FIELD EVALUATION OF TEMEPHOS AND AGNIQUE® MMF AGAINST IMMATURE STAGES OF ANOPHELES ARABIENSIS PATTON (DIPTERA: CULICIDAE) THE VECTOR OF MALARIA IN KHARTOUM, SUDAN

BASHIR ADAM ISMAIL M. AHMED

UNIVERSITI SAINS MALAYSIA
2007

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By

BASHIR ADAM ISMAIL M. AHMED

Thesis submitted in fulfillment of the requirements for the degree of Master of Science

July 2007

DEDICATION

To

My beloved father Adam and uncle Osman

My lovely wife Nafisa and son Emad

Brothers and sisters

Bashir
July 15, 2007

ACKNOWLEDGEMENTS

Grateful acknowledgment to the Khartoum State, Ministry of Health, for giving the opportunity and fully sponsored me to do my postgraduate studies.

I would like to express my sincere gratitude and deepest appreciation to my supervisor, Dr. Abu Hassan Ahmad, Dean and Professor of Entomology, School of Biological Science, USM, for being kind and helpful, his step-by-step follow up, guidance, advices, and encouragement were of great importance for me to carry out this study. My sincerely thanks also due to Co- supervisor, Dr. Abd Wahab Rahman, Professor of Parasitology, School of Biological Science, USM, for his critical revision and valuable comments.

My special thanks and warmest congratulate to my local supervisor, Dr. Osman Abd Elnour, Professor of Medical Entomology, the head of National Health Laboratory, for his being supportive, constructive, kind and wonderful cooperation during the various phases, organized, designed, and until accomplishment of this work.

I am greatly indebted to, Dr. Talal F. Mahdi, the D. G. of KSMOH, for his support and warmest welcome to my supervisor, Dr. Abed Alhameed D. Nugud, of Sudan National Medical Entomology lab., Dr. Arshad Ali, Professor of Aquatic Entomology & Ecology, University of Florida and Dr. El-fadol O. M. Ali, the Director of Environmental Health (FMOH), Dr. Tarig A. Abdu Rahiem, the Director of Preventive Medicine (KSMOH) for their valuable discussions, advices and constructive comments.

I would also like to thanks Mr. Salah, M. Al-Khalifa the director of Khartoum Malaria Free Initiative, Mr. Ibrahim H., Mr. Mohamed A., Mr. Musab B., Mr. Jamal A., Mr. Yasir G., Mr. Osam M., Al tuhami, A. & all my colleagues in KMFI for their various forms of help, assistance and encouragement to accomplish this study.

I am gratefully appreciated the role and efforts of technical team of the Medical Entomology, Mr. Osman, N., Gasim, M., Mahmoud, A., Gallal, M. & Hassan, A., for the wonderful help during the field and laboratory works. Thank to Dr. Hamza & Miss Hind from KMOH Public Health Laboratory especially for kindly analysis of water samples. A special thanks to Dr. El-Hameem M., the General Manager, of El-Seilate Agricultural Scheme who kindly give me space & permission to conduct this study, and his staff Abd Elrahman and Bakhat, for their continuous co-operation. Special thanks towards Mr. Hazrie from USM Entomological Laboratory, for his various kinds of assistance.

I would also like to express my sincere and grateful appreciation to my wife and son for their kindness, hospitality and sacrifices during my extended leave away which are of great meaning and value for me to go a head. My profound appreciations also extended to my father and brothers Hamza, Mohammed and my sisters Asmahan, Rugeia, Uncle Osman Abd Allah and all my extended family for their encouragement, moral support and prayers.

Finally, an overall I thank God "Allah" who bestowing me health, and availing me the strength and patience to complete this study.

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ABSTRACT

Two field experiments on mosquitoes were conducted in stimulated ponds at Al-sielate Agricultural Scheme of Bahary locality, Khartoum, Sudan, for a period 13 weeks (1st trial) and 9 weeks (2nd trial). A total of 30 and 25 ponds were utilized in completely randomized block design for first and second trial, respectively. Mosquito larvae and pupae were sampled using the dipping method.

The main objectives of these trials were to evaluate the efficacy and residual activity of temephos (Terminate® 50% EC, Zeolite 1% W/W) and Agnique® MMF for controlling mosquito immature stages in the field, and determine laboratory susceptibility status of *An. arabiensis* to temephos.

Anopheles arabiensis was the most prevalent species during the transit dry-wet season (54%) and in the winter season (68%). *Culex* spp were also common, but were much higher during the transition period. Despite, continuous use of temephos for more than two decades in control operations, *An. arabiensis* is highly susceptible to temephos LC_{50} =0.00271 ppm (95% CL: 0.00138 to 0.00411ppm).

In first field study the weekly reapplication of temephos 50% EC, at a rate of 0.5ppm reduced *An. arabiensis* larvae by 99.9% (range: 99.7 to 100%) and *Culex* species 99.1% (range: 95.5 to 100%). At the higher dosage of 1ppm, temephos completely reduced *An. arabiensis* larvae and also gave 99.8% (range: 97.9 to 100%) control of *Culex* spp larvae.

In the second field study, temephos 0.5ppm EC and 1ppm ZG treatments resulted in 100% control of third and fourth instar larvae of *An. arabiensis* up to 12 days, and similar control of *Culex* spp. up to 7days post-treatment. Meanwhile, treatment with 1ppm EC provided 100% control of up to 20 days for 3rd and 4th instar *An. arabiensis* larvae and up to 18 days for 3rd and 4th instar *Culex* larvae.

Agnique® MMF acts faster on 3rd and 4th instar larvae and pupae and affects populations of *Anopheles* spp more rapidly than those of *Culex* spp. However, 6-7 days re-treatment with Agnique® MMF at 0.25ml/m^2 completely eliminated pupae within 24 hour post-treatment, and provided a control ranging between 79.8% and 92.6% of *An. arabiensis* 3rd and 4th instar and 57.5% to 65.6% of *Culex* larvae after 7 days post-treament.

PENILAIAN LAPANGAN TEMEPHOS DAN MMF KE ATAS PERINGKAT IMATUR *ANOPHELES ARABIENSIS* PATTON (DIPTERA: CULICIDAE) VEKTOR MALARIA DI KHARTOUM, SUDAN

ABSTRAK

Dua kajian lapangan telah dilakukan dalam kolam terstimulasi di Skim Pertanian Al-sielate Bahary, Khartoum, Sudan, selama 13 minggu (percubaan 1) dan 9 minggu (percubaan ke-2). Sejumlah 30 dan 25 kolam digunakan dalam rekabentuk blok sepenuh rawak bagi kedua-dua percubaan. Larva dan pupa diambil sampelnya dengan menggunakan penceduk.

Tujuan utama kajian ini adalah untuk menilai keberkesanan, aktiviti temephos (Terminate® