investigators have also used pyrethrum spray catches (PSCs) to determine the HBR, although this method might underestimate the human–vector contact and consequently the intensity of malaria transmission because many indoor-fed mosquitoes will leave houses before and during spraying (Garrett et al., 1980).

The annual *P.falciparum* and *P.vivax* EIR of *An. arabiensis* was calculated from CDC light traps by using the standard method, 1.605 X (no. circumsporozoite-positive ELISA results from CDC light trap / no. mosquitoes tested) X (no. mosquitoes collected from CDC light trap/no. catches) X 365 days, and the alternative method, 1.605 (no. positive ELISA/no. catches) X 365 days (Drakeley et al., 2003). The monthly EIR was determined as a product of the EIRs for each day of the month. The EIR was also estimated from the PSC as described by the World Health Organization WHO (2003) by using the formula: (human biting rate) X (CSP rate).

The HBR was calculated by dividing the total number of freshly fed *A.arabiensis* caught by PSC by the total number of occupants who slept in the houses the night before collection and multiplied by the HBI. The HBI was calculated as the proportion of *Anopheles* mosquitoes that fed on humans (including mixed blood meal origins) of the total *Anopheles* analyzed for blood meal origin (Garrett, 1964).

**2.10.2. Methods of age determination in female *Anopheles* mosquitoes**

One of the entomological determinants in the transmission of malaria in an area is the age status of individual females in the population of the vector. Older females are responsible for much of malaria transmission, as age also indicates the efficacy level of VC interventions in an area (Warrell and Gilles, 2002).

Most current control programmes aim at shifting the age-structure of the vector population towards a younger age since the young vectors are incapable of transmitting

Mosquito age-grading methods include morphological changes in the skeletal apodemes, ovarian dissection, cuticular hydrocarbon, transcriptional profiling and pteridine fluorescence (Wang et al., 2013 and Wu and Lehane., 1999). The growth of the layers of the skeletal apodemes of the Anopheles can be observed on a daily basis. This provides the actual calendar age, and is a better estimate than the physiological age determination. The length of the thoracic apodemes is directly related to the size of the thoracic muscles. Therefore, the length of the apodemes reflects the amount of growth of the thoracic muscles and hence the calendar age of anophelines up to the age of 13 calendar days (WHO, 1975b and Schlein and Gratz, 1973).

The physiological age of female mosquitoes can be determined by counting the number of dialations or follicular relics in the ovary as either 0-parous (nulliparous) or 1-parous, 2-parous, 3-parous, etc… based on the number of ovipositions. Yet, it is technically difficult and labourious, and may be of limited value as the proportion of diagnostic ovarioles decline with age (Detinova and Gillies, 1964, and Detinova et al., 1962). The tracheation method distinguishes between nulliparous (tightly coiled tracheols) and parous (stretched tracheols) (Ungureanu, 1974), which is relatively faster and easier to use in the field. The pteridine fluorescence method is unreliable due to the difference in the concentration of the pteridine with respect to the mosquito’s physiological condition (Cook et al., 2006, and Wu and Lehane., 1999).

Transcriptional profiling, a method of age-grading based on genes that display an age-dependent expression in mosquitoes, was found to determine the chronological age of mosquitoes under field conditions. It can determine the age of adult mosquitoes to a much higher degree of accuracy and precision than the previous methods used (Cook et al., 2006). This method was found to be consistent with the ovarian dissection method and also valuable for the determination of the age of A.gambiae mosquitoes in two malaria endemic areas in western Kenya. It may therefore be useful for the determination of the age, vectorial capacity and survivorship of a population of a vector where VC interventions are ongoing (Wang et al., 2013).
2.10.3. Methods of host-preference in *Anopheles* mosquitoes

The identification of the blood meal source of freshly fed female mosquitoes remains important to understand their host-preference and vectorial role. Techniques such as the precipitin test, the enzyme-linked immunosorbent assay (ELISA) and the polymerase chain reactions (PCR) have been used in mosquito blood meal identifications (WHO, 1975b, Service, 1986, Kent and Norris, 2005 and Beier *et al*., 1988).

The precipitin test has been the most commonly used serological technique to identify the blood meal source of mosquitoes. However, the test is neither sensitive nor specific, which results in the underreporting of feeding habits of arthropods that take small blood meals. The test also demonstrates multiple blood meals, indicating a lack of specificity. As a result, other serological methods have been adapted for mosquito blood meal source identification, among which the ELISA is most preferred (Tempelis, 1975, and Washino and Tempelis,1983).

The advantages of the ELISA technique over the precipitin test are that blood meals can be rapidly identified in microtiter plates, the test results are more objective and the sensitivity is very high. The ELISA can be quantified and automated, and the automated equipment is relatively cheap, compact and easy to operate (Beier *et al*.,1988, and Washino and Tempelis,1983). A single mosquito can be tested, both by the blood meal ELISA and the malaria sporozoite ELISA, for host-preference and infection, respectively. An experienced technician can more easily diagnose the blood meal sources of larger number of mosquitoes with accuracy when using ELISA than precipitin (Beier, *et al*., 1988).

Although the ELISA method has been used to determine the blood meal sources of *Anopheles* mosquitoes, it still has its own limitations, including the difficulties of obtaining specific antisera against a broad diversity of host species (Martínez-de,*et al*., 2013). The PCR-based technique overcomes the limitations of the serological tests in identifying mosquito blood meal sources, especially for laboratories using DNA-based techniques. In this technique, individual DNA extracts serve multiple purposes, such as species confirmation, the determination of blood meal sources, the infection status for
various pathogens, insecticide resistance mechanisms and vector population genetic studies. Fed mosquitoes can also be preserved dry, stored for long periods of time and tested at facilities physically distant from the point of collection (Kent and Norris, 2005). Human-specific genetic markers within fresh fed anophelines may allow for identification of the individual human host (Gokool et al., 1993). A multiplexed PCR targeting cytochrome b was found to identify the mammalian blood host in engorged vector mosquitoes two–seven months after collection in Zambia. The host DNA was detectable in frozen mosquito abdomens 24–30 hrs post-feeding. This test is advantageous, as multiple blood hosts can be directly identified by size-specific fragments (Kent and Norris, 2005).

The proportion of *Anopheles* giving a positive reaction for human blood is the result of the human blood index (HBI), which is a valuable guide to the potential importance of an *Anopheles* mosquito species as a malaria vector. Sampling bias must be taken into consideration in interpreting the results as, e.g. it is to be expected that a high proportion of adults caught from houses will have fed on humans, and most of those caught from cattle sheds will have fed on cattle (Warrell and Gilles, 2002).

### 2.10.4. The sporozoite rate (SR) and its detection methods

The sporozoite rate is the proportion of vectors that carry *Plasmodium* sporozoites in their salivary glands. The sporozoite infection (SI) status of anophelines can be detected using methods including dissecting salivary glands, ELISA and PCR. The SI status of *Anopheles* mosquitoes has been detected by dissecting and observing salivary glands using a microscope (WHO, 1975b, and Coleman et al., 2000). Although the microscopic evaluation of dissected salivary glands is the gold standard for the determination of mosquito infection, it is labour intensive, requires a trained and experienced technician and may not differentiate among the *Plasmodium* species (Coleman et al., 2000, and Bass et al., 2008).

Microscopical examination often fails to differentiated oocysts and sporozoites of human *Plasmodium* parasites because they are morphologically similar. Thus, it is primarily replaced by the circumsporozoite protein enzyme-linked immunosorbent assay (CSP ELISA) (Burkot et al., 1984a, and Burkot et al., 1984b). This test is species-
specific and can detect *P. falciparum, P. vivax, P. ovale, P. malariae* or mixed species (Burkot et al., 1984a; Wirtz et al., 1985, and Beier et al., 1991), and also serves in testing single and pooled mosquito specimens. Testing pooled specimens is a highly efficient and economically cheap method for determining sporozoite rates, particularly when vector infection rates are low (Gu, 1995). A CSP ELISA may overestimate the true salivary gland infection rate as a result of the spread of the circumsporozoite protein throughout the mosquito after being shed (Bass et al., 2008, and Beier and Koros, 1991) or detecting the CSP from the oocysts bursting, which occurs two to three days before the sporozoites actually reach the salivary glands (Fontenille et al., 2001). The CSP ELISA may be less sensitive than the microscopic examination of dissected salivary glands for detecting low-level sporozoite infections in the *Anopheles* mosquitoes (Beier et al., 1991). The long-term storage of mosquitoes in chemicals, such as in ethanol or isopropanol for later analysis, makes the specimens unsuitable for ELISA testing. A mosquito must be separately subjected to four assays each for one *Plasmodium* species that causes human malaria, but in practice many studies only test for the presence of one or two of the species. Additionally, neither microscopy nor ELISA assays allow for the detection of genetic diversity in the *Plasmodium* sporozoites. Furthermore, CSP ELISA overestimates the mosquito infectivity rate by detecting the circulating sporozoites, even in non-infective mosquitoes (Bass et al., 2008; Beier et al., 1991; Beier and Koros, 1991, and Marie, *et al.*, 2013).

The limitations of the microscopy and the ELISA-based detection of *Plasmodium* parasites in the thorax and salivary glands of vector mosquitoes led to the adoption of molecular tools, including the multiplex PCR, Real-time PCR and duplex Real-time PCR. These methods helped to improve the sporozoite detection rate of all four *Plasmodium* species much better than the ELISA and microscopy, but still have their own limitations (Bass *et al.*, 2008, and Marie, *et al.*, 2013). The Real-time quantitative PCR assay is probably highly-sensitive and more specific than multiplex and duplex PCRs in detecting *P. falciparum* in the salivary glands of vectors (Marie *et al.*, 2013).
The proportion of *Anopheles* mosquitoes positive for *Plasmodium* sporozoites is the SR, which is a valuable guide to incriminate mosquitoes as malaria vectors and to describe the epidemiology of the disease. Sporozoite detection is also useful for defining the season of transmission and in evaluating the effect of mass drug administration (WHO 1975b). The presence of oocysts in a mosquito indicates that the mosquito is a potential vector, but not that it is infective. When sporozoites are found in the salivary glands, the mosquito is assumed to be capable of transmitting malaria. There may be considerable seasonal variations in SR, reflecting, in part, changes in adult survival rates of the mosquitoes (Warrell and Gilles, 2002).

### 2.10.5. Living conditions and exposure to infectious *Anopheles* bites

In Africa, the primary malaria vectors are nocturnal, endophilic or exophilic and endophagic or exophagic. They mainly transmit the disease at home while interacting with humans to imbibe blood. However, all homes are not equally accessible for the mosquitoes (Howell and Chadee, 2007). Malaria transmission is heterogeneous in that it varies among villages, households and individuals due to factors, including altitude, vector distribution and abundance, household distance from nearby mosquito breeding site, house construction, household crowding and personal protection methods employed against mosquito bites (Trape *et al.*, 1992; Gamage-Mendis *et al.*, 1991; Ghebreyesus *et al.*, 1999, and Ghebreyesus *et al.*, 2000).

During an epidemic season in a highland area of western Kenya, malaria transmission was found to be clustered in low-altitude areas due to a relatively high temperature, which affects the development and survival of the vector and also the development of *Plasmodium* parasites within the vector (Brooker *et al.*, 2004). An increase in the number of households in an area increases the number of human-made breeding habitats, consisting mainly of broken pipes, roadside ditches and potholes, and temporary pools of water along unpaved roads and paths within and around family compounds, drainage or abandoned swimming pools, tire tracks and shallow garden wells, which expose inhabitants to an increased risk of malaria infection (Keating *et al.*, 2003). In a dry area of Kenya (Baringo), the odds of *A. arabiensis* occurrence increased with a decreasing distance to the animal shelter and the nearest larval habitat and an
increasing number of houses, sleepers and the size of eaves. *An. arabiensis* was also more likely to be encountered in grass-thatched- than in metal-roofed houses and in the absence rather than the presence of animals (Mala *et al.*, 2011). Human activities also increased human-vector contact. In the study, lightly-dressed residents who stayed out late in the evening to irrigate their farms exposed themselves to mosquito bites. In Sri Lanka, the risk of getting malaria was greater for inhabitants of poorly constructed houses compared to complete brick, plaster walled and tiled-roof houses. Such houses might be a preferred resting place as they offer dark and cool microenvironments, such as crevices in mud walls and thatched roofs (Gamage-Mendis *et al.*, 1991).

In a north-central dry zone of Sri Lanka, Konradsen *et al.* (2003) found that houses closer than 750 m to a breeding stream had a 4.7-fold higher risk of harbouring *A. culicifacies* and a 1.5-fold higher risk of harbouring *A. subpictus* than houses at least 750 m away. Rooms where more than two people slept the night before PSC had increased risk of having *A. culicifacies*.

On the south bank of the River Gambia, some 180 km inland, Lindsay *et al.* (1995) found that children’s exposure to *A. gambiae* s.l. bite increased in houses adjacent to mosquito breeding sites, with open eaves and sleeping in a room without a ceiling in the wet-season. In the dry-season, the group found an increased number of *A. gambiae* s.l. related to living next to a mosquito breeding site, living in an unfenced compound, sleeping in a room without a ceiling and using no insecticide aerosol (Njie *et al.*, 2009). In Guinea Bissau, significantly greater numbers of indoor-resting *Anopheles* mosquitoes were present in rooms with open eaves, in houses with a well on the compound and in houses where pigs were present. In addition, an abundance of female *Anopheles* mosquitoes increased with increasing human biomass / m$^2$ of bedroom area (Palsson *et al.*, 2004).

The mean malaria incidence in Adama, a city located 100 km southeast of Addis Ababa Ethiopia, among children below the age of 18 in houses at a distance of 150 m from a nearby breeding site was 1,374/1,000 populations, compared with 373/1,000 population residing at 350 m. Household level factors significantly associated with
malaria were age, distance from vector breeding site and the number of adults with indoor jobs (Peterson et al., 2009).

In northern Ethiopia, Ghebreyesus et al. (2000) found that the use of irrigated land, animals sleeping inside houses, open eaves, no separate kitchen and one sleeping room were all significantly associated with malaria infection. In this area, children living closer to a dam had a seven-fold increase in the risk of malaria infection compared to those living farther away from dams. In Eritrea, houses having a mud wall were positively associated with malaria infection (Sintasath et al., 2005). In a holoendemic area of North West Burkina Faso, the prevalence of *P. falciparum* infection among inhabitants of iron sheet-roofed houses (12.7%) was two times less than the prevalence among residents of mud-roofed houses (25.6%) (Ye et al., 2006). In a highland area of western Kenya, livings close to swamp and forest and at a lower elevation were associated with greater risk of malaria infection (Ernst et al., 2006). An increase in malaria risk was also associated with a low education level of female household heads, overnight travel, living near a channeled swamp and keeping livestock near a residential house at night. Living in a house with a metal roof, no ceilings or a separate kitchen was also related to higher risk of infection (Ernst et al., 2009). In addition to the overall variation in vector abundance, biting density is influenced by factors such as local climate, topography, house design, and house proximity to mosquito breeding site, host availability, personal protection methods and mosquito avoidance behaviour (Lindsay et al., 1995).

Improved housing generally protects the entry of indoor-feeding and indoor-resting mosquitoes that transmit malaria, filariasis and arboviruses (Ogoma et al., 2010). Scant consideration has been given to house design and construction as an environmental strategy in controlling malaria. House screening reduced the indoor densities of *An. gambiae* s.l. as well as *Mansonia* species both of which are vectors of several tropical diseases in rural areas of Africa and some parts of Asia. As a control tool against house-entering mosquitoes, blocking eaves and screening houses may help reduce nuisance mosquitoes and thus encourage the uptake of control interventions that
which rely on acceptance, participation and even investment by the community (Ogoma et al., 2010).

The mosquito proofing of a house therefore offers the advantage of equitably protecting all the members of the household, including those who are not sleeping under a bed net (Ogoma et al., 2009). House proofing may be used for protecting households against mosquito bites, and hence for a community-level suppression of malaria transmission. In Tanzania, an increased coverage of screens and ceilings was associated with a decline in malaria prevalence between April 2004 and March 2007 (Ogoma et al., 2010).

2.11. Malaria vector control

Mosquito control activities are mostly employed at the local level depending on the season, environmental conditions, biology and behaviour of both mosquitoes and humans. It is therefore important to have a basic knowledge of the bionomics of the mosquitoes. The basic knowledge of their bionomics includes the development of immature stages (egg, larva, and pupa) and the life of the adults under the influence of the local environment, since VC is directed against the larval and adult stages (WHO, 1975b).

2.11.1. Larval habitat management (LHM)

Since the discovery of Anopheles mosquitoes as vectors of malaria, LHM has been used in reducing and eliminating malaria transmission (Walker and Lynch, 2007). LHM refers to the planning, organizing, carrying out and monitoring of activities for the modification and/or manipulation of mosquito breeding habitats or their interaction with man, with a view towards preventing or minimizing vector propagation, thereby reducing man-vector-pathogen contact. LHM primarily includes habitat modification (Hm), habitat manipulation (HM), larviciding and biological control (BC) (WHO, 2013b).

2.11.1.1. Habitat modification (Hm)

Habitat modification is a form of environmental management consisting of a physical transformation that is permanent or long-lasting of land, water and vegetation,
which is aimed at preventing, eliminating or reducing the habitats of vectors without causing unduly adverse effects on the quality of the human environment (WHO, 2013b). It was also the major VC method before the advent of pesticides (WHO, 2013b), causing a physical change to mosquito breeding areas and help in preventing, eliminating or reducing vector density (VD). It includes drainage, filling, land levelling and transformation and impoundment margins. The work of Mm is usually permanent in nature; however, proper design and adequate maintenance are essential for their effectiveness. Draining includes creating ditches or drains to keep water moving and to carry water used as mosquito breeding sites in a managed way. Drains may be lined or unlined and located at the surface or on the subsoil level (Walker and Lynch, 2007),
involves techniques for drainage and surface water management that depend on the local topography (WHO, 2013b).

Habitat modification also involves the elimination of wetlands, thereby creating channels to increase water flow in areas of standing water, filling small ponds or water collecting depressions, or changing banks of water impoundments to help reduce mosquito populations. As slow-moving rivers and streams create larval breeding habitats for certain vector species, straightening their banks reduces vector populations. Modification can also involve human-made vector breeding habitats associated with water-holding structures in mini-dams and small-scale irrigation projects. The creation of a larval habitat can be avoided through careful design and collaborations with other sectors such as agriculture and construction (WHO, 2013b).

2.11.1.2. Habitat manipulation (HM)

Habitat manipulation refers to producing temporary conditions that are unfavourable to the breeding of vectors in their habitats (WHO, 2013b, and WHO, 1982), it refers to activities that reduce vector abundance through a temporary change of aquatic environments. HM must be repeated to remain efficacious and is primarily directed at a specific vector species (WHO, 2013b). Water salinity change, stream flushing, the regulation of water levels in reservoirs, the dewatering or flooding of swamps or boggy areas, vegetation removal and management, shading and exposure to sunlight, and intermittent irrigation to agricultural fields, are all examples of the activities. This is appropriate where permanent habitat modification is not feasible or in areas of irrigated agriculture (Walker and Lynch, 2007, and WHO, 1982).

With proper planning, design and maintenance, LHM can reduce or eliminate mosquitoes. LHM offers a number of advantages over other VC methods, including long-term effects, low-cost, mutual benefit for agriculture and health, only a slight environmental impact, a low level of exposure to chemicals, prevention and the control of other vector-borne and water associated diseases. Nonetheless, habitat management should be preceded by in-depth ecological studies to help avoid undesired environmental change (WHO, 1982).

In recent years, there has been a renewed interest in VC using LHM alone or by employing LHM as a supplement to existing strategies (WHO, 2013b, and Fillinger, et al., 2003). Larval control played a major role in the eradication of \textit{A. gambiae} from northeast Brazil in the 1930s and early 1940s (Killeen \textit{et al.}, 2002), and
suppressed malaria transmission significantly in Zambia and Tanzania (Bang et al., 1977). Larval habitat modification was important for malaria eradication in the USA, Israel, and Italy. Effective larval control measures depend on locally derived ecologic concepts that can be adapted to each vector species and applied continuously without any time limit. An. gambiae and An. arabiensis occur in diverse types of habitats (Minakawa et al., 1999 and 2012), thus creating difficulty for environmental management. The lack of basic sanitary installations, proper use and maintenance can produce several breeding habitats that may go undetected and escape environmental management. In developing countries, high population growth is associated with unreliable services, frequent breakdowns and leakages of water supply. As a result, people store water in their houses, underground cisterns, roof tanks, water jars and other vessels, most of which are usually left uncovered and then become suitable habitats for vectors, such as A. dthali, A. stephensi, A. claviger and A. varuna. Such habitats can be managed by covers such as a plastic floating mesh screen (WHO, 1982).

2.11.1.3. Larviciding
Larviciding can be achieved through treating breeding habitats with chemical or biological agents, which is feasible and effective when habitats are relatively few in number and are easily accessible (Walker and Lynch, 2007). Chemical and biological larvicides were important to malaria control programmes in the early 20th century, and played the primary role in the eradication of A. gambiae s.l. from rural Brazil in the 1930s (Walker and Lynch, 2007, Killeen et al., 2002).

Chemicals used as larvicides include petroleum oils, Paris Green (copper acetato-arsenite), monolayer surface films, DDT, organophosphate (OPs), e.g. temephos, synthetic pyrethroids and insect growth regulators (IGRs). Their efficacy depends on factors including formulation, water quality, habitat size and speed, and susceptibility of the target larvae (Walker and Lynch, 2007). Although chemicals are effective in reducing mosquitoes (Walker and Lynch, 2007; Killeen et al., 2002, and WHO, 2006a), part of them exhibit mammalian toxicity, a high persistence and non-target effects, a lower effectiveness as an adulticide through a selective pressure for resistance, and a high toxicity to aquatic non-target organisms (WHO, 2006a). Larvicides are used on breeding sites that cannot be drained or filled, and where the use of larvivorous fish is expensive or impossible (Rozendaal, 1997).
Larvicidal oils kill larvae when they rise to the surface to breathe, either by suffocation or by poisoning with toxic vapour (Chwatt, 1980). Different grades of oil may be suitable for larval mosquito control, depending on local conditions. At higher temperatures thicker oil is required, e.g. crude or fuel oil, while in the presence of vegetation lighter oil with a greater spreading power, e.g. kerosene or diesel oil, is necessary (Chwatt, 1980). Oils kill larvae quickly, but only last between a few hours and several days. Because of their relatively high-cost and limited persistence, their use for mosquito control has decreased. They can be used in situations where mosquitoes are resistant to insecticides and in small-scale applications by individual households or communities (Rozendaal, 1997).

In the 1940s, the organochlorine (OC) insecticides were adopted for the spraying of breeding sites, but were resisted in the 1950s. They persist in soil and in the tissues of plants and animals. The OPs, the carbamates and the pyrethroids are less persistent, breaking down quickly in the environment, and are recommended as larvicides. However, the pyrethroids are very toxic to fish and crustaceans. Temephos (Abate®) is the current insecticide of choice for larviciding because of its reduced persistence and relative safety for non-target organisms (Rozendaal, 1997).

2.11.1.4. Biological control (BC)

Biological control is the introduction of the natural enemies of larvae, including predatory fish, predatory invertebrates, bacteria, fungi and viruses, into their habitats (WHO, 2013b). Such a method can be considered as an alternative in areas where mosquito larvae develop a resistance to insecticides, and adults are exophilic and exophagic (Rozendaal, 1997). However, an effective use of the biological methods requires a good knowledge of the bionomics of the vectors and the local ecological conditions. The method can be most effective when used in combination with the others (Walker and Lynch, 2007 and Rozendaal, 1997).

Several viruses have been studied in mosquitoes, but have shown little practical applicability. The bacteria *Bacillus thuringiensis* H-14 and *B. sphaericus* form spores that produce toxins, which poison the gut of mosquito larvae when ingested. They tend to be more specific in terms of which mosquito species they can control and what habitats. Their short persistence of activity often requires repeated applications, which increases costs and logistical complications (Walker and Lynch, 2007. and WHO, 2006a). Field trials of *B. thuringiensis var. israelensis* (Bti) and *B.
shape to control *A. gambiae* s.l. larvae exhibited a good control, but a short duration of efficacy (Fillinger *et al.*, 2003 and Nicolas, *et al.*, 1987). In addition, several genera of fungi, nematodes of the family Mermithidae, predatory mosquitoes of the genus *Toxorhynchites*, dragonflies, small crustaceans and *Azolla*, a freefloating fern that can completely cover water surfaces and prevent breeding by mosquitoes, showed a strong biological activity against *Anopheles* larvae (WHO, 2006a, and Rozendaal, 1997). In comparison to chemical control, biological agents can be effective at relatively low-doses, are safe to humans and non-target wildlife, have a low toxicity and a simple application procedures, are low-cost and have a lower-risk of resistant development (Walker and Lynch, 2007, and WHO, 2006a). However, of all the BC interventions, the use of larvivorous fish has been most successful in different areas of the world (Warrell and Gilles, 2002, and Rozendaal, 1997).

The most successfully used fish species are *Gambusia affinis* and *Poecilia reticulate*, of which the first is efficient in clean water, while the second can be used in organically polluted water (Castleberry and Cech, 1990). The annual killifishes, *Cynolebias*, *Nothobranchius* and *Aphyosemion*, have dry-resistant eggs that make them useful in mosquito breeding habitats that temporarily dry out (Rozendaal, 1997).

Exotic fish species should be evaluated for their suitability to the local vector species and ecology. Imported fish species may cause unwanted side-effects in the natural habitats by replacing local species or affecting other aquatic animals. The practice of importing *G. affinis*, a freshwater species native to the southeastern USA, has been discouraged as the efficacy is highly variable and negative impacts of this voracious and aggressive fish on native fauna have been quite significant (WHO, 1982). The introduction of *Gambusia* has actually led to the elimination of native fish from certain habitats (Rupp, 1996). However, it can be freely used in man-made breeding habitats such as water tanks and cisterns for the storage of drinking water, swimming pools, garden ponds and water reservoirs in desert locations without a risk of escaping into the natural environment (Warrell and Gilles, 2002, and Rozendaal, 1997).
2.11.2. Adult control
2.11.2.1. Indoor residual spraying (IRS)

IRS is the application of chemical insecticides on the walls and roofs of houses and domestic animal shelters, with the purpose of killing the adult vector mosquitoes resting on these surfaces. IRS reduces the lifespan and density of the vector. In some cases, the insecticides repel mosquitoes, thereby reducing human-vector contact in sprayed rooms (WHO, 2006a).

IRS saved hundreds of millions of lives between the 1940s and the 1980s in Europe, Asia and the Americas (WHO, 2013c), as well as contributing to malaria eradication from Europe, the former USSR and several countries in Asia and the Caribbean (WHO, 2006b). IRS is effective when properly applied, but requires capacity, structures and systems (WHO, 2013c).

In Africa, malaria eradication pilot projects, initiated from the 1950s to the 1970s, demonstrated a significant reduction of malaria and the vectors following the application of IRS. Subsequent evidence over several decades has confirmed the effectiveness of IRS in reducing the level of infection and incidence of malaria, but was not fully implemented in large parts of SSA (WHO, 2006b). The scaled-up implementation of IRS, together with LLINs and case treatment, brought a remarkable decline in the malaria burden during the last decade in Africa (WHO, 2013a).

The consistent application of IRS has altered the vectors and epidemiological pattern of malaria in Botswana, Namibia, South Africa, Swaziland and Zimbabwe, as *A. funestus* has been eliminated or reduced to negligible levels. *A. gambiae s. s.* and *A. arabiensis* are also well-controlled in some areas of Africa.

The IRS requires detailed and rigorous planning, management and supervision, and the strategy in IRS management and its implementation has been changed in recent years. The changes are associated with universal LLIN coverage, insecticide resistance management and the reorientation of many national malaria control programmes towards an integrated vector management (IVM) approach. Effective IRS operations require an adequate socio-political commitment, a health system capable of delivering good-quality implementation, information on local vectors, indoor versus outdoor feeding and resting behaviours and sustainable financial, logistical and human resources. Twelve insecticides that belong to the four
chemical classes (OCs, OPs, carbamates and pyrethroids) have been used in IRS. Nonetheless, insecticide resistance has been reported to most of these chemicals in the African malaria vectors (WHO, 2013c).

2.11.2.2. Space sprays:

The residual insecticides are usually applied to specific habitats where the adult vectors may come to rest in their normal life inside the houses. Such applications may take several days or weeks before they become effective, and it may take considerable time before an area can be completely treated using this method. A quick action formulation is thus required, which permits the coverage of a large area within a short period. Space spray is ideal for such a purpose. This approach can be especially considered when the vector is *exophilic* and/or *exophagic*, when introdomiciliary residual spraying is not sufficiently effective or when dense populations in urban areas are involved, where residual insecticides or larvicidal applications are operationally difficult and costly. Two forms of space sprays are generally utilized, namely thermal fogs and Ultra–Low–Volume (UL) aerosols or mists (Wernsdorfer and Gerger 1988).

2.11.2.3. Insecticide treated nets (ITNs)

The use of mosquito nets as physical barriers against mosquitoes, flies and other arthropods has been practiced from early times. During the past 30 yr, the protective effect of the nets against mosquitoes has been enhanced by treating them with insecticides. The insecticides have killing and excito-repellent effects against mosquitoes that add a chemical barrier to the physical one, thus further reducing human-vector contact. Sleeping under ITNs protects humans from the night-biting malaria vectors, hence reducing transmission of the disease (Atieli et al., 2011). When properly made and used, they prevent disease-transmitting and annoying mosquitoes. In areas where most people sleep under ITNs, large numbers of mosquitoes are killed and do not survive long enough to transmit the disease (WHO, 2006a). High ITN coverage provides community-level malaria protection through prevention for both users and non-users (Hawley et al., 2003). ITN use by the majority of entire populations could protect all children, even those not actually covered by achieving existing personal protection targets (Killeen et al., 2007). Even so, the day-biting habits of some mosquito species, inadequate maintenance/use of the nets, a simple
lack of care, and resistance of the vector to insecticides in the net fabrics, ecology and the population genetics of the vectors can all help reduce their value (WHO, 1982).

ITNs are considered to be effective in all types of malarious areas where mosquito biting patterns coincide with the time when most people are likely to be sleeping under a net (WHO, 2006a). The use of the LLIN (Long lasting impregnated net), a factory-treated mosquito net that is expected to retain its biological activity for at least 20 WHO standard washes under laboratory conditions and three yr of recommended use under field conditions, has been distributed over the last 10 yr. It avoids the need for visits by a re-treatment team and re-treatment by the owners (WHO, 2006a).

In Africa, this tool is effective against A.gambiae s.l. and A. Funestus, which prefer to bite at night when people are in bed. The species are efficient malaria vectors, because of their anthropophagic, endophagic and endophilic characteristics, their longevity and their abundance. LLINs reduce the vector populations by mass killing, leading to a significant reduction in the lifespan, reducing human contact and the malaria sporozoite rate of Anopheles mosquitoes, and the use of excito-repellent insecticides that cause mosquitoes to leave rooms for outdoors (Sougoufara et al., 2014).

The insecticides commonly used for net impregnation are the pyrethroids, in particular permethrin, deltamethrin and lamdacyhalothrin, because of their low toxicity hazard and good residual effect. However, pyrethroid resistance is reported in most malaria vectors (WHO, 2012a). Moreover, a low level of ITN usage (Animut et al., 2014 and Gobena et al., 2012), the increased outdoor feeding tendency of vectors following ITN use (Russell et al., 2011), the feeding behaviour of mosquitoes as a result of ITN use immediately after sunset and before sunrise (Fornadel et al., 2010) and a low level of ownership and the misuse of ITNs (Sena et al., 2013) are among the major problems in using ITNs as a malaria VC strategy.

2.11.2.4. Improved housing

Houses located away from nearby breeding habitats experience a low density of indoor-biting malaria vectors to its occupants. The maximum active flight range of most Anopheles species from their breeding places does not exceed 2 km (Thomas et al., 2013), with the great majority occurring within a radius of 1 km from their breeding site (Staedke et al., 2003).
The few species that may fly up to five km or more, supported by environmental factors such as wind and vehicles, can be controlled by other measures or may be too small to establish disease transmission.

Houses on high ground and exposed to wind currents harbour a lower mosquito density and experience a facilitated draining of rain water to lower lands, thereby reducing potential breeding habitats (WHO, 1982). Houses located on the leeward side of breeding places, and at the foot of hills or in enclosed valleys, experience a high density of *Anopheles* mosquitoes due to calmer air and more abundant water. Sandy and porous soils that do not easily become waterlogged are preferred village sites compared to clay and impermeable soils which form water pools (WHO, 1982).

Well-designed and mosquito-proof houses contribute to a significant reduction in malaria transmission, as wire mesh cloth screens designed to give adequate protection, maximum ventilation and long-life are preferable and sustainable mosquito screening strategies. In humid areas, wire screens could be exposed to the corrosive action of air and to vibrations induced by strong winds; for such situations, a plastic mesh is less liable to deteriorate, although it may require backing with a welded mesh of thick wire to prevent sagging under the wind pressure. In the early 20th century, house improvement and screening were the methods given a priority for the control of malaria (Lindsay *et al.*, 2003). People living in poor houses are more exposed to malaria than those occupying complete rick and plastered houses. House screening was found to reduce human biting rates of mosquitoes and malaria infections in the USA, Greece and Italy, with clinical trials showing that house screens and ceilings alone provide protection against anaemia and exposure to malaria infection in rural parts of The Gambia. Window screening, closed eaves and ceilings prevent the entry of mosquitoes into houses in Africa (Ogoma *et al.*, 2009 and Kampango *et al.*, 2013).

In southwest Ethiopia, screening windows and doors with a metal mesh, in addition to closing all openings with mud, reduced the overall indoor densities of *An. arabiensis* by 40% (Massebo and Lindtjorn, 2013).

In the tropics, people may remain outdoors until late at night and become exposed to infectious mosquito bites, consequently leading to a minimal effect of house screening on malaria transmission (WHO, 1982).
2.11.2.5. Repellents

Repellents are products that are applied to the skin or clothing to repel mosquitoes and other biting insects and prevent them from biting human (WHO, 2006a). They can be used in the outdoors, in the early evenings and in the mornings in places where IRS and LLINs cannot be used (Sangoro et al., 2014). Spatial repellents are chemicals that change the behaviours of mosquitoes resulting in driving away mosquitoes from a potential human host and reduce human–vector contact and, therefore, offer personal protection (Achee et al., 2012).

Depending on efficacy and application modality, it creates a vector-free area both in- and outdoors. The protected space range depends on the active ingredient, application platform and environmental conditions such as air-flow, temperature and humidity (Achee et al., 2012). Spatial repellents discourage mosquitoes from entering a space occupied by a potential human host, thus reducing encounters between humans and vectors (WHO, 2006a, and Achee et al., 2012).

Repellents delay the emergence of insecticide resistance and reduce the toxic effects of insecticides to human and non-target organisms. They can reduce malaria transmission by forcing mosquitoes to either feed upon non-human hosts or to search for an alternative blood source. A longer exposure period of a vector species to outdoor conditions, e.g. predation, stressful environments and excessive energy expenditure during host-seeking, or identifying a resting or oviposition site, reduces the longevity and size of the mosquito population. The reduction in human-vector contact could ultimately lead to reduced numbers and the survival of older mosquitoes that transmit mature infectious stage parasites (Achee et al., 2012, and Killeen and Smith, 2007).

According to WHO (1982), repellents can be either synthetic or plant-based products. In the USA alone, 7,000 synthetic organic chemicals were being tested at one point. The mixture of compounds has produced repellents several times more effective than single-molecule chemicals. At present, dimethyl phthalate (DMP), dibutyl phthalate (DBP) and diethyltoluamide (DEET) are those most commonly used. Metofluthrin, a newly synthesized pyrethroid, has offered a strong knockdown and lethal activity against mosquitoes. It has high vapour pressure that enables itself to vaporize at normal temperatures without heating compared to the other pyrethroids, which require heating for evaporation. A multilayer paper strip impregnated with
metofluthrin caused a significant spatial repellency effect against mosquitoes in laboratory- and open-field conditions for a month at a 200 mg concentration in Indonesia (Kawada, et al., 2004). Metofluthrin (5% w/w) -impregnated polyethylene latticework plastic strips (approx. 9 x 18 cm$^2$; thickness, 0.2 cm; and weight, 10.7 g.) significantly reduced the density index of A. gambiae complex resting inside houses in Tanzania (Kawada et al., 2008)

Maia and Moore (2011) stated that plants contain natural chemicals to prevent themselves from the attacks of predator insects. These chemicals may serve as repellents, feeding deterents, toxins or growth regulators (GRs). Mankind has been using plant repellents since time immemorial by hanging, fumigating or as oil formulations applied to the skin or clothes to drive away nuisance mosquitoes, which is still practiced in most poor rural communities of the tropics. There are a diverse amount of plant species containing repellent chemicals. Although plant-based repellents are better for the environment than synthetic ones, they may contain compounds that need adequate formulation and monitoring (Maia and Moore, 2011).

A repellent might also be used in combination with other intervention tools in view of seeking a radical reduction in malaria transmission. In a community-based study in the Bolivian Amazon, a significant reduction in the episodes of P. vivax, P. falciparum and reported fever was observed in the group that used treated nets and a repellent (PMD) (Hill et al., 2007). Yohannes and Boeele (2012) reported that the use of repellents with LLINs or IRS may contribute to a significant reduction of the disease in areas where vectors feed in the early evening and outdoors.

2.12. Problem of insecticide resistance and management:

Insecticides play a central role in controlling major disease vectors (DVs) such as mosquitoes, sandflies, fleas, lice, tsetse flies, bedbugs, ticks and triatomid bugs. However, IR has been documented in insect vectors from every genus. IRs refers to the situation in which DVs are no longer killed by the standard dose of insecticide or manage to avoid coming into contact with the insecticide. The resistance of anopheline mosquitoes has been documented in almost all countries with ongoing malaria transmission to most of the available insecticides (WHO, 2012a).

Insecticide resistance is a growing concern in many countries, which requires immediate attention because of the limited chemical resources available for VC. Countries in west and central Africa (particularly Benin, Burkina Faso, Cameroon,
Côte d’Ivoire, Mozambique, South Africa and Ghana) (WHO, 2012a) have long been reporting high frequencies of resistance to the four classes of insecticides, such as OCs, Pyrethroids, Carbamates and OPs. In Ethiopia, resistance has been reported to all four classes of insecticides, including DDT and Pyrethroids, with a similar condition documented in Uganda, Kenya, Tanzania, Malawi and Zambia). In Eastern Mediterranean Region resistance to pyrethroids has been reported in Afghanistan, the Islamic Republic of Iran, and Oman. In addition, there is DDT resistance in Yemen. There have been reports of resistance to three of the four classes of insecticides in Afghanistan. Somalia and Sudan have reported resistance to all four classes of insecticide, including widespread resistance to DDT and an increasing frequency of resistance to pyrethroids (WHO, 2012a). In West Kordofan state of Sudan Tarig et al. (2018) reported the resistance of A. arabiensis to the deltamethrin 0.05% and DDT 4%. IR leads to a reduced efficacy of chemical based interventions or the possibility of control failure (WHO, 2012a). However, the pattern of resistance is very heterogeneous, even over relatively small distances (Ranson et al., 2009), which is possibly due to a misuse of insecticides and a cross-resistance with the locally used insecticides, as well as herbicides.

Tokponnon et al. (2014) stated that the widespread use of LLINs resulted in the development of vectors resistant to insecticides impregnated within the net fabrics. This problem could be exacerbated in households owning damaged nets. In addition, the benefit of a community-wide reduction in the number of infectious vectors could be reversed. However, the authors added, the LLINs can maintain their physical protective effect, against malaria vectors, as long as they are not damaged and used properly.

In order to minimize the increasing trend of IR in malaria vectors, the WHO and its partners have developed a Global Plan for Insecticide Resistance Management (GPIRM) (WHO, 2012a). The plan is developed to serve as the basis for a national VC strategy, including the use of IRS. The basis of the plan is the building of capacity and systems for basic epidemiological and entomological monitoring, including bioassays for the insecticide susceptibility of vectors to insecticides in order to delay the further development of resistance. This remains important for the pyrethroids, the only class of insecticide that can be used on nets.
Because pyrethroids are safe for close contact and have a rapid, persistent effect on mosquitoes at low doses, they are recommended for the treatment of nets (Zaim et al., 2000). Understanding of the IR mechanism in malaria vectors has been revolutionized by biochemical and molecular biological approaches, which opened up the exciting possibility of novel methods of maintaining insecticide susceptibility in the major DVs (Hemingway, 1999). For instance, the knock-down resistance (Kdr) mutation involved in pyrethroid resistance has been found widely distributed in the Savannah form of the A. gambiae (S. S.), but not in the wild population of the Mopti form or A. Arabiensis, even in areas where both occur. The Savanna form was fully able to interbreed, and the Kdr mutation was transmissible from one to another (Chandre et al, 1999). Vatandoost et al., (2007) found that adults of A. dthali were susceptible to DDT 4%, dieldrin 0.4%, malathion 5%, fenitrothion 1%, propoxur 0.1%, bendiocarb 0.1%, permethrin 0.75%, deltamethrin 0.05%, lambda-cyhalothrin 0.05% and cyfluthrin 0.15%. The larvae of this species were susceptible to different larvicides including malathion, temephos, fenitrothion and chlorpyrifos. Irritability level of A. dthali to lambda-cyhalothrin, permethrin, cyfluthrin and deltamethrin revealed that this species is more irritant to permethrin than other pyrethroids. Nevertheless, effective non-pyrethroid alternatives are being sought because of the consequence that the emergence of a strong resistance to pyrethroids would have on the effect of ITNs (WHO, 2006a). The GPIRM recommends that pyrethroids need to be “protected” through judicious use and through a rotation among the four classes of insecticides that can be used for IRS (WHO, 2013c).

The four main strategies for managing insecticide resistance are:
1) Rotating insecticides, with different modes of action, from one year to the next,
2) Using two or more insecticide-based VC interventions in a house (e.g. pyrethroids on nets and an insecticide of a different class on the walls),
3) Using one compound in one geographic area and a different compound in neighbouring areas, the two being in different insecticide classes, and
4) Using a mix of two or more compounds of different insecticide classes in a single product or formulation, so that the mosquito is guaranteed to come into contact with the two classes at the same time (WHO, 2012a).

2.13. Integrated vector management (IVM)
The reliance on a single method of VC may be challenging in several respects (e.g. IR or the outdoor- and early biting behaviour of the vectors), hence making single-intervention method such as IRS or ITNs is useless. For this reason, a combination of two or more methods of VC may have to be worked out for effective control depending on the local condition of the area. Such an approach is the concept of IVM, which is a rational decision-making process designed to optimize the use of resources for VC based on evidence and integrated management, promoting the use of a range of interventions – whether alone or in combination – selected on the basis of local knowledge about the vectors, diseases and disease determinants (WHO, 2012b). In Zambia, an IVM that integrated chemical and non-chemical approaches resulted in a marked reduction in malaria-related morbidity and mortality, while ensuring a better protection of the environment (Chanda et al., 2008).
CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Study Area:

Jazan area is about 11670 km$^2$ located in south-western part of Saudi Arabia with a coastal boundary 250 km along the Red Sea and a 120 km border with the Republic of Yemen (Map 3.1). The population according to the 2010 census was 1.37 million. This region includes over 100 islands located in the Red Sea, including the Farasan islands. The Al-Sarawat Mountains rise to over 3000 m. The highland area of the Fayfa Mountains lead to plains that provide rich escarpment agriculture, including coffee, millets, mangoes and citrus fruits (AlFarhan et al., 2005). The coastal regions are part of the Tihama straights that extend down through Yemen and are hot and arid.

The study was carried out in two different locations in Jazan Region, viz Harob and Eledabi Malaria control center (Map 3.1). These areas have different altitudes and geographical characteristics, but the demographic, educational, health system, cultural, housing, environmental and agricultural characteristics are almost similar. In these sites, the population lives mostly in villages, few of which have >500 inhabitants. Typically, the population of the villages ranges between 50 and 150 inhabitants. These small hamlets are widely scattered in the plain and foothill area. Nearly all the villages in the plain are situated along valleys and seldom lie further than 1km from the valleys. The total malaria cases recorded during the study period were 240 and 45 cases in Eledabi and Harob respectively; out of these only two cases were recognized as indigenous cases in Harob (Kingdom of saudi Arabia (KSA), Ministry of Health (MOH) reports 2014 and 2015).

Traditional houses of wattle and daub with thatched roof were previously common but these are being replaced by cement block buildings, or stone and mud structures. Health services (primary health care centre, PHC, and one general hospital) are also available and health care offered free of charge. Most centers where the first line treatment of malaria cases is sought are provided with a laboratory.
3.2. Selection Criteria:
The following criteria was applied for selection of the study area meeting the requirements established by the WHO in terms of being representative and sensitive for predicting, forecasting and monitoring purposes:
- The presence of high (Eledabi) and moderate (Harob) malaria endemicity.
- Capture stations have the highest density of *A. dthali*.
- The topography of the areas is facilitating the accessibility of these localities throughout the year.
- The identification of dwellings close to breeding sites (at least in three directions).
- The types of houses and the materials from which these houses are constructed are homogeneous.
- People occupy the residences at night.

3.3. Data Collection Methods:
- The data was collected according to the standard entomological techniques, tools, surveys and investigation as determined by the WHO (1975a and b) manual on practical entomology in malaria and manual on entomological laboratory techniques for malaria control (WHO, 1994 part l).
- The entomological activities were planned and carried out in representative areas with different patterns of distribution of the disease. The dynamics and behavior of the *A. dthali* population, and the roles played in the transmission of malaria were studied in relation to the breeding potentialities, contact with people, change of environment and vectors’ behavior. The susceptibility test was carried out to determine any change or existence of resistance to insecticides.
Map (3.1) The topography and boundaries covered by malaria centres in Jazan Region. 1 Aldarab, 2 Bayish, 3 Sabya, 4 Harob, 5 Eledabi (Iban), 6 Abu Arish, 7 Al Aridah, 8 Al khoba, 9 Samtah, and 10 Jazan/ Farasan Islands. DEM= Digital elevation data in meters above sea level
3.4. Entomological Survey:

3.4.1. Identification of mosquito's species

All adults and larvae stage of Anopheline mosquitoes collected by different techniques were identified up to the species levels, using the pictorial taxonomic identification keys prepared by Mattingly and Knight (1956), DuBose and Curtin (1965), Gillies and De- Meillon (1968), and Glick (1992). The identified A. dthali adults samples and other Anophelines were preserved in plastic bags containing silica gel for further analyses. In the laboratory, mosquitoes were identified to the species level using morphological keys as described earlier. A. dthali females were classified into different abdominal stages as unfed, fed, half-gravid and gravid. Then they were separated into two portions; abdomens were separated from heads and thoraxes, all parts were preserved in plastic tube at -80°C in refrigerator for subsequent PCR tests. DNA of the identified A. dthali (abdomens) specimens was extracted for the blood meal determination. Morphologically identified A. dthali member species were sent to Macrogenie Laboratory in South Korea for De novo whole genome sequencing. Head and thorax of A. dthali females were squashed and DNA was extracted and sporozoite species was also determined with the nested-PCR assay of Snounou et al. (1993a and b).

3.4.2. The collection of immatures (Larval collection and density assessment):

The techniques for collection and preservation of immature stages of mosquitoes were performed according to the procedure described by WHO in the Manual of Practical Entomology in Malaria (WHO, 1975b) and Entomological Laboratory Techniques for Malaria Control (WHO, 1994) unless the procedures are otherwise described.

This section addresses the detection of potential breeding sites of Anopheles in the study area during the period (Oct. 2014 to Sep. 2015). All potential breeding sites were regularly visited in a circle of a radius of about 1-2 km, including the surrounding farms and valleys, target houses were surveyed and inspected every week for potential breeding sites, in particular drinking water storage containers. The presence of Anopheline mosquito’s larvae were checked and carefully inspected using naked eyes or hand lens. The standard dipping method was utilized for obtaining
regular weekly samples that contain the various aquatic stages of *A. dthali* from the fixed entomological stations (Plate 3.1). Standard dipping methods were used to sample the larvae from various positive breeding sites. Various devices were employed:

a) A ladle which had a dipper of 8cm in diameter and 5 cm in depth (used for small water collections),

b) An enamel bowl (for sampling larvae from accessible large swamps, pools, ditches, irrigation paddles, and rivers pools),

c) A round frying pan about 25cm. in diameter and 5cm. in depth with a small handle which is normally attached to a long wooden stick (which is utilized for sampling larvae from more inaccessible potential breeding places)

d) A dip net attached to a long stick was used for sampling larvae from inaccessible wells, and a rope of a length of less or more than 3m. was attached when necessary (WHO, 1992). The mean number of each aquatic stage/10dips was calculated and noted. The samples were then transferred to large plastic bottles with half-full of water, so as to protect the sample and to minimize shaking of water. The characteristics of breeding sites were recorded.

3.4.3 Adult collection

a) Pyrethrum Spray Collection (PSC)

The pyrethroid space spray collection (Plate 3.2) was done every week in the mornings between 6.30 a.m. and 10.00 a.m. from all stations. This method consisted of the collection of indoors resting mosquitoes on white cotton sheets after being knocked-down by the spraying of the solution of pyrethroid (Aerosols manufactured by Arabian Company for Chemical Products, KSA).

The sample of house coverage was about 25 rooms weekly, and the total coverage of the rooms (about 100) was rotated every month. Observations of home, the number of occupants during the night before collection, temperature, relative humidity (R.H.), and the room sizes were recorded.
b) Dispersion of Adult Population of *A. dthali*

The target houses in the fixed entomological stations were categorized into four groups according to the distance from their main potential breeding sites (valleys). The first group distance was 200m or less, the second was 500m, the third was 1000m and the fourth was 1500m. The Pyrethrum Spray method was used to collect the samples. The number of mosquitoes collected from each house was recorded, and the total number of mosquitoes collected from each group was determined.

c) Collecting by means of human-baited trap nets

The direct catch of mosquitoes using human baits is not generally recommended because of ethical concerns over the exposure of collectors to malaria infection and other viral infection. In this case an alternative method of collection was used which gave a representative sample of the vector population that would bite humans.

A CDC light trap was installed in the bedroom beside a bed with a mosquito net. The human bait sleeps under the net, while the light trap attracts female Anophelines that have entered the room to bite the person under the net. The trapped mosquitoes used as a proxy to estimate the biting rate. In the house, one volunteer acting as human bait slept alone during the night. One CDC light trap was positioned indoors, fitted with incandescent bulbs, and placed close to the human volunteer sleeping under an untreated bed net in his/her usual sleeping place. The light trap was installed at about 1.5 m above the floor next to the foot of the bed. Trapped mosquitoes were removed the next morning (WHO, 2013d).

The collection was taken once every four wk, performed in the five houses as a fixed entomological station both indoors and outdoors near the human dwelling and the other inside the sleeping room. This field activity consisted of 12 hr, all night catches commencing at sun set (6.30 p.m.) and terminating at sunrise (6.30 a.m.) The number of mosquitoes/light trap/night was obtained.

The rooms in the houses for night collection were regularly inhabited on the night during or before collection. During the intervention period, the rooms used as catching stations were sprayed like other rooms.
Plate (3.1) Collection of mosquito Larvae using a dipper at the study areas
Plate (3.2) Bed sheet collection of adult mosquitoes
3.4.4. Transportation and Handling of Specimens

The various containers with immature stages and adults mosquitoes were placed in box with ice bags folded by a wet towel to reduce mortality during transportation of the species to the laboratory. Mosquitoes captured by PSC method were placed either in plastic containers or in vials plugged with cotton wool and they were labeled according to the place, station serial number, date, name of collector and time of collection.

3.4.5. Killing of specimens

Mosquitoes were killed with pyrethroid, for example, the females knocked down by a spray catch). The female Anophelineae was killed by stunning them in a test tube (Entomological Laboratory Techniques for Malaria Control (WHO, 1994). They were kept in refrigerators overnight, or kept for some hours in plastic containers with a moist cotton cloth at the bottom.

3.4.6. Dissection of Adult Females Mosquitoes

Adult females ovarian dissection was done according to that described in the entomological laboratory techniques for malaria control (WHO, 1994), while parity rate was similar to those described in the manual of practical entomology in malaria (WHO1975 b).

3.4.7. Blood–Meal Identification:

The blood–feed of A.dthali collected by the knock down and CDC collection methods was selected to determine the source of their blood meals. After removing the legs, wings, thorax and head, the mosquitoes' abdomen were preserved individually in 1.5 ml plastic tube, labeled, capped, and stored at -86 until further investigation to determine blood meal using the PCR technique.

3.4.8. Susceptibility Test of Adult and Larvae of A. dthali to Insecticides

The susceptibility level of A.dthali to the common insecticides used in the region was tested according to the WHO standards susceptibility test procedures described in the manual on practical entomology in malaria (WHO, 1975b; WHO, 2013e). Unfed female and larvae of A.dthali mosquitoes were exposed to diagnostic concentration of selected insecticides (methoprine, dilubenzuron, pyriproxyfen, temephos, permethrin 0.75%, deltamethrin 0.05%, cyfluthrin 0.15%,

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lambdacyhalothrine 0.05, DDT 4%, malathion 5%, fenitrothion 1%, dieldrin 4% and bendiocarb 0.1%). The survivors obtained after the discriminating exposure period and mortality after holding period for 24-hour were observed and recorded (Plate 3.3A).

The temperatures during the test and relative humidity were measured by the electronic thermometer.

The insecticides used in this study were provided by the WHO Malaysia manufacturer (Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia).

Interpretation of results was carried according to the WHO (2016) guidelines.

3.4.9. Susceptibility test of A.dthali Larvae to methoprine, dilubenzuron and pyriproxyfen

3.4.9.1. Methods:
This method is designed to determine the susceptibility or resistance of mosquito larvae to Insect Growth Regulator insecticides (IGRs). The effect of chitin synthesis inhibitors occurs at the end of each larval instar and interferes with normal development up to the time of adult emergence.

3.4.9.2. Equipments:
The equipments and insecticides were ordered separately from the WHO.

Composition of the test kit:

A) 2 1-ml pipettes with two rubber suction bulbs.

B) 2 eye droppers with rubber suction bulbs.

C) The following materials used in making a strainer: two wire loops, one piece of nylon netting (30cm²) and one tube of cement. The net cut into two pieces and cemented to the opposite side of the large end of the wire loop. More cement was applied around the outside of the loop to join the two pieces of netting. After dry the netting was trimmed with scissors.

3.4.9.3. Insecticides Procedure:
Standard solutions used for each insecticide (methoprine, dilubenzuron and pyriproxyfen) were; 20 mg/l, 4 mg/l, 0.8 mg/l, 0.16 mg/l and 0.032 mg/l in alcohol (ethanol). All these alcoholic solutions were supplied in 50 ml bottles. One 50 ml
bottle of alcohol was supplied for the control (Denatured by the addition of 2% Butanone).

Procedure:

a) Sufficient numbers of larvae of the *A.dthali* were collected from the field any larvae showed abnormalities were discarded from the test. Lots of 20 third-instar larvae were placed in 12 small beakers, each containing 25 ml of water.

b) Into each of 24 cups approx. 7.5-10 cm in dia., 225 ml of distilled water was placed. The average temperature of the water and pH were 25.1 °C and 7 respectively (Plate 3.3B).

c) A series of test concentrations were prepared by pipetting 1 ml of each standard solution (concentration) just above the surface of the water in each cup and stirred vigorously with a glass rod for 30 sec (most diluted concentration was prepared first). There were four replicates for each concentration, in addition to four control replicates. The four untreated controls were prepared by adding 1 ml of alcohol to the water in each cup.

d) Within 15 min of the preparation of the test concentrations, the *A. dthali* larvae were added to them by tipping the contents of the small beakers into the cups.

e) Larvae were lifted in continuous exposure to insecticides. Pinch of Cerilac was added to the exposure cups and held in dim light until mortality counts were made.

f) Larvae in the control cups were fed at the same time and in the same manner as those of the treated batches. They were kept until they had developed into pupae and adult.

g) The duration of the observation period was that required for complete adult emergence in the control batches.

h) At the end of the observation period the IGRs effect was expressed in terms of larvae not developed successfully into adults. In recording percentage effect for each concentration, moribund and dead larvae and pupae as well as adult having not completely emerged from the pupal case and flown out, were considered as “affected”.
Plate (3.3A). Susceptibility test of Adult A. dthali to insecticides
3.4.10. Estimation of the Duration of Sporogonic Cycle:

The sporogonic period of *Plasmodium falciparum* in the malaria vector was computed using the following formula developed by Moskkovsky (c.f. Detinova, 1962):

\[ N = \frac{111^\circ \text{C}}{(T - 16)} \]

Where \( N \) = duration of sporogonic cycle of *Plasmodium falciparum*;
\( 111^\circ \text{C} \) is the Sum of heat in degrees necessary for the sporogonic cycle of *P. falciparum*;

Plate (3.3B). Susceptibility test of *A. dthali* Larvae
$7^\circ\text{C}$ is the average temperature prevalent at the study area, estimated as an average of the daily mean month’s temperature;

$16^\circ\text{C}$ is the estimated lowest development threshold for the $P.\ falciparum$ every 24 hr.

The 16 degrees are constant for $P.\ falciparum$.

3.4.11. Estimation of longevity of $An.\ dthali$

There is no known way of measuring directly the expectation of life of the mosquito population. A simple technique for distinguishing between parous and nulliparous females Anophelines is by examination of the tracheoles of the ovaries as to whether they were coiled skeins or they had become stretched. This permits assessment of the nulliparous and parous, from which the probability of survival of mosquitoes and therefore their average longevity was calculated. To estimate longevity of females $A.\ dthali$, in particular the survival rate (probability of mathematical expression of the likelihood of female Anophelines remaining alive for a specified period), Detinova’, (1962) method was used. From the formula $p^n = M$, the daily survival rate was computed, where

$n =$duration of gonotrophic cycle;

$M =$ proportion of parous females; and,

$p =$ daily survival rate.

From the known value of $n$ and $M$, the survival rate was calculated using $p = n \sqrt[n]{M}$ where $n = 2$ days; thus, $P$ is calculated as the proportion parous.

3.4.12. Estimation of Vectorial capacity for $A.\ dthali$:

The vectorial capacity ($C$) is defined as the "daily rate at which future inoculations arise from a currently infective case".

It is directly related to the:

- number of bites / person / day (or man-biting rate)
- feeding habits (anthropohilic vs. zoophilic)
- life expectancy of the mosquito

The incidence of malaria inoculations / case / day, is influenced by all the above parameters. Vectorial capacity is expressed by the following formula (Macdonald, 1957; Garrett-Jones, 1964):\[ C = \frac{ma^2 p^n}{-\log_{ep}} \]

Where:
C = Vectorial Capacity
Ma = man-biting rate
A = man-biting habit
p^n = probability of vector survival through the sporogonic period of parasite
n = sporogonic period of parasite

3.5. Molecular Test:

3.5.1. Detection of Sporozoites:

Anopheles dthali specimens were collected from indoor human dwellings of 26 villages distributed in two Malaria control stations (Eledabi and Harob) from Oct. 2014 to Sept. 2015. The collection of specimens was performed using Pyrethroid Knockdown (PKD) collections as described by WHO(1992). Collected mosquitoes were brought to the National Center for Vector-Borne Diseases in Jazan for morphological identification, and sporozoite rate determination.

3.5.1.2. Preparation of Mosquitoes:

A total of 300 A.dthali females were preserved individually in 1.5 ml plastic tube, labeled, capped, and stored at -86 until further investigation. After removing the legs, wings, and abdomen, the mosquito Thorax and head were homogenized individually in a mortar and pestle (mini borosilicate glass chamber length 60 mm / pestle diameter 9.0mm 3.0 ml, Fisherbrand) in 100 μL of Minimum Essential Media (MEM; manufactured Euro Clone, UK). The homogenate was saved in -86 degree till next procedure.

3.5.1.3. DNA extraction:

DNA was extracted from the stored homogenate using RealLine DNA – Extraction 2 (BIORON Diagnostic, Germany) following the manufacture’s recommendations: 300 μL of lysis Reagent with sorbent (magnetic particles) added to homogenate in 1.5 ml tubes and placed into the thermo shaker for five minutes at 65°C, 1300rpm. Then 400 μL of DNA precipitation solution was added to each tube and mixed for 15 seconds in a vortex. Samples were then centrifuged at 13000 rpm for five minutes at room temperature then the supernatant discarded and the pellet was washed twice and dried for 2-3 minutes at room temperature. Specimen solution used
to re-suspend the DNA. The extracted DNA stored at -86°C till next procedure (plate 3.4).

3.5.1.4. Detection of Plasmodium (Sporozoite) in *A. dthali* by nested-PCR:

Nested PCR procedures were carried out for detection and identification of *Plasmodium* species in *A.dthali* as described by Snounou, *et al.* (1993a). DNA samples were amplified by oligonucleotide primers obtained from Integrated DNA Technology (Belgium), targeting the *Plasmodium* small subunit ribosomal RNA (ssRNA) genes (Waters and Mccutchan, 1989; Table 3.1). Primer pair's rPLU5 and rPLU6 used to detect *Plasmodium* genus in Primary amplification and species-specific primers rFAL1/rFAL2 (*P. falciparum*) and rVIV1/rVIV2 (*P. vivax*) for nested PCR in 2 separated reactions.

In brief, primary and nested PCR were carried out in total 25 µl reaction volume, each containing 12.5 µl GoTag®G2 green master mix ready to use from Promega and 25µM of each primer. Five µl of extracted DNA was used as a sample for the primary amplification and 2 µl of the PCR product for the nested PCR. In each run, negative and positive controls were included. Thermal cycling was done in T100 thermal cycler (Bio-Rad, USA). PCR primers and conditions are shown in Table (3.1). The PCR products of nested amplification were analyzed by gel electrophoresis (1.5 agarose in Tris-Acetate EDTA buffer) staining with ethidium promide. The visualization was carried out using Gel Doc XR Imaging System (Bio-Rad).
Plate (3.4). PCR Techniques for *A. dthali*
3.5.2. Blood–Meal Identification:

3.5.2.1. Preparation of Mosquito:

After removing the legs, wings, head and Thorax, the blood-fed-mosquito, abdomen was homogenized individually in a mortar and pestle (mini borosilicate glass chamber length 60 mm/pestle diameter 9.0mm 3.0ml, Fisherbrand) in 100 μL of MEM. The homogenate was saved in -86 °C till next procedure.

3.5.2.2. DNA extraction:

As mentioned previously in detection of Plasmodium.

3.5.2.3. Detection of blood meal:

PCR Primers of human and sheep blood were obtained from previously published primer sequences (Table 3.2).

PCR was carried out in total 25 μl reaction volume, each containing 12.5 μl GoTag®G2 green master mix ready to use from Promega and 25μM of each primer. Five μl of extracted DNA was used as a sample for the primary amplification and 2 μl of the PCR product for the nested PCR. In each run, negative and positive controls were included. An initial denaturation of 3min at 95°C was followed by 30 cycles at 95 °C for 30 sec, 55°C for 1 min and 72°C for 1min. The final extension step was 72°C for 5 min.

Thermal cycling was done in T100 thermal cycler (Bio-Rad, USA). The PCR products were analyzed by gel electrophoresis (1.5 agarose in Tris-Acetate EDTA buffer) staining with ethidium promide. The visualization was carried As 3.5.1.4 above

3.5.3. Detection of West-Kdr (1014F) & East-Kdr (1014S):

Mosquitoes tested by the WHO susceptibility kits were subjected to PCR test for the confirmation of the results. The protocol used for the detection of the L1014S or L1014F kdr alleles was adapted from the protocols developed by Martinez et al., 1998 and Ranson et al. 2000.

Primers Agd1 (5'-ataagatccccgaccatg-3'), Agd2 (5'-agacaaggatgatgaacc-3'), Agd3 (5'-aatttgccattttcagaca-3') and Agd4 (5'-cttgatgtgtagggatatta-3') were used to detect the L1014F allele (AS-PCR Agd3), whereas primers Agd1, Agd2, Agd4 and Agd5 (5'-tttgccattttctgactg-3') were used to detect the L1014S allele (AS-PCR
Agd5). Amplification was performed in a 50 μl reaction containing 1 μl of template DNA, 1 × Qiagen PCR buffer, 0.5 mM MgCl2, 100 nM of each primer, 200 μM of dNTP's, and 1 U of Taq DNA polymerase (Taq PCR core kit, Qiagen, Hilden, Germany). The cycling conditions were: initial 94°C denaturation for 5 min, 10 cycles of one min denaturation at 94°C, 30 sec annealing at 54°C and 30 sec extension at 72°C, followed by 30 cycles of 1 min denaturation at 94°C, 30 sec annealing at 47°C and 30 sec extension at 72°C, and a final extension at 72°C for 10 min. Amplification products were checked on a 2% agarose gel and visualized after ethidium bromide staining.
Table (3.1) Primers used to detect sporozoite in *A. dthali* and their PCR conditions

<table>
<thead>
<tr>
<th>Species</th>
<th>Primer Name</th>
<th>Sequence (5'-3')</th>
<th>PCR Product Size (BP)</th>
<th>PCR Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Plasmodium sp.</em></td>
<td>rPLU5</td>
<td>CCTGGTTGGTTGCGCTTAAACTTC</td>
<td>1100</td>
<td>Initial denaturation at 94°C for 3 min, 35 cycles of denaturation at 94°C for 60 seconds, annealing at 60°C for 90 seconds, extension at 72°C for 90 seconds and final extension for 5 minutes</td>
</tr>
<tr>
<td></td>
<td>rPLU6</td>
<td>TTAAAATTGGTTGCGTTAAACG</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. falciparum</em></td>
<td>rFAL1</td>
<td>TAAACTGGTTTGGGAAAACC AAATATATT</td>
<td>205</td>
<td>Initial denaturation at 94°C for 3 min, 35 cycles of denaturation at 94°C for 60 seconds, annealing at 55°C for 90 seconds, extension at 72°C for 90 seconds and final extension for 5 minutes</td>
</tr>
<tr>
<td></td>
<td>rFAL2</td>
<td>ACACAATGAACTCAATCATGA CTACCCGTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. vivax</em></td>
<td>rVIV1</td>
<td>CGCTTCTAGCTTAATCCACAT AACTGATAC</td>
<td>120</td>
<td>Initial denaturation at 94°C for 3 min, 35 cycles of denaturation at 94°C for 60 seconds, annealing at 55°C for 90 seconds, extension at 72°C for 90 seconds and final extension for 5 minutes</td>
</tr>
<tr>
<td></td>
<td>rVIV2</td>
<td>ACTTCCAAGCCGAAGCAAAGA AAGTCCTTA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table (3.2) Primers used for determination of blood meal

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Product Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human R</td>
<td>GGTTGTCCCTCCAATTCAATGTTA</td>
<td></td>
</tr>
<tr>
<td>Human HUF</td>
<td>GGCTTACTTCTCTCATTCCTCCTCCT</td>
<td>335 bp</td>
</tr>
<tr>
<td>Animal F</td>
<td>5-GACCTCCCAGCTCCATCAAAC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ATTCATCTTTGATGAAA-3</td>
<td></td>
</tr>
<tr>
<td>Sheep R</td>
<td>5-CTATGAATGGCTTGAGCTATTGTCGCA-3</td>
<td>331 bp</td>
</tr>
</tbody>
</table>

3.5.3. Whole Genome Denovo Sequencing for A. dthali:

Genomic DNA was extracted using TNES-UREA method. The integrity of the extracted DNA was checked by running an agarose gel electrophoresis and gDNA were quantified using Quant-IT PicoGreen (Invitrogen).

The sequencing libraries were prepared according to the manufacturer’s instructions of TruSeq DNA PCR-free Sample Preparation Kit (Illumina, Inc., San Diego, CA, USA). Briefly, Fragmentation of 1ug of genomic DNA was performed using adaptive focused acoustic technology (AFA; Covaris) and the fragmented DNA is end-repaired to create 5’-phosphorylated, blunt-ended dsDNA molecules. Following end-repair, DNA was size selected with bead-based method. These DNA fragments go through the addition of a single ‘A’ base, and ligation of the truseq indexing adapters. The purified libraries were quantified using qPCR according to the qPCR Quantification Protocol Guide (KAPA Library Quantification kits for Illumina Sequencing platforms) and qualified using the high-sensitivity DNA chip (Agilent Technologies, Waldbronn, Germany). And then the paired-end (2×100 bp) sequencing was performed by the Macrogen using the HiSeq4000 platform (Illumina, San Diego, USA).

The whole genome sequencing for A.dthali was conducted by Macrogen Incorporation, Korea (www.macrogen.com, http://dna.macrogen.com) and was registered in National Center for Biotechnology Information (NCBI-https://www.ncbi.nlm.nih.gov/)
CHAPTER FOUR

4. RESULTS

4.1 Entomological Investigations of Anopheles Larvae:

4.1.1 Species composition of Anopheline larvae:

A total of 111241 Anopheles mosquitoes larvae were collected in the period from Oct.2014 to Sept.2015 from Herob and Eledabi area. Out of these 104260 (93.7%) were A.dthali. Larvae of A.dthali were found in highest density in the study area; comprised about 92.5% and 94.8 of the total larvae of Anopheles population collected from Harob and Eledabi, respectively. Other Anopheles species collected were A.arabiensis, A.pretoriensis and A.multicolor which represented low percentage in the study area. More details about the presence of Anopheles species larvae in the two areas are presented in tables (4.1 and 4.2) and appendix (1.1 and 1.2).

4.1.2 A.dthali abundance and seasonal patterns:

The larval density was determined from different breeding sites at the two study areas during different months of the year and the pattern of the average larval density /dip/month is shown in table (4.3). Potential breeding sites of A.dthali in Eledabi and Harob area were weekly visited. The mean larval density/10dips/month shows no significant difference between the two study areas, so the density of larvae were almost similar (P˂ 0.856). There was one major peak of the larval density at the two areas in December (wet season). The breeding sites existed throughout the year but this was not statistically confirmed when the Pearson coefficient correlation between precipitation and the density of larvae was used (P value = 0.379). The average larval density/dip/month gradually increased in the second half of the year starting from August and reached its peak in December (15.2 and 14.5 larvae/dip in Harob and Eledabi, respectively). The larval density then declined from March to reach the minimum density in May (3.2 and 3 larvae per dip) in Eledabi and Harob, respectively, (Appendix 1.3).
Table (4.1) Species composition of *Anopheles* mosquitoes larvae collected from Harob area (Oct. 2014 – Sept. 2015)

<table>
<thead>
<tr>
<th>Species</th>
<th>Month</th>
<th>A. dthali No. (%)</th>
<th>A. arabiensis No. (%)</th>
<th>A. pretoriensis No. (%)</th>
<th>A. multicolor No. (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td></td>
</tr>
<tr>
<td>Oct. 2014</td>
<td></td>
<td>4493 (95.9)</td>
<td>193 (4.1)</td>
<td>0</td>
<td>0</td>
<td>4686</td>
</tr>
<tr>
<td>Nov. 2014</td>
<td></td>
<td>6682 (96.1)</td>
<td>271 (3.9)</td>
<td>0</td>
<td>0</td>
<td>6953</td>
</tr>
<tr>
<td>Dec. 2014</td>
<td></td>
<td>7752 (98.6)</td>
<td>110 (1.4)</td>
<td>0</td>
<td>0</td>
<td>7862</td>
</tr>
<tr>
<td>Jan. 2015</td>
<td></td>
<td>5602 (99.3)</td>
<td>42 (0.7)</td>
<td>0</td>
<td>0</td>
<td>5644</td>
</tr>
<tr>
<td>Feb. 2015</td>
<td></td>
<td>4998 (96.1)</td>
<td>0</td>
<td>207 (3.9)</td>
<td>0</td>
<td>5205</td>
</tr>
<tr>
<td>Mar. 2015</td>
<td></td>
<td>2500 (78.2)</td>
<td>0</td>
<td>698 (21.8)</td>
<td>0</td>
<td>3198</td>
</tr>
<tr>
<td>Apr. 2015</td>
<td></td>
<td>2479 (78.5)</td>
<td>72 (2.3)</td>
<td>607 (19.2)</td>
<td>0</td>
<td>3158</td>
</tr>
<tr>
<td>May 2015</td>
<td></td>
<td>1434 (94.5)</td>
<td>83 (5.5)</td>
<td>0</td>
<td>0</td>
<td>1517</td>
</tr>
<tr>
<td>June 2015</td>
<td></td>
<td>1862 (91.1)</td>
<td>183 (8.9)</td>
<td>0</td>
<td>0</td>
<td>2045</td>
</tr>
<tr>
<td>July 2015</td>
<td></td>
<td>2299 (67.2)</td>
<td>214 (6.3)</td>
<td>909 (26.5)</td>
<td>0</td>
<td>3422</td>
</tr>
<tr>
<td>Aug. 2015</td>
<td></td>
<td>3661 (94.1)</td>
<td>218 (5.6)</td>
<td>0</td>
<td>12 (0.3)</td>
<td>3891</td>
</tr>
<tr>
<td>Sept. 2015</td>
<td></td>
<td>4824 (97.7)</td>
<td>115 (2.3)</td>
<td>0</td>
<td>0</td>
<td>4939</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>48586 (92.5)</td>
<td>1501 (2.85)</td>
<td>2421 (4.6)</td>
<td>12 (0.05)</td>
<td>52520</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>3882.2</td>
<td>125.1</td>
<td>201.8</td>
<td>1</td>
<td>4376.7</td>
</tr>
<tr>
<td>SE±</td>
<td></td>
<td>584</td>
<td>26</td>
<td>96.8</td>
<td>1</td>
<td>544.4</td>
</tr>
<tr>
<td>C.V.%</td>
<td></td>
<td>52.1</td>
<td>71.9</td>
<td>166</td>
<td>346</td>
<td>43.1</td>
</tr>
<tr>
<td>Month</td>
<td>A. dthali No. (%)</td>
<td>A. arabiensis No. (%)</td>
<td>A. pretoriensis No. (%)</td>
<td>A. multicolor No. (%)</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>-------------------</td>
<td>-----------------------</td>
<td>-------------------------</td>
<td>-----------------------</td>
<td>--------</td>
<td></td>
</tr>
<tr>
<td>Oct. 2014</td>
<td>5994 (96.0)</td>
<td>253 (4.0)</td>
<td>0</td>
<td>0</td>
<td>6247</td>
<td></td>
</tr>
<tr>
<td>Nov. 2014</td>
<td>7187 (92.0)</td>
<td>347 (4.4)</td>
<td>281(3.6)</td>
<td>0</td>
<td>7815</td>
<td></td>
</tr>
<tr>
<td>Dec. 2014</td>
<td>8092 (97.3)</td>
<td>228 (2.7)</td>
<td>0</td>
<td>0</td>
<td>8320</td>
<td></td>
</tr>
<tr>
<td>Jan. 2015</td>
<td>6314 (97.5)</td>
<td>159 (2.5)</td>
<td>0</td>
<td>0</td>
<td>6473</td>
<td></td>
</tr>
<tr>
<td>Feb. 2015</td>
<td>5292 (98.7)</td>
<td>71 (1.3)</td>
<td>0</td>
<td>0</td>
<td>5363</td>
<td></td>
</tr>
<tr>
<td>Mar. 2015</td>
<td>3290 (94.5)</td>
<td>64 (1.8)</td>
<td>127 (3.7)</td>
<td>0</td>
<td>3481</td>
<td></td>
</tr>
<tr>
<td>Apr. 2015</td>
<td>2261 (91.4)</td>
<td>19 (0.8)</td>
<td>193 (7.8)</td>
<td>0</td>
<td>2473</td>
<td></td>
</tr>
<tr>
<td>May 2015</td>
<td>1723 (86.4)</td>
<td>17 (0.9)</td>
<td>254 (12.7)</td>
<td>0</td>
<td>1994</td>
<td></td>
</tr>
<tr>
<td>June 2015</td>
<td>2609 (92.1)</td>
<td>223 (7.9)</td>
<td>0</td>
<td>0</td>
<td>2832</td>
<td></td>
</tr>
<tr>
<td>July 2015</td>
<td>3460 (92.8)</td>
<td>267 (7.2)</td>
<td>0</td>
<td>0</td>
<td>3727</td>
<td></td>
</tr>
<tr>
<td>Aug. 2015</td>
<td>4087 (92.9)</td>
<td>313 (7.1)</td>
<td>0</td>
<td>0</td>
<td>4400</td>
<td></td>
</tr>
<tr>
<td>Sept. 2015</td>
<td>5365 (95.9)</td>
<td>216 (3.9)</td>
<td>0</td>
<td>15 (0.2)</td>
<td>5596</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>55674(94.81)</td>
<td>2177(3.7)</td>
<td>855(1.45)</td>
<td>15(0.04)</td>
<td>58721</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4639.5</td>
<td>181</td>
<td>71.3</td>
<td>1.3</td>
<td>4893.4</td>
<td></td>
</tr>
<tr>
<td>SE±</td>
<td>589.1</td>
<td>32.8</td>
<td>32.1</td>
<td>1.3</td>
<td>599.4</td>
<td></td>
</tr>
<tr>
<td>C.V.%</td>
<td>44</td>
<td>62.7</td>
<td>156</td>
<td>346</td>
<td>42.4</td>
<td></td>
</tr>
</tbody>
</table>
Table (4.3). Abundance of *A. dthali* larvae in Harob and Eledabi area of Jazan Region (Oct. 2014 – Sept. 2015)

<table>
<thead>
<tr>
<th>Station Month</th>
<th>Eledabi Density of <em>An. dthali</em> Larvae/10 dips</th>
<th>Harob Density of <em>An. dthali</em> Larvae/10 dips</th>
<th>Average Density of <em>An. dthali</em> Larvae/10 dips</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct. 2014</td>
<td>10.8</td>
<td>9.3</td>
<td>10.1</td>
</tr>
<tr>
<td>Nov. 2014</td>
<td>13.0</td>
<td>13.8</td>
<td>13.4</td>
</tr>
<tr>
<td>Dec. 2014</td>
<td>14.5</td>
<td>15.2</td>
<td>14.9</td>
</tr>
<tr>
<td>Jan. 2015</td>
<td>11.3</td>
<td>11.5</td>
<td>11.4</td>
</tr>
<tr>
<td>Feb. 2015</td>
<td>8.6</td>
<td>9.4</td>
<td>9.0</td>
</tr>
<tr>
<td>Mar. 2015</td>
<td>5.8</td>
<td>5.2</td>
<td>5.5</td>
</tr>
<tr>
<td>Apr. 2015</td>
<td>4.1</td>
<td>5.1</td>
<td>4.6</td>
</tr>
<tr>
<td>May 2015</td>
<td>3.2</td>
<td>3.0</td>
<td>3.1</td>
</tr>
<tr>
<td>June 2015</td>
<td>4.7</td>
<td>3.8</td>
<td>4.3</td>
</tr>
<tr>
<td>July 2015</td>
<td>7.2</td>
<td>4.7</td>
<td>6.0</td>
</tr>
<tr>
<td>Aug. 2015</td>
<td>8.3</td>
<td>7.5</td>
<td>7.9</td>
</tr>
<tr>
<td>Sept. 2015</td>
<td>9.4</td>
<td>9.0</td>
<td>9.7</td>
</tr>
<tr>
<td>Mean</td>
<td>8.4</td>
<td>8.1</td>
<td></td>
</tr>
<tr>
<td>SD±</td>
<td>3.58</td>
<td>3.96</td>
<td></td>
</tr>
<tr>
<td>SE±</td>
<td>1.03</td>
<td>1.14</td>
<td></td>
</tr>
<tr>
<td>CV%</td>
<td>42.62</td>
<td>48.89</td>
<td></td>
</tr>
<tr>
<td>t-test Sig. (2-tailed)</td>
<td>P value (0.856)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.1.3. Ecological characteristics of *A. dthali* habitat:

Out of the 111241 *Anopheles* larvae pough, 93.72% were *A. dthali* that was found in the two study areas (Table 4.1 and 4.2). Among the larval nests in Eledabi containing this species, 63% and 37% were found in permanent and temporary habitats, respectively. Additionally, 53% of them were of slow running water; and 47% were of the stagnant water. The larval nests with vegetations, sunny, sandy bottom, and clean water formed 81, 81, 52 and 45% of the total larval habitats, respectively. Most of the natural and artificial larval nests were located at the edge of the valleys (62%) and in the swamps (21%), respectively (Table 4.4).

In Harob this species represented 43.7% of the larvae caught from the study area. They were collected from all the breeding sites under investigation. *A.dthali* larvae were seen almost equally in the clear and turbid waters. More than 83% of larval habitats of this species had been exposed to the sunlight. Likewise, its larval nests more than 96% situated in the shallow water. The species often breeds in stagnant permanent water (76%). It has been found that more than 73% of its natural larval nests were on the valleys sides and on 51% of the plots of cultivated sorghum (Table 4.4).

In general, this species was found in permanent, shallow and slow-running water with or without algae and vegetation. It prefers sunny water for egg-laying and it was also collected from sandy, muddy, rocky and brackish water substrata. More than 70% of natural larval habitats were in the edge of the valleys (Table 4.4 and plate 4.1 A to H).

4.1.4. Chemical and physical properties of breeding sites:

The results of salinity tolerance of *A.dthali* showed that the survival rate of *A.dthali* began to decline at 20% salinity and no larvae survived salinities greater than 30% (Table 4.5).

*A.dthali* larval mortality was recorded with diluted seawater its range between 1% - 40% concentration *i.e.* 0.317 - 12.68 gm/L NaCl (WHO defines seawater as containing 31.7gm NaCl/L which is equivalent to 100% seawater) *A.dthali* showed that they could tolerate NaCL at levels of 0.317 to 9.51gm/L (Table 4.5), but they showed total mortality if the concentration exceed (12.68gm/L). Larval mortality
increased as the NaCl concentration increased. Mean concentration is 6.4 with standard deviations of ±4.9.

Analysis of water from the main potential breeding sites of the *A. dthali* showed the range of pH was 7.2 to 7.9 (the average pH was 7.6). The mean water temperature was 27.5 and the total dissolved solids (TDS) ranged from 224 to 1145 ppm. The results showed that there was no sign of extreme alkalinity or acidity at the main breeding sites of *A. dthali* in the two study areas.

Table (4.4) Percentage of the presence of *A. dthali* larvae at different ecological habitats in Eledabi and Harob areas (Oct 2014 - Sept. 2015)
<table>
<thead>
<tr>
<th>Breeding sites</th>
<th>Characteristics</th>
<th>Eledabi</th>
<th>Harob</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Depth</td>
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<td>98</td>
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<tr>
<td></td>
<td>Deep</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Type of water</td>
<td>Permanent</td>
<td>63</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>Temporary</td>
<td>37</td>
<td>24</td>
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<td>Status of water</td>
<td>Clear</td>
<td>45</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Turbid</td>
<td>55</td>
<td>50</td>
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<tr>
<td>Vegetation</td>
<td>Out of water</td>
<td>23</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>Water surface</td>
<td>36</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Under water</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Without plant</td>
<td>19</td>
<td>21</td>
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<tr>
<td>Stream of water</td>
<td>Slow-running water</td>
<td>53</td>
<td>57</td>
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<tr>
<td></td>
<td>Stagnant</td>
<td>47</td>
<td>43</td>
</tr>
<tr>
<td>Surface debris</td>
<td>Present</td>
<td>50</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>50</td>
<td>49</td>
</tr>
<tr>
<td>Algae</td>
<td>Present</td>
<td>65</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>35</td>
<td>46</td>
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<tr>
<td>Sunlight</td>
<td>Sunny</td>
<td>81</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Shade</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>Substrate</td>
<td>Muddy</td>
<td>27</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Sandy</td>
<td>52</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>Rocky</td>
<td>21</td>
<td>28</td>
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<tr>
<td>The natural habitats</td>
<td>Edge of valley</td>
<td>62</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>Wetland</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Footprint</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Swamps</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>Artificial Habitats</td>
<td>Plot of sorghum</td>
<td>46</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>cultivation</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Irrigation channel</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Cement Water Tank</td>
<td>42</td>
<td>46</td>
</tr>
</tbody>
</table>
Plate (4.1A to H) Showing types of *A.dthali* breeding sites

Plate (4.1A) Water surface vegetation
Plate (4.1B) Rocky breeding site
Plate (4.1C) Sandy substrate breeding site
Plate (4.1D) Algae breeding site
Plate (4.1E) Sunny breeding site
Plate (4.1F) Under surface water vegetation
Plate (4.1G) Partially shaded breeding site
Plate (4.1H) Rocky breeding site
### Table (4.5) Effect of Salinity on *A. dthali* larvae.

| Concentration of Seawater (g/L) | Control | 1%  
|                                | 0.317   | 10%  
|                                | 3.17    | 20%  
|                                | 6.34    | 30%  
|                                | 9.51    | 40%  
| Replicate                      | 4       | 1%  
| Exposed                        | 80      | 10% 
| Dead                           | 0 (0%)  | 20% 
|                                | 16 (20%)| 20% 
|                                | 16 (20%)| 50% 
|                                | 40 (50%)| 66.3% 
|                                | 53 (66.3%)| 100% 
| No. Tolerant                   | 80      | 1%  
|                                | 64      | 10% 
|                                | 64      | 20% 
|                                | 40      | 30% 
|                                | 27      | 40% 
| % Tolerant                     | 100     | 1%  
|                                | 80      | 10% 
|                                | 80      | 20% 
|                                | 50      | 30% 
|                                | 21.6    | 40% 
|                                | 0       | 40% |
4.2. Entomological Investigation of Adult Anopheles Mosquitoes

4.2.1. Relative Abundance of Adult Anopheline:

A total of 2226 adult female Anopheles mosquitoes were collected from the two study areas throughout the study period. The mean A. dthali population density was 1.9 mosquito/house. Anopheline mosquitoes caught from Eledabi area accounted 1144 (51.4%) and from Harob the total collected was 1082 (48.6%) (tables 4.6 and 4.7).

4.2.2. Species composition:

All of Anopheles mosquitoes collected fall into three major species: A. dthali 1384 (62.2%), A. arabiensis 718 (32.2%) and A. pretoriensis 124 (5.6%) (tables 4.6 and 4.7). Of the 1082 Anopheles mosquitoes collected from Harob in Oct. 2014 to Sept. 2015, 60.4% belonged to A.dthali, 28.7% belonged to A.arabiensis and 10.9% belonged to A. pretoriensis (table 4.6) Appendix(1.4). For in Eledabi 1144 Anopheles mosquitoes were collected and morphologically identified as A.dthali (63.9%), A.arabiensis (35.7%) and A. pretoriensis (0.4%) (table 4.7) Appendix (1.5). The pattern of the density of A. dthali collected from the two study areas is almost similar (P<0.608).
### Table (4.6). Species Composition of Adult Anophelinae Mosquitoes collected from Harob area of Jazan Region (Oct. 2014-Sept. 2015)

<table>
<thead>
<tr>
<th>Station Month</th>
<th>A. dthali No. (%)</th>
<th>A. arabiensis No. (%)</th>
<th>A. pretoriensis No. (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct. 2014</td>
<td>99 (62.6)</td>
<td>42 (26.6)</td>
<td>17 (10.8)</td>
<td>158</td>
</tr>
<tr>
<td>Nov. 2014</td>
<td>80 (48.8)</td>
<td>65 (39.6)</td>
<td>19 (11.6)</td>
<td>164</td>
</tr>
<tr>
<td>Dec. 2014</td>
<td>63 (48.9)</td>
<td>47 (36.4)</td>
<td>19 (14.7)</td>
<td>129</td>
</tr>
<tr>
<td>Jan. 2015</td>
<td>60 (58.8)</td>
<td>29 (28.4)</td>
<td>13 (12.8)</td>
<td>102</td>
</tr>
<tr>
<td>Feb. 2015</td>
<td>44 (68.8)</td>
<td>13 (20.3)</td>
<td>7 (6.9)</td>
<td>64</td>
</tr>
<tr>
<td>Mar. 2015</td>
<td>37 (69.8)</td>
<td>12 (22.6)</td>
<td>4 (7.6)</td>
<td>53</td>
</tr>
<tr>
<td>Apr. 2015</td>
<td>16 (64)</td>
<td>7 (28)</td>
<td>2 (8)</td>
<td>25</td>
</tr>
<tr>
<td>May 2015</td>
<td>9 (60)</td>
<td>5 (33.3)</td>
<td>1 (6.7)</td>
<td>15</td>
</tr>
<tr>
<td>June 2015</td>
<td>17 (44.7)</td>
<td>16 (42.1)</td>
<td>5 (13.2)</td>
<td>38</td>
</tr>
<tr>
<td>July 2015</td>
<td>56 (67.5)</td>
<td>20 (24.1)</td>
<td>7 (8.4)</td>
<td>83</td>
</tr>
<tr>
<td>Aug. 2015</td>
<td>77 (69.4)</td>
<td>23 (20.7)</td>
<td>11 (9.9)</td>
<td>111</td>
</tr>
<tr>
<td>Sept. 2015</td>
<td>95 (67.9)</td>
<td>31 (22.1)</td>
<td>14 (10.0)</td>
<td>140</td>
</tr>
<tr>
<td>Total</td>
<td>653 (60.4)</td>
<td>310 (28.7)</td>
<td>119 (10.9)</td>
<td>1082</td>
</tr>
<tr>
<td>Mean</td>
<td>54.4</td>
<td>25.8</td>
<td>9.9</td>
<td>90.2</td>
</tr>
<tr>
<td>SE±</td>
<td>8.8</td>
<td>5.2</td>
<td>1.9</td>
<td>14.9</td>
</tr>
<tr>
<td>C.V.%</td>
<td>56.1</td>
<td>69.6</td>
<td>65.2</td>
<td>57.3</td>
</tr>
</tbody>
</table>
Table (4.7). Species Composition of Anophelinae Mosquitoes collected from Eledabi area of Jazan Region (Oct. 2014-Sept. 2015)

<table>
<thead>
<tr>
<th>Station Month</th>
<th>A.dhali No.(%)</th>
<th>A.arabiensis No.(%)</th>
<th>A.pretoriensis No.(%)</th>
<th>Total No.(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct. 2014</td>
<td>98 (60.5)</td>
<td>64 (39.1)</td>
<td>0</td>
<td>162</td>
</tr>
<tr>
<td>Nov. 2014</td>
<td>97 (58.1)</td>
<td>66 (39.5)</td>
<td>4 (2.4)</td>
<td>167</td>
</tr>
<tr>
<td>Dec. 2014</td>
<td>83 (58.5)</td>
<td>59 (41.5)</td>
<td>0</td>
<td>142</td>
</tr>
<tr>
<td>Jan. 2015</td>
<td>73 (68.9)</td>
<td>32 (30.2)</td>
<td>1 (0.9)</td>
<td>106</td>
</tr>
<tr>
<td>Feb. 2015</td>
<td>55 (65.5)</td>
<td>29 (34.5)</td>
<td>0</td>
<td>84</td>
</tr>
<tr>
<td>Mar. 2015</td>
<td>41 (69.5)</td>
<td>18 (30.5)</td>
<td>0</td>
<td>59</td>
</tr>
<tr>
<td>Apr. 2015</td>
<td>13 (72.2)</td>
<td>5 (27.8)</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>May 2015</td>
<td>18 (78.3)</td>
<td>5 (21.7)</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>June 2015</td>
<td>23 (58.98)</td>
<td>16 (41.02)</td>
<td>0</td>
<td>39</td>
</tr>
<tr>
<td>July 2015</td>
<td>66 (75.0)</td>
<td>22 (25.0)</td>
<td>0</td>
<td>88</td>
</tr>
<tr>
<td>Aug. 2015</td>
<td>74 (66.7)</td>
<td>37 (33.3)</td>
<td>0</td>
<td>111</td>
</tr>
<tr>
<td>Sept. 2015</td>
<td>90 (62.1)</td>
<td>55 (37.9)</td>
<td>0</td>
<td>145</td>
</tr>
<tr>
<td>Total</td>
<td>731 (63.9)</td>
<td>408 (35.7)</td>
<td>5 (0.4)</td>
<td>1144</td>
</tr>
<tr>
<td>Mean</td>
<td>60.9</td>
<td>34</td>
<td>0.4</td>
<td>95.3</td>
</tr>
<tr>
<td>SE±</td>
<td>8.9</td>
<td>6.4</td>
<td>0.3</td>
<td>15.2</td>
</tr>
<tr>
<td>C.V.%</td>
<td>50.4</td>
<td>65.4</td>
<td>277</td>
<td>55.2</td>
</tr>
</tbody>
</table>
4.2.3. Seasonal Population incidence of *A. dthali* Mosquitoes:

The density of *A. dthali* mosquitoes increased rapidly following the beginning of the rainy-season in Aug., during which 151(10.9%) of the total *A. dthali* were caught. This number reached a peak of 197 (14.2%) in the month of Oct., and fell only to 27 (1.95%) in May (table 4.8).

4.2.4 Resting Preference of *A. dthali* Mosquitoes

The outdoor collection was significantly higher than the indoor collection with a noticeable preference to animal shelter (P < 0.01) (Table 4.9). No mosquitoes were collected from the moderate building with Freon air conditioners. *A. dthali* in the two study areas was highly exophilic with some degree of tendency to being endophilic.

The highest number of the *A. dthali* was found resting outdoors in animal shelters and animal's food storage room (Table 4.9) and Appendix (1.6).

4.2.5. Dispersion of *A. dthali*:

Table (4.10) shows the dispersion of adult female *A. dthali* according to the distance from their breeding sites in the study area. Results indicated that 76.7% of the females were found in houses close to the breeding sites, (at a distance of < 200m), 18.7% in houses far from breeding sites (at distances between 200m-500m), and 4.6% in houses further than that (at distances of 500m -1000m). There were none observed in the houses at a distance of >1000m (Appendix 1.7). This result shows that the adult population of *A. dthali* was distributed in the area according to distance from breeding sites, and in clusters around the breeding sites (P < 0.046).

As for the collection methods, the bed sheet collection method yield about 72.33% of the total *A. dthali* collected from the two study areas while the light trap collected 27.67% (Table 4.11).
Table (4.8) Abundance of adult *A. dthali* mosquitoes in Eledabi and Harob
(Oct. 2014-Sept. 2015)

<table>
<thead>
<tr>
<th>Station Month</th>
<th>Adult of <em>An. dthali</em> No. (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eledabi</td>
<td>Harob</td>
</tr>
<tr>
<td>Oct. 2014</td>
<td>98 (13.41)</td>
<td>99 (15.16)</td>
</tr>
<tr>
<td>Nov.2014</td>
<td>97 (13.27)</td>
<td>80 (12.25)</td>
</tr>
<tr>
<td>Dec. 2014</td>
<td>83 (11.35)</td>
<td>63 (9.65)</td>
</tr>
<tr>
<td>Jan. 2015</td>
<td>73 (9.99)</td>
<td>60 (9.19)</td>
</tr>
<tr>
<td>Feb. 2015</td>
<td>55 (7.52)</td>
<td>44 (6.74)</td>
</tr>
<tr>
<td>Mar. 2015</td>
<td>41 (5.61)</td>
<td>37 (5.67)</td>
</tr>
<tr>
<td>Apr. 2015</td>
<td>13 (1.78)</td>
<td>16 (2.45)</td>
</tr>
<tr>
<td>May 2015</td>
<td>18 (2.46)</td>
<td>9 (1.38)</td>
</tr>
<tr>
<td>June 2015</td>
<td>23 (3.15)</td>
<td>17 (2.60)</td>
</tr>
<tr>
<td>July 2015</td>
<td>66 (9.03)</td>
<td>56 (8.58)</td>
</tr>
<tr>
<td>Aug. 2015</td>
<td>74 (10.12)</td>
<td>77 (11.79)</td>
</tr>
<tr>
<td>Sept.2015</td>
<td>90 (12.31)</td>
<td>95 (14.55)</td>
</tr>
<tr>
<td>Total</td>
<td>731</td>
<td>653</td>
</tr>
<tr>
<td>Mean</td>
<td>60.9</td>
<td>54.4</td>
</tr>
<tr>
<td>SE±</td>
<td>8.9</td>
<td>8.8</td>
</tr>
<tr>
<td>C.V.%</td>
<td>50.4</td>
<td>56.1</td>
</tr>
</tbody>
</table>

*Percentages shown are calculated in relation to the total number of *An. dthali* collected from each area
Table (4.9) Resting Habit of *A. dthali* in Harob and Eledabi of Jazan area

<table>
<thead>
<tr>
<th>Station</th>
<th>Animal keeper Hut No. (%)</th>
<th>Animal shelter No. (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harob</td>
<td>237 (36.29)</td>
<td>416 (63.71)</td>
<td>653</td>
</tr>
<tr>
<td>Eledabi</td>
<td>239 (32.69)</td>
<td>492 (69.31)</td>
<td>731</td>
</tr>
<tr>
<td>Total</td>
<td>476 (34.39)</td>
<td>908 (65.61)</td>
<td>1384</td>
</tr>
</tbody>
</table>

Table (4.10) Dispersion range of Adult *A. dthali* from the main breeding sites (Oct. 2014-Sept. 2015)

<table>
<thead>
<tr>
<th>Distance from breeding site (m)</th>
<th>% of collected <em>An. dthali</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 200</td>
<td>76.7</td>
</tr>
<tr>
<td>200 - 500</td>
<td>18.7</td>
</tr>
<tr>
<td>500 – 1000</td>
<td>4.6</td>
</tr>
</tbody>
</table>

Table (4.11) Comparison between Bed Sheet collection and Light trap collection of *A. dthali* in Harob and Eledabi of Jazan area (Oct. 2014-Sept. 2015)

<table>
<thead>
<tr>
<th>Station</th>
<th>BSC</th>
<th>Light trap</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harob</td>
<td>479 (73.35%)</td>
<td>174 (26.65%)</td>
<td>653</td>
</tr>
<tr>
<td>Eledabi</td>
<td>522 (71.41%)</td>
<td>209 (28.59%)</td>
<td>731</td>
</tr>
<tr>
<td>Total</td>
<td>1001 (72.33%)</td>
<td>383 (27.67%)</td>
<td>1384</td>
</tr>
</tbody>
</table>
4.2.6. Examination of the Abdominal State of A. dthali

Percentage of fed A. dthali mosquitoes in Harob and Eledabi was 47.5% (657) in the two areas, mean was 18.4 and 19.9, and standard deviation was 11.2 and 10.4, respectively) (Tables 4.12 and 4.13). On other hand, the total number of unfed mosquitoes collected from the two stations was 460 (33.2%), mean (25.3 and 29.4) and standard deviation was 15.4 and 17.3 at Harob and Eledabi, respectively. There was highly significant difference (p <0.001) between fed (F) and unfed (UF) mosquitoes in the two study areas. While, no significant differences in the abdominal status of A. dthali mosquitoes across the two study areas were found (P values of UF, FF, HG and G were 0.751, 0.623, 0.977 and 0.409, respectively).

4.2.7. Feeding Preferences of the A. dthali Mosquitoes in Jazan

Results of the PCR on blood meal analysis as shown on table (4.14) and plates (4.2 and 4.3) indicate that 147 (73.5%) of A. dthali were positive for animal (sheep) blood and only 27 (13%) of the A. dthali had taken human blood (Appendix 1.8). The Human Blood Index (HBI) was 0.13 indicating low anthropophilic behaviors.

4.2.8. PCR Test for detection of Plasmodium (Sporozoite) species and EIR:

Plasmodium species in the infected mosquito was determined using the PCR method. Out of 300 randomly selected A. dthali mosquitoes only four (1.3%) were positive for P. falciparum sporozoite; three from Eledabi (sporozoite rate= 0.02) and one from Harob (sporozoite rate= 0.01). The results are shown in table (4.15), plate (4.4) and Appendix (1.9).
Table (4.12) Genotrophic cycle of *A. dthali* collected from Harob area
(Oct. 2014-Sept. 2015)

<table>
<thead>
<tr>
<th>Status Month</th>
<th>No. of occupants</th>
<th>U</th>
<th>F</th>
<th>HG</th>
<th>G</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct. 2014</td>
<td>53</td>
<td>23</td>
<td>57</td>
<td>10</td>
<td>9</td>
<td>99</td>
</tr>
<tr>
<td>Nov. 2014</td>
<td>46</td>
<td>43</td>
<td>32</td>
<td>2</td>
<td>3</td>
<td>80</td>
</tr>
<tr>
<td>Dec. 2014</td>
<td>43</td>
<td>22</td>
<td>41</td>
<td>0</td>
<td>0</td>
<td>63</td>
</tr>
<tr>
<td>Jan. 2015</td>
<td>30</td>
<td>23</td>
<td>19</td>
<td>12</td>
<td>6</td>
<td>60</td>
</tr>
<tr>
<td>Feb. 2015</td>
<td>30</td>
<td>20</td>
<td>21</td>
<td>3</td>
<td>0</td>
<td>44</td>
</tr>
<tr>
<td>Mar. 2015</td>
<td>45</td>
<td>17</td>
<td>19</td>
<td>1</td>
<td>0</td>
<td>37</td>
</tr>
<tr>
<td>Apr. 2015</td>
<td>35</td>
<td>6</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>May 2015</td>
<td>32</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>June 2015</td>
<td>39</td>
<td>5</td>
<td>8</td>
<td>3</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>July 2015</td>
<td>37</td>
<td>20</td>
<td>23</td>
<td>9</td>
<td>4</td>
<td>56</td>
</tr>
<tr>
<td>Aug. 2015</td>
<td>24</td>
<td>13</td>
<td>25</td>
<td>22</td>
<td>17</td>
<td>77</td>
</tr>
<tr>
<td>Sept. 2015</td>
<td>47</td>
<td>27</td>
<td>43</td>
<td>14</td>
<td>11</td>
<td>95</td>
</tr>
<tr>
<td>Total</td>
<td>461</td>
<td>221</td>
<td>304</td>
<td>77</td>
<td>51</td>
<td>653</td>
</tr>
<tr>
<td>Mean</td>
<td>38.4</td>
<td>18.4</td>
<td>25.3</td>
<td>6.4</td>
<td>4.3</td>
<td>54.4</td>
</tr>
<tr>
<td>SE±</td>
<td>2.5</td>
<td>3.2</td>
<td>4.5</td>
<td>2.0</td>
<td>1.6</td>
<td>8.8</td>
</tr>
<tr>
<td>C.V.%</td>
<td>22.4</td>
<td>60.7</td>
<td>60.9</td>
<td>108.5</td>
<td>130</td>
<td>56.1</td>
</tr>
</tbody>
</table>

U = Un Fed, F = Full Fed, HG = Half Graved and G = Graved
Table (4.13) Genotrophic cycle of *A. dthali* collected from Eledabi area
(Oct. 2014-Sept. 2015)

<table>
<thead>
<tr>
<th>Status Month</th>
<th>No. of occupants</th>
<th>U</th>
<th>F</th>
<th>HG</th>
<th>G</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nov. 2014</td>
<td>42</td>
<td>40</td>
<td>47</td>
<td>5</td>
<td>5</td>
<td>97</td>
</tr>
<tr>
<td>Dec. 2014</td>
<td>42</td>
<td>34</td>
<td>42</td>
<td>3</td>
<td>4</td>
<td>83</td>
</tr>
<tr>
<td>Jan. 2015</td>
<td>22</td>
<td>21</td>
<td>22</td>
<td>18</td>
<td>12</td>
<td>73</td>
</tr>
<tr>
<td>Feb. 2015</td>
<td>18</td>
<td>13</td>
<td>17</td>
<td>14</td>
<td>11</td>
<td>55</td>
</tr>
<tr>
<td>Mar. 2015</td>
<td>23</td>
<td>16</td>
<td>15</td>
<td>7</td>
<td>3</td>
<td>41</td>
</tr>
<tr>
<td>Apr. 2015</td>
<td>21</td>
<td>7</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>May 2015</td>
<td>19</td>
<td>8</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>June 2015</td>
<td>23</td>
<td>10</td>
<td>12</td>
<td>1</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>July 2015</td>
<td>25</td>
<td>21</td>
<td>43</td>
<td>0</td>
<td>2</td>
<td>66</td>
</tr>
<tr>
<td>Aug. 2015</td>
<td>31</td>
<td>15</td>
<td>46</td>
<td>9</td>
<td>4</td>
<td>74</td>
</tr>
<tr>
<td>Sept. 2015</td>
<td>45</td>
<td>26</td>
<td>41</td>
<td>10</td>
<td>13</td>
<td>90</td>
</tr>
<tr>
<td>Total</td>
<td>348</td>
<td>239(32.7%)</td>
<td>353(48.3%)</td>
<td>74(10.1%)</td>
<td>65(8.9%)</td>
<td>731</td>
</tr>
<tr>
<td>Mean</td>
<td>29</td>
<td>19.9</td>
<td>27</td>
<td>6.2</td>
<td>5.4</td>
<td>60.9</td>
</tr>
<tr>
<td>SE±</td>
<td>2.9</td>
<td>3.0</td>
<td>4.8</td>
<td>1.7</td>
<td>1.4</td>
<td>8.9</td>
</tr>
<tr>
<td>C.V.%</td>
<td>34</td>
<td>52.5</td>
<td>61.7</td>
<td>93.5</td>
<td>91.9</td>
<td>50.4</td>
</tr>
</tbody>
</table>

U = Un Fed, F = Full Fed, HG = Half Graved and G = Graved
Table (4.14) PCR identification of feeding preference (Blood meal) of *A. dthali* collected from Harob and Eledabi areas

<table>
<thead>
<tr>
<th>Area</th>
<th>Total examined</th>
<th>Human blood</th>
<th>Animal (Sheep blood)</th>
<th>Mixed</th>
<th>HBI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harob</td>
<td>100</td>
<td>11 (11%)</td>
<td>74 (74%)</td>
<td>15 (15%)</td>
<td>0.11</td>
</tr>
<tr>
<td>Eledabi</td>
<td>100</td>
<td>15 (15%)</td>
<td>73 (73%)</td>
<td>12 (12%)</td>
<td>0.15</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>26 (13%)</td>
<td>147 (73.5%)</td>
<td>27 (13.5%)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Plate (4.2) A typical example of a gel with positive samples for the human blood in *A. dthali*

Lane 1 represents the 100bp Ladder, lane 2 positive control, lanes 3, 5, 6 and 7 positive samples, lane 4 negative sample, lane 8 negative control.

Lane 1 represents the 100bp Ladder, lane 2 positive control, lanes 8 negative controls, lanes 3, 5, 6 and 7 samples were identified as positive for human blood.
Plate (4.3) A typical example of a gel with positive samples for the Sheep blood meal in *A. dthali*.
Lane 1 represents the 100 bp Ladder, lane 2 positive control, lanes 3 negative controls, lanes 4, 5, and 7 samples were identified as positive for sheep blood and lane 6 and 8 were negative sample.
Table (4.15) Detection of Sporozoite and annual EIR in *A. dthali* collected from Eledabi and Harob area of Jazan Region

<table>
<thead>
<tr>
<th>Station</th>
<th>No. examined</th>
<th>No. +ve (Sporozoite)</th>
<th>SR</th>
<th>Annual EIR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eledabi</td>
<td>150</td>
<td>3</td>
<td>0.02</td>
<td>1.8</td>
</tr>
<tr>
<td>Harob</td>
<td>150</td>
<td>1</td>
<td>0.01</td>
<td>0.7</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>4</td>
<td>0.013</td>
<td>1.1</td>
</tr>
</tbody>
</table>

SR=Sporozoite rate

EIR = Entomological Inoculation Rate

Plate (4.4) Stained agarose gel for the detection of sporozoite in *A. dthali*.

Lane 1 represents the 100bp Ladder, lane 2 positive control, lanes 7 negative controls, lanes 3, 4, 5, and 6 samples were identified as *plasmodium falciparum* sporozoite
4.2.9. Whole Genome De novo sequencing of the *Anopheles dthali*

The whole genome sequencing for *An. dthali* was conducted by Macrogen Incorporation, Korea ([www.macrogen.com](http://www.macrogen.com), [http://dna.macrogen.com](http://dna.macrogen.com)) and was registered in National Center for Biotechnology Information ([NCBI](https://www.ncbi.nlm.nih.gov/)) under the following code numbers:

- **Run:** SRR5585848
- **Biosample:** SAMN06909325
- **Bioproject:** PRJNA385755

4.2.9.1 Results of Data preprocessing

4.2.9.1.1 Quality filtered data

<table>
<thead>
<tr>
<th>Sample</th>
<th>Filter</th>
<th>Total Base</th>
<th>Read count</th>
<th>GC (%)</th>
<th>Q20 (%)</th>
<th>Q30 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. <em>dthali</em></td>
<td>Pre Filtered</td>
<td>53343180502</td>
<td>528150302</td>
<td>39.72</td>
<td>94.71</td>
<td>88.42</td>
</tr>
<tr>
<td></td>
<td>Post Filtered</td>
<td>46159505204</td>
<td>457024804</td>
<td>37.45</td>
<td>96.97</td>
<td>92.09</td>
</tr>
</tbody>
</table>

- **Total Bases:** The total number of bases in reads.
- **Reads Count:** The number of reads identified.
- **GC (%):** The percentage of bases on a DNA that are either guanine or cytosine.
- **Q20 (%):** The percentage of bases called that have a quality score of 20 or above.
- **Q30 (%):** The percentage of bases called that have a quality score of 30 or above.

4.2.9.1.2 Average Base Quality at Each Cycle after Trimming:

![Average base quality of A.dthali Read1 at each cycle after trimming](image)

**Fig. 4.1** Average base quality of *A.dthali* Read1 at each cycle after trimming
Fig. 4.2 Average base quality of *A. dthali* Read2 at each cycle after trimming

- Yellow box: Interquartile range (25-75%) of phred score at each cycle
- Red line: Median of phred score at each cycle
- Blue line: Average of phred score at each cycle
- Green background: Good quality
- Orange background: Acceptable quality
- Red background: Bad quality

4.2.9.2. Results of analysis
4.2.9.2.1 K-mer analysis

Fig. 4.3 K-mer species
**Fig. 4.4** K-mer individuals

**Table (4.17) K-mer analysis**

<table>
<thead>
<tr>
<th>K-mer size (bp)</th>
<th>K-mer individual sum</th>
<th>Approximate peak depths</th>
<th>Estimate genome size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>38,841,355,315</td>
<td>32</td>
<td>1,213,792,354</td>
</tr>
<tr>
<td>21</td>
<td>37,012,910,680</td>
<td>84</td>
<td>462,397,087</td>
</tr>
<tr>
<td>25</td>
<td>35,184,567,026</td>
<td>78</td>
<td>474,524,496</td>
</tr>
<tr>
<td></td>
<td></td>
<td>73</td>
<td>481,980,370</td>
</tr>
</tbody>
</table>
4.3. Vectorial Capacity Parameters of *A. dthali*

The survival of a female mosquito after a blood meal (probability of surviving one day after blood meal, denoted as \( p \) and expectation of life for \( n \) days (\( n \) being the number of days for a sporogonic cycle to be completed).

The result of ovarian dissections of *A. dthali* in Eledabi and Harob station was as follows:

- Eledabi (82/250) = 0.328
- Harob (68/250) = 0.272

Given an interval of two days between blood meals, the probability of surviving one day (denoted as \( p \)) can be estimated as:

\[ p = \sqrt{\text{Proportion parous}} \]

Thus, \( p = \sqrt{0.328} = 0.573 \) in Eledabi and \( \sqrt{0.272} = 0.522 \) in Harob.

It is also possible to calculate the probability of surviving through \( n \) days. As \( p \) is the probability of surviving one day, \( p^n \) is the probability of surviving \( n \) days. At an average daily temperature of 27°C, it would take about 10 days for *P. falciparum* to complete the sporogonic cycle in the vector. The probability that this parasite can be transmitted by *An. dthali* in Harob = 0.522\(^{10}\) (0.002) and in Eledabi= 0.573\(^{10}\) (0.004) (Table 4.18).

\[
C = \frac{ma^2 p^n}{-\ln p}
\]

Where,
- \( C \) = Vectorial Capacity
- \( ma \) = density of vectors in relation to man
- \( a \) = number of blood meals taken on man / vector / day, = HBI x 0.5 (gonotrophic cycle of two days is assumed)
- \( p \) = daily survival probability (or proportion of vectors surviving / day)
- \( n \) = incubation period in the vector (days)

\( \ln \) means “natural logarithm” and \( p \) is the daily survival rate of a mosquito.

(The formula is the inverse of minus the natural log of \( p \), that is, 1/-\( \ln p \)).
Table (4.18). Vectorial Capacity (VCap) parameters of *A. dthali* in Eledabi and Harob (Oct. 2014-Sept. 2015)

<table>
<thead>
<tr>
<th>Indices</th>
<th>Eledabi</th>
<th>Harob</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density / man</td>
<td>2.1</td>
<td>1.42</td>
</tr>
<tr>
<td>Parous rate</td>
<td>0.573</td>
<td>0.522</td>
</tr>
<tr>
<td>HBI</td>
<td>0.15</td>
<td>0.11</td>
</tr>
<tr>
<td>A</td>
<td>0.15 x 0.5</td>
<td>0.11 x 0.5</td>
</tr>
<tr>
<td>p(^n)</td>
<td>0.004</td>
<td>0.002</td>
</tr>
<tr>
<td>1/-Log(_p)</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td>VCap</td>
<td>0.0004</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
4.4. Bioassay:

4.4.1. Organochlorines insecticides

   *A. dthali* showed full susceptibility to DDT in Harob and Eledabi (mortality 100%), (Tables 21 and 22) it was also showed possibility of resistance and resistance to dieldrin (91% and 89% in Harob and Eledabi, respectively).

4.4.2. Pyrethroid insecticides:

   Mosquitoes from the two areas were found fully susceptible to all pyrethroids tested. Mortality was recorded between 98% and 100% after 24 hr post-exposure period.

   *A. dthali* populations showed full susceptibility to permethrin and deltamethrin in the two study areas (mortality 100%). While it was susceptible to lambda-cyhalothrin and cyfluthrin in the two areas although with little variations in mortality (Tables 4.19 and 4.20).

4.4.3. Organophosphate insecticides

   The data revealed a full susceptibility of *A. dthali* mosquitoes to malathion in the two study areas, whereas it exhibited possibility of resistance to fenitrothion (mortality 91.25% and 93% in Harob and Eledabi, respectively tables 4.19 and 4.20) Appendix (1.10).

4.4.4. Carbamates

   *A. dthali* populations were found fully susceptible to bendiocarb (mortality 100%).

4.4.5. PCR test for detection OF *kdr* mutation

   A total of 100 *A. dthali* mosquitoes preserved after susceptibility test were analyzed for presence of *kdr* mutation by PCR technique (Plate 4.5). No gene resistant-mutations were found among the specimens analyzed for *kdr* (L1014F or L1014S).
Table (4.19) Susceptibility level of *A. dthali* mosquito exposed to various insecticides in Harob area

<table>
<thead>
<tr>
<th>Insecticides</th>
<th>Replicate</th>
<th>No. of mosquitoes tested</th>
<th>No. Dead</th>
<th>% Mortality (Status)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permethrin 0.75%</td>
<td>4</td>
<td>100</td>
<td>100</td>
<td>100 (S)</td>
</tr>
<tr>
<td>Deltamethrin 0.05%</td>
<td>4</td>
<td>80</td>
<td>80</td>
<td>100 (S)</td>
</tr>
<tr>
<td>Cyfluthrin 0.15%</td>
<td>4</td>
<td>80</td>
<td>79</td>
<td>98.75 (S)</td>
</tr>
<tr>
<td>Lambda-cyhalothrine 0.05</td>
<td>4</td>
<td>80</td>
<td>80</td>
<td>100 (S)</td>
</tr>
<tr>
<td>DDT 4%</td>
<td>4</td>
<td>100</td>
<td>100</td>
<td>100 (S)</td>
</tr>
<tr>
<td>Malathion 5%</td>
<td>4</td>
<td>100</td>
<td>100</td>
<td>100 (S)</td>
</tr>
<tr>
<td>Fenitrothion 1%</td>
<td>4</td>
<td>80</td>
<td>73</td>
<td>91 (PR)</td>
</tr>
<tr>
<td>Dieldrin 4%</td>
<td>4</td>
<td>100</td>
<td>91</td>
<td>91 (PR)</td>
</tr>
<tr>
<td>Bendiocarb 0.1%</td>
<td>4</td>
<td>80</td>
<td>80</td>
<td>100 (S)</td>
</tr>
<tr>
<td>Total tested</td>
<td></td>
<td></td>
<td>800</td>
<td></td>
</tr>
</tbody>
</table>

S= Susceptible; PR= Possibility of Resistance; R= Resistance.

* Status of susceptibility according to WHO guide lines (2016).
Table (4.20) Susceptibility level of *A. dthali* mosquito exposed to various insecticides in Eledabi area

<table>
<thead>
<tr>
<th>Insecticides</th>
<th>Replicate</th>
<th>No. of mosquitoes tested</th>
<th>No. Dead</th>
<th>% Mortality (Status)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permethrin 0.75%</td>
<td>4</td>
<td>80</td>
<td>80</td>
<td>100 (S)</td>
</tr>
<tr>
<td>Deltamethrin 0.05%</td>
<td>4</td>
<td>80</td>
<td>80</td>
<td>100 (S)</td>
</tr>
<tr>
<td>Cyfluthrin 0.15%</td>
<td>4</td>
<td>100</td>
<td>100</td>
<td>100 (S)</td>
</tr>
<tr>
<td>Lambda-cyhalothrine 0.05</td>
<td>4</td>
<td>100</td>
<td>98</td>
<td>98 (S)</td>
</tr>
<tr>
<td>DDT 4%</td>
<td>4</td>
<td>100</td>
<td>100</td>
<td>100 (S)</td>
</tr>
<tr>
<td>Malathion 5%</td>
<td>4</td>
<td>100</td>
<td>100</td>
<td>100 (S)</td>
</tr>
<tr>
<td>Fenitrothion 1%</td>
<td>4</td>
<td>100</td>
<td>93</td>
<td>93 (PR)</td>
</tr>
<tr>
<td>Dieldrin 4%</td>
<td>4</td>
<td>100</td>
<td>89</td>
<td>89 (R)</td>
</tr>
<tr>
<td>Bendiocarb 0.1%</td>
<td>4</td>
<td>80</td>
<td>80</td>
<td>100 (S)</td>
</tr>
<tr>
<td>Total tested</td>
<td>-</td>
<td>840</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

S = Susceptible; PR = Possibility of Resistance; R = Resistance.

* Status of susceptibility according to WHO guide lines (2016).
Plate (4.5) Show the absence of *kdr* mutation gene responsible for Pyrethroid resistance

Lane 1 represents the 100bp Ladder, lane 2 positive control 200bp, lane 8 negative control, lanes 3,4,5,6 and 7 samples were identified as *negative KDR gene*
4.5. Laboratory Evaluation of Diflubenzuron, Methoprene, Pyriproxyfen and Temephos against *An. dthali* larvae:

Susceptibility of *A. dthali* larvae to the various IGRs and temephos larvicides varied considerably (Table 4.21 and Appendix 1.11). Larvae tested from Harob and Eledabi area were most susceptible to diflubenzuron (LC\_50 = 0.001 and 0.004 ppm, respectively) and least susceptible to methoprene (LC\_50 = 0.250 and 0.32 ppm respectively), respectively.

Diflubenzuron in Harob was 47x and 250x more active than pyriproxyfen and methoprene, respectively (LC\_50 = 0.001 ppm). However, in Eledabi it was 14.75x and 80x more active than pyriproxyfen and methoprene, respectively (LC\_50 = 0.004 ppm).

Pyriproxyfen in Eledabi and Harob was approx. 5x more active than methoprene (LC\_50 = 0.059 and 0.047 ppm, respectively). However methoprene in general had low level of activity against *A. dthali* in the two study areas.

Temephos shows equal toxicity (equitoxic) in the two study areas as indicated by LC\_50 = 44.566 ppm in Eledabi and 44.266 ppm in Harob, a finding that reflected the resistance of *A. dthali* to this chemical.
Table (4.21) Regression Line Parameters of *A. dthali* exposed to various insecticides in Harob and Eledabi areas of Jazan region

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Intercept</th>
<th>Slope</th>
<th>Probit equation (y=a+bx)</th>
<th>LC50 (95cl)</th>
<th>LC90 (95cl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diflubenzron Eledabi</td>
<td>0.97</td>
<td>0.26</td>
<td>Y = 0.97+0.26X</td>
<td>0.004</td>
<td>14.54</td>
</tr>
<tr>
<td>Diflubenzron Harob</td>
<td>0.979</td>
<td>0.314</td>
<td>Y = 0.98+0.3X</td>
<td>0.001</td>
<td>9.19</td>
</tr>
<tr>
<td>Methoprene Eledabi</td>
<td>0.186</td>
<td>0.378</td>
<td>Y = 0.19+0.39X</td>
<td>0.322</td>
<td>794.48</td>
</tr>
<tr>
<td>Methoprene Harob</td>
<td>0.217</td>
<td>0.359</td>
<td>Y = 0.22+0.36X</td>
<td>0.250</td>
<td>922.91</td>
</tr>
<tr>
<td>Pyriproxyfen Eledabi</td>
<td>0.93</td>
<td>0.761</td>
<td>Y = 0.93+0.8X</td>
<td>0.059</td>
<td>3.99</td>
</tr>
<tr>
<td>Pyriproxyfen Harob</td>
<td>1.03</td>
<td>0.80</td>
<td>Y = 1.03+0.8X</td>
<td>0.047</td>
<td>2.608</td>
</tr>
<tr>
<td>Temephos Eledabi</td>
<td>-0.918</td>
<td>0.559</td>
<td>Y = -0.92+0.56X</td>
<td>44.566</td>
<td>10665.89</td>
</tr>
<tr>
<td>Temephos Harob</td>
<td>-0.956</td>
<td>0.575</td>
<td>Y = -0.96+0.58X</td>
<td>44.266</td>
<td>8982.971</td>
</tr>
</tbody>
</table>
CHAPTER FIVE

5. DISCUSSION

5.1 Abundance and Distribution of Anopheline Larvae

The study of biology, ecology and species composition of Anopheline mosquitoes is pretty important for the malaria surveillance and control programs. The fauna of Anopheline larvae species in Jazan region in the present study revealed four Anopheline species, which includes *A.dthali*, *A.arabiensis*, *A.pretoriensis* and *A. multicolor*.

*A. dthali* Larvae were found to be the predominant Anopheline species in Jazan region, where they represent 94.8 and 92.5% of the total Anopheline larvae collected from Eledabi and Harob areas, respectively. This finding is reported by ALsheikh (2011) who mentioned that the *A.arabiensis* and *A.dthali* were the predominant species in Jazan region. *A. dthali* has wide distribution throughout the plain and mountainous areas of the two study areas, a finding which is in consistence with Hamid *et al.* (2014) and Vatandoost *et al.*, (2007) who reported the spread of the species in the costal and mountain areas of Iran.

The present study revealed that drainage valleys, foot prints, sorghum farms and water tanks, were important mosquito breeding habitats. The results indicated that the Anopheline larval abundance in the two study areas is depending on the habitat suitability, habitat stability, vegetation covering the habitat and rainfall intensity. In contrast, habitats along the valleys created by human activities such as construction of water-retaining structures (Oqoum) and stagnant pools were suitable for mosquito breeding during the dry-season when the water levels and water flow were reduced. In addition, when it rains heavily, flooding would occasionally occur especially in habitats along the valleys rendering the habitats unsuitable for mosquito breeding. This was more facilitated by the topography of the area which is descended from Southern Mountain extended from Yemen border to Northern part of the Jazan region.

Results also showed that in Eledabi and Harob the permanent habitats constituted about 63% and 76% of breeding site, respectively and were more preferred breeding habitats for *A. dthali*. Conversely, in clear and turbid water *A. dthali* larvae occupied
temporary and permanent habitats equally. These results are similar to reports by Fillinger et al. (2004), who found that semi-permanent and permanent habitats were suitable for both Anophelines and Culicines. The temporary habitats due to their transient nature hold water for a shorter period of time whereas permanent habitats hold water for a long period of time and after the rains, and were the most preferred habitats by mosquitoes.

In the present study, when different ecological habitats, e.g. natural and artificial breeding site for Anopheline mosquito were visited, the highest density of larvae was observed in valleys. In these habitats, A. dthali was found active throughout the year with one peak in December. In spite of the natural role of rainfall which is known to be the primary source of the larval habitat (Minakawa et al., 1999), the abundance of A. dthali was not correlated with rainfall it was found all over the year. This was most likely because of two reasons; firstly the breeding sites for this species were sustained by perennial flowing of water from high area (mountains) in Yemen passed through valleys which ends with the red sea; and the second reason is that the rainfall can negatively affect mosquitoes by flushing their eggs and larvae, and also by killing them directly (Paaijmans et al., 2007). In Eritrea, Shililu et al. (2007) stated that mosquito breeding significantly decreases with an increase in rainfall.

It is well known that each Anopheline species has its own preferred breeding sites for oviposition depending on factors, e.g. weather conditions, physical geography and human activity (Liu et al., 2012). It was observed that the A. dthali larvae prefer to breed in valleys edge and plots of sorghum cultivation. This observation is similar to those raised by Patton (1905), Gill (1916) and Vatandoost et al. (2007). In the present study, more than 83% of A. dthali larval habitats were found continuously exposed to the sunlight which provides suitable habitats for larval growth and oviposition by gravid females (Mutuku et al., 2006 and Minakawa et al., 2006).

Vegetations cover or under the water surface of breeding habitats were not found to affect the presence of A. dthali when compared to habitats in the two study areas this suggested that grass and vegetation in general protect mosquito larvae from being swept or flushed away by running water and/or predation (Minakawa et al., 2004 and Mutuku et al., 2009). These results are supported by experiments of Paaijmans et al. (2007) who reported that precipitation flushed ejected and killed A. gambiae larvae in a semi natural environment. However, these results contradicted
the results of Amani et al. (2014) who found that *A. dthali* preferred mostly larval nests without vegetations.

Regarding the pH, *A. dthali* larvae were found in water with pH ranging from 7.2 to 7.9, TDS ranged from 224 to 1145 ppm and temperature of 27.5 ºC. The mosquito also showed tolerance to salt water in the laboratory. These findings coincide with the work of Manouchehri and Rohani (1975) and Azari-Hamidian (2011) who reported the presences of *A. dthali* in water with high salinity (180–392 mg/L) and temperature ranging between 13 ºC and 28 ºC with pH of 6.9-8.0.

Few mosquitoes breed almost exclusively in brackish or salt water, while some species are less specific in their requirements and can inhabit a wide range of breeding habitats (Abeku et al., 2003; Minakawa, 2012, and WHO, 1975b).

In the two study areas, *A. arabiensis* was found abundant and widespread with *A.dthali*; both species were co-existed in the same habitats. In a previous study, AlSheikh (2011) had collected *A. dthali* larvae from valleys in Jazan region from the same habitat of *A. arabiensis*. The suitability of the available surface water for breeding of different Anopheline species may further be affected by light, shade, emerging vegetation, temperature, salinity and other physiochemical characteristics, competitors, predators and parasites (Wernsdorfer and Gerger, 1988).

Generally speaking, as an Anopheline larva, *A. dthali* was collected from clear or turbid water, sunny stagnant permanent water, sandy, muddy and rocky bottom breeding site, and saline water. It was also found in shallow and slow current water with or without algae and vegetations. This result agreed with the findings of Patton (1905), Gill (1916), Manouchehri et al. (1975) and Service (1989). Comparison of the present study results with the aforementioned researches suggests the differences or similarities in the characteristics of the larval habitats of *A. dthali*.

5.2 Abundance of Adult Anopheline mosquitoes

Information about the Anopheline species and their biology, abundance and behaviour are essential in understanding their role in malaria transmission and for malaria surveillance and control programs. Entomological investigation of Adult Anopheline in the two study areas revealed the presence of three Anopheline species; *A. dthali* (62.2%), *A. arabiensis* (32.2%) and *An. pretoriensis* (5.6%). This result is relevant to the finding of Alsheikh (2011) who reported the presence of the adult *A.
A. dthali and A. pretoriensis in the Jazan region. In spite of the presence of A. multicolor larvae in the two study areas, yet, no catch of its adult were recorded.

Results indicated that A. dthali is the predominant species in the two study areas where it comprised 63.9% and 60.4% of the total adult Anopheline mosquitoes collected from Eledabi and Harob, respectively. This abundance in the two study areas is governed by the availability of breeding sites and favorable environmental conditions such as rainfall, temperature and R.H.

The mean amount of rain that fell in Jazan during the study period was 10.1 mm/year. The maximum amount of rains recorded in Oct. 2014 (63.2mm) and the minimum was recorded in Feb. 2015 (0.001mm). The mean temperature recorded during the study period was 31.2°C, while the maximum temperature was 39°C recorded in Aug. 2014 and the minimum temperature (22.5°C) in Feb. 2015. The mean R. H. recorded during the study period was 64.7%. The maximum (91%) was recorded in Sept. 2015 and the minimum (30%) in June 2015 (Appendix 2).

A. dthali spread in plain and mountain areas of the two study areas throughout the year and peaked in Oct.

A. dthali is widespread in semi-arid parts of the WHO Eastern Mediterranean region. It is found in north Ethiopia and Somalia, Socotra, north Africa to north west of Pakistan, Southwest of Saudi Arabia, around the Red sea and Adan Gulf (Christophers, 1933; De Meillon, 1947; Stone et al.,1959; Gillies and Meillon, 1968), in Iran (Manouchehri and Rohani, 1975), Eritrea (Shililu et al., 2003), and Sudan (Eltayeb et al., 2011).
5.3. Dispersion of *A. dhali*

Results of the dispersion of *A. dhali* indicated that 76.7% of the *A. dhali* females were found in houses close to the breeding sites, (at a distance of < 200m), 18.7% in houses far from breeding sites (between 200m-500m), and 4.6% in houses further than that (500m -1000m) table 12. There was none of *A. dhali* mosquitoes observed in the houses at a distance >1000m. Thus, it can be concluded that the adult population of *A. dhali* was distributed in the area according to distance from breeding sites, and in clusters around the breeding sites ($P < 0.05$). This finding is in agreement with the results of Service (1993) who mentioned that the population size of *Anopheles* mosquitoes decreases with an increasing distance from their source of breeding places or release points. The main reason governing the dispersion of *A.dhali* in these sites may be the habit of the population live mostly in villages or small hamlets which are widely scattered and situated along the valleys, and seldom lie further than 1km from the valleys which enhanced the dispersion of emerging female *Anopheles* mosquito and oriented by hosts' stimuli to the areas where hosts are available. This result is relevant to WHO (1975b) findings which indicated that the tropical *Anopheles* mosquitoes apparently fly within a range of 1-3 km. As most of villages are located in a buffer of 1km from the permanent/seasonal valleys, close contact of human-*Anopheles* is inevitable. Thus the two study areas with specific geographical, topographical and economical situations have great potentials for the development of mosquitoes and malaria transmission.

Flight distance differs among the *Anopheles* species. In Senegal for example, a significant decrease in the density of *A.arabiensis* with an increase in distance up to 910m from a permanent marshy area was observed (Trape *et al*., 1992). In Sri Lanka, a marked number of *A.culicifacies* were caught at 498 m from a release point within a day after marking (Curtis and Rawlings, 1980), while in Burkina Faso, the mean distance moved by individual *A.arabiensis* and *A.gambiae s.s.* ranged from 350-650m (Costantini *et al*., 1996). In contrast, under a condition with a very low wind speed, mosquitoes can detect air-carried, host-specific odors from a distance and orient themselves to the host by flying upwind (WHO, 1975b).

Knowledge regarding the dispersal of adult vectors from their breeding sites helps to identify areas where control methods are better applicable.
The movement of mosquitoes is governed by number of factors, including temperature, humidity, host attractiveness and the attractiveness of breeding sites depending on their physiological conditions. The flight of gravid *Anopheles* female mosquitoes to breeding places is stimulated by fully developed ovaries and the characteristics of the breeding site, as they disperse in the direction of post emergence or oviposition, resting, feeding, daytime resting and breeding sites (Service, 1993).

### 5.4 Abdominal appearance of *A. dthali*

The percentage of the fed *A. dthali* females collected from the two study areas was higher (47.5%) than unfed ones (33.2%), which is mainly due to the availability of the host both indoor and outdoor without any protecting barriers. Campbell *et al.* (1987) and Sloof (1989) reported that the regular use of IBN which acts as a biting barrier was an effective means of reducing mosquito bite on sleeping persons. The proportion of gravid female to the fed female (116/657) in the two study areas was 1:5.7 (>1/5th of the fed females). This may be due to the fact that the female avoiding mechanism for the impact of IRS by changing their behaviour to outdoor feeding (exophagy) and outdoor resting (exophily) and/or due to the killing effect of IRS and space spraying targeting the resting places of mosquitoes. This result agree with the findings of Russell *et al.* (2013), Reddy *et al.* (2011) and Bugoro *et al.* (2011).

### 5.5 Resting and feeding behaviour of *A. dthali*

Under natural circumstances where the majority of the hosts (Human and animal) is domestics and kept in or near human dwellings at night, a high proportion of *A. dthali* females feed and remains there to rest for whole gonotrophic period (Manouchehri and Rohani 1975).

In this study, outdoor collection was significantly higher than the indoor collection with a noticeable preference to animal shelter (P <0.01). These results indicate that the *A. dthali* was highly exophilic and zoophilic. It prefers to feed outdoor on animal and human (animal keepers used to sleep out their hut near the sheep shelter) thus mosquitoes feeding behaviour may be governed by human sleeping behavior. This finding is in line with Service (2008), who stated that the behaviour of both people and mosquitoes is relevant in malaria transmission. During the hot-and dry-seasons, a substantial number of people may sleep outdoors and as a
result, be bitten more frequently by exophagic mosquitoes. Some mosquitoes bite predominantly in forests or wooded areas, so people will only get bitten when they visit these places (Service, 2008). Gillies and De Meillon (1968) reported that in southern Arabia, Mauritania and Somalia the adults are not uncommon indoors.

Although *A. dthali* adults in the two study areas were highly exophilic, there was some degree of tendency to being endophilic (Table 4.9). This phenomenon of the *A. dthali* in the two study areas exhibited the highest number of mosquitoes resting outdoors in animal shelters and animal’s food store room. This finding is in agreement with the work of Manouchehri and Rohani (1975) who found *A. dthali* to be more prevalent in animal shelters, tent and human dwellings. No mosquitoes were collected from the moderate building with Freon air conditioners.

**5.6 Host-preference**

The identification of the blood meal source of freshly fed female mosquitoes remains important to understand their host-preference and vectorial role (WHO 1976b). Many female *Anopheles* mosquitoes bite humans to obtain a blood meal, and a few feed on humans in preference to animals (Chwatt 1980 and Pates *et al.*, 2001). According to Takken and Verhulst (2013), host-preference is defined as the trait to preferentially select certain host species above others. This selective behaviour has a great influence on disease transmission.

Results of the PCR for identification of the *A. dthali* blood meal sources indicated that 73.5% had taken sheep blood (high zoophilic) and the human blood meal was only 13% (Low anthropophilic behaviour). The mixed meal (human and sheep blood) was 13.5% of the tested *A. dthali*.

The human blood index (HBI) in Harob and Eledabi is 11% and 15%, respectively. This result showed a low anthropophilic behaviour of *A. dthali* in the two study areas, a result which agreed with the findings of Bruce-Chwatt *et al.* (1966) who reported 4 - 18.7% HBI of *A.dthali* from Morocco and Saudi Arabia. The results were also relevant with those reported from Iran; for instance Edrissian *et al.* (1985) reported 12.5% HBI in south of Iran and Manouchehri *et al.* (1972) reported 20.8% HBI from north Bandar Abbas. This result confirmed that the host-preference in the two study areas depends not only on the innate host-preference of the mosquito species, but also is determined by other factors including; the human outdoor sleeping behavior in the vicinity to the sheep shelters, favorable climatic conditions in the two
study areas, and vector control interventions. This result was in agreement with the work of Takken and Verhulst (2013) and Smallegange et al. (2010) who stated that host-preference resulting from selective behaviour may be attributed to extrinsic and intrinsic factors.

5.7 Entomological Inoculation Rate (EIR)

The results showed that the annual EIR was higher in Eledabi (1.8 ib/p/yr) by 2.6 folds than in Harob (0.7 ib/p/yr). The calculation of the annual EIR in the two study areas was 1.1. It is worthy to mention that only the annual EIR of less than one could reduce parasite rates to a level that could interrupt malaria transmission (Beier et al., 1999). Based on that, it can be speculated that Eledabi area is more at risk of malaria transmission than do Harob area. This necessitates the prompt preventive measures by the public health authorities in Eledabi to avoid high malaria transmission and hyper-endemicity in the area.

5.8 Longevity of A. dthali:

The tracheation method distinguishes between nulliparous (tightly coiled tracheols) and parous (stretched tracheols) (Ungureanu., 1974), which is relatively faster and easier was used in this study. The proportion of paraous mosquito was expressed as noted by MacDonald (1957).

In Eledabi, the proportion of paraous A. dthali females was found as 1.2 fold more than in Harob, and the probability of the survival in Eledabi was found double that of Harob area.

One of the entomological determinants in the transmission of malaria in an area is the age status of individual females in the population of the vector. Age also indicates the efficacy level of VC interventions in an area (Warrell and Gilles 2002).

5.9 The sporozoite rate (SR):

The results showed that only four mosquitoes were found infected with sporozoite (3 from Eledabi and only one from Harob) giving SR of 0.02 and 0.01 in Eledabi and Harob, respectively.

The potentiality of A. dthali to complete the extrinsic cycle of malaria parasite was confirmed by several studies, i.e. in Somalia, Rishikesh (1961) found one specimen of A. dthali with sporozoite-positive glands among 14 dissected females. In Bander Abbas IPHR (1965) reported two positive females of A. dthali to sporozoites
infection. Manoochehri, et al. (1972) and Manouchehri and Rohani (1975) stated that A. dthali was repeatedly found infected during 1965-1967, with SR reported in three different areas as 1%, 2.1% and 7.7%. Also one specimen with sprozoite-positive was reported in each of Anseba and Gash-Barka (SR = 0.45) areas of Eretria (Shililu et al., 2003; Shililu et al., 2004).

5.10 Vectorial Capacity of A. dthali:

This is the first study done on the vectorial capacity of A. dthali in Saudi Arabia as well as over the entire world. The previous studies were concentrated on the presence of the sporozoite in the salivary gland of the A. dthali. Determining the vectorial capacity of A. dthali in the present study can fill the gap of information and knowledge in this important field of applied medical entomology and malaria epidemiology.

The vectorial capacity for A. dthali in this study was found to be 0.0004 and 0.0001 for Eledabi and Harob, respectively. This low vectorial capacity of A. dthali in the two study areas could be attributed to the extensive VC activities using IRS and ITNs as recommended by the WHO.

Considering the paucity of work ever done to determine the vectorial capacity of anopheles species in the world, especially A. dthali, these findings could serve as a reference point for any further researches.

5.11. A. dthali whole genome sequencing:

This is the first whole genome sequencing of the A.dthali established in all over the world and it was registered in the National Center for Biotechnology Information (NCBI- https://www.ncbi.nlm.nih.gov/) under the following code numbers:

Run: SRR5585848
Biosample: SAMN06909325
Bioproject: PRJNA385755

Establishing the whole genome sequencing of A. dthali in the current study is intended to fill the gap of knowledge in this important field of applied molecular biology, medical entomology, and malaria epidemiology.

The importance of the A. dthali whole genome sequencing is that it will give the answers to what is the gene responsible for disease transmission, making primers
for identification of *A. dthali*, studying its behaviour, investigating the genes responsible for insecticide resistance, and it could be considered as strong base for other genetic studies and researches on *A. dthali*.

### 5.12. Profiling of adult *A. dthali* resistance test to various insecticides

In this study, *A. dthali* showed full susceptibility to DDT in Harob and Eledabi (100% mortality). These results contradicted the results of the efficacy of some pesticides to *A. dthali* in Iran. For example, *A. dthali* was found to be tolerant to DDT and propoxur in some parts of Iran (Fathian *et al*., 2015), while, the adults were resistant in other parts of the country (WHO, 1992a).

The results also exhibited the possibility of resistance and resistance to dieldrin (91 and 89% in Harob and Eledabi, respectively). This finding agrees with the previous studies in Saudi Arabia and Pakistan, which reported resistance of other Anopheline species (*A. fluviatilis*) to dieldrin (WHO, 1992a).

The present study revealed the full susceptibility of *A. dthali* to permethrin and deltamethrin in the two study areas (mortality 100%). The same results were reported for deltamethrin in Iran, whereas Permethrin had high irritancy effect on *A. dthali* (Fathian *et al*., 2015 and Vatandoost *et al*., 2005).

Results also showed that *A. dthali* was found susceptible to Lambda-cyhalothrin and cyfluthrin in the two areas although with little variations in mortality. This finding completely coincides with the results obtained from Iran (Fathian *et al*., 2015).

The bioassay results of susceptibility of *A. dthali* to pyrethroids in this study were further confirmed by the *kdr* results of PCR which showed that no gene resistant-mutations were found among the specimens analyzed for *kdr* (L1014F or L1014S).

The current study revealed a full susceptibility of *A. dthali* mosquitoes to malathion in the two study areas similar to that attained in Iran (Fathian *et al*., 2015). Whereas the mosquito exhibited possibility of resistance to fenitrothion (mortality 91.25 and 93% in Harob and Eledabi, respectively; table 21 and 22), a finding which agrees with the results obtained by Vatandoost *et al.* (2005) who reported resistance of *A. dthali* to fenitrothion.

Globally, *A. dthali* is found resistant to chlorpyrifos, fenitrothion, bromofos and some carbamate insecticides in Egypt, and temephos in Jordan (WHO, 1992a).
The enhanced resistance status of *A. dthali* to dieldrin and fenitrothion in this study may be due to previous intensive use of insecticides in control programs against malaria vectors and insecticide application in agriculture (Cross and multiple resistance). However, more investigation for determination of resistance mechanisms is needed. Furthermore, regular monitoring of resistance status by standard bioassay tests and other complementary methods especially in active foci of malaria transmission is suggested.

**5.13. Laboratory Evaluation of Diflubenzuron, Methoprene, Pyriproxyfen and Temephos against *An. dthali* larvae**

Susceptibility of *A. dthali* larvae to the various IGRs larvicides used in this study varied considerably (Table 4.21). However, the order of efficacy for the three IGRs was as follows: diflubenzruon >pyriproxyfen>Methoprene. It seems that methoprene had low level of activity against *A. dthali* in the two study areas.

Diflubenzruon is among the chitin synthesis inhibitors (CSI) which is highly active against mosquitoes and can provide practical control at the rates of less than 1-2 g/h of active ingredient. Treated larvae die during ecdysis; the molting larvae fail to completely shed the old cuticle. Apparently, due to inhibition of chitin deposition, the larvae do not have the rigidity to get out of the old cuticle (Mulla, 1995).

Pyriproxyfen is a juvenile hormone analogue (JHA) that acts as an IGR. Pyriproxyfen generally inhibits adult emergence of target insects (Anon., n.d.).

Methoprene is considered among the juvenoids hormones or JHAs analogues and mimics have been evaluated in VC programs in USA. Its use in the earlier years was geared primarily to control *Aedes* larvae. Due to its low stability in aquatic habitats, its use against *Culex* and *Anopheles* species was minimal (Mulla, 1995).

One common characteristic of IGRs is that they do not induce rapid mortality in the treated larvae. The active ingredients enter the insect body either through the cuticle or by ingestion or by both modes. Larvae receiving lethal doses do not die outright. With some compounds such as the juvenoids and their analogues, the larvae survive and suffer mortality in the pupal stage or if they do survive, then mortality may occur in the adult stage during eclosion (Mulla, 1995).
Results of Temephos against *A. dthali* larvae showed an equal toxicity in the two study areas as indicated by LC$_{50} = 44.566$ppm in Eledabi and 44.266ppm in Harob, a finding that reflected the resistance of *A. dthali* to this chemical. The prolonged use of Temephos as a larvicide in Jazan region since 1986 could be one possible reason. Resistance to Temephos was detected in the region at 2003 (Alsheikh *et al.*, 2016).

Pyrethroid insecticides are used as indoor residual sprays in Jazan region. As our results showed that the *A. dthali* as a secondary malaria vector in the region is still susceptible to pyrethroid insecticides, therefore, it is proposed that the use of pyrethroids with low irritancy effect in rotation with carbamate insecticides (*e.g.* bendiocarb) or IGRs (*e.g.* diflubenzuron) in the interval of seasonal peaks of malaria transmission in the region.
CHAPTER SIX

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

- *A. dthali* was found to be the most predominant Anopheline species in the two study areas and it was often collected from valleys with slow current water.

- This species also proved to be a secondary vector of malaria for the first time in Jazan region and the KSA. Thus, the incriminating of the *A. dthali* as a secondary vector of malaria, along with its high density in vast areas of the region would create a burden to the health authorities and necessitate the periodical surveillance of the *A. dthali* and prompted preventive and control measures.

- The vectorial capacity of *A. dthali* was determined in the current study for the first time in Saudi Arabia and over the entire world.

- The vectorial capacity was found to be very low in the two study areas, nonetheless, and considering the paucity of work done to determine the vectorial capacity of *Anopheles* species in the world, this finding could serve as a reference for the future researches.

- Due to the epidemicity of malaria in the two study areas, some cases of local transmission and focal epidemics have occurred during past years. Based on the findings of this study, the correct time for IRS in the areas under vector control measures can be determined. This will timely suppress the mosquito population and reduce the potential of malaria transmission.

- Also, based on activity of exophilic vectors, using long lasting insecticide treated nets, as well as larviciding should be considered accordingly.

- The importance of establishing the *A. dthali* whole genome sequencing and registering it in the gene bank for the first time in the world is that it will give the answers to some raised questions, e.g. what is the gene responsible for disease transmission? Facilitate the provision of primers for the identification of *A. dthali*, studying its behaviour, investigating the genes responsible for insecticide resistance, and it could be considered for other genetic studies and researches on *An. dthali*. It would also help the researchers to deeply understand the role of this species in the transmission and epidemiology of vector-borne diseases.
6.2. Recommendations

- Because the low current-water valleys serve as the major A. dthali and other Anopheline mosquitoes breeding habitats during the dry-and wet-seasons, larval source reduction (LSR) and management (LSM) alongside those valleys should be adopted in any VC strategies and/or interventions.
- Distribution of LLIN in the epidemic areas and improving animal keeper houses may play a considerable role in minimizing the densities and the bites of A. dthali, and other nuisance biting mosquitoes.
- A study that involves A.dthali whole genome sequencing could be important in helping to describe the genetic role of A.dthali in malaria transmission, assessing the vector genetic role in insecticides resistance or susceptibility, and feeding and resting behavior.
- The vectorial capacity of A.dthali should be noted as they are prevalent in the two study areas and could possibly play a role in malaria transmission in the Jazan region.
- Most of the Anopheles catches were made from animal shelter, animal's food store and animal keeper shelter or huts. This indicates the exophilic habit of A. dthali, so space spray should be oriented to those resting places.
- Studies on EIRs, as well as the resting- and biting behaviors of A.dthali should be considered in control strategies, since they are directly associated with the risk of malaria transmission.
- Seasonal workers from Yemen, Ethiopia and other African countries are recruited in the farming operations and animal rearing in the region. Some of them are malaria parasites' carriers and can infect the local vector mosquitoes and spread the epidemics; special attention should be paid through passive detection and treatment of the infected immigrant population through quarantine regulations at the borders plus the provision of LLIN.
- The use of pyrethrins with low irritancy effect in rotation with the carbamate insecticides (bendiocarb) or IGR (diflubenzuron) in the interval seasonal peaks of malaria transmission in the region is highly recommended.
• It is highly recommended to periodically evaluate the susceptibility status of the malaria vectors against different larvicides for resistance monitoring.
7. References


Garnham, P.C.C., 1966. Malaria parasites and other haemosporidia. *Malaria Parasites and Other Haemosporidia*. 161


IPHR (Institute of Public Health Research), 1965. Annual entomological studies in Hormozgan province. Annual reports of IPHR, Bandar Abbas, souyh of Iran.


**Malaria:** Life Cycle of the Malaria Parasite. At [http://www3.niaid.nih.gov/topics/Malaria/lifecycle.htm](http://www3.niaid.nih.gov/topics/Malaria/lifecycle.htm).


**Minakawa, N., Mutero, C.M., Githure, J.I., Beier, J.C. and Yan, G., 1999.** Spatial distribution and habitat characterization of anopheline mosquito larvae in


Newton, R.C., 1895. Some Observations which Appear to Establish the Aerial Transportation of Malarial Germs. *Transactions of the American Climatological Association*, 11, p.91-111.


prevalence of Plasmodium falciparum among children under five years in areas with highly resistant malaria vectors. *Malaria journal, 13*(1), p.76.

**Torr, S.J., Mangwiro, T.N.C. and Hall, D.R., 2006.** The effects of host physiology on the attraction of tsetse (Diptera: Glossinidae) and Stomoxys (Diptera: Muscidae) to cattle. *Bulletin of entomological research, 96*(01), pp.71-84.


WHO, 1975b. Manual on Practical Entomology in Malaria. Part (II). Methods and
Techniques. Division of Malaria and Other Parasitic Diseases, World Health

WHO, 1982. Manual on environmental management for mosquito control, with
special emphasis on malaria vectors.

WHO. 1992. Entomological field techniques for malaria control. Part I, Learners

committee on vector control. WHO Tech Rep Ser. No. 818.

In Entomological laboratory techniques for malaria control.


Organization.

malaria/docs/MCT_workingpaper.pdf.

WHO, 2006a. Pesticides and their application: for the control of vectors and pests of
public health importance.

WHO, 2006b. Indoor residual spraying: use of indoor residual spraying for scaling up


Organization.


WHO, 2013e. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes.

WHO, 2016. Monitoring and managing insecticide resistance in Aedes mosquito populations Interim guidance for entomologists


8. Appendices

Appendix (1)

Appendix (1.1) Species composition of Anophelinae mosquitoes larvae in (Harob)
Appendix (1.2) Species composition of *Anophelinae* mosquitoes larvae in (Eledabi)

Appendix (1.3) Abundance of *A.dthali* larvae in Eledabi and Harob
Appendix (1.4) Species Composition of Adult Anophelinae Mosquitoes collected from Harob

Appendix (1.5) Species Composition of Adult Anophelinae Mosquitoes collected from Eledabi
Appendix (1.6) Resting Habit of *A. dthali* in Harob and Eledabi

Appendix (1.7) Dispersion of *A. dthali* from breeding sites
Appendix (1.8) Feeding preference (Blood meal) of A. dthali
Appendix (1.9) Detection of Sporozoite and annual EIR in *A. dthali* collected from Eledabi and Harob
Appendix (1.10) Susceptibility test of Adult An. dthali

Appendix (1.11) LC₅₀ of IGRs tested against A. dthali larvae
### Appendix (2) Metrological data of Jazan (Oct. 2014 – Sept. 2015)

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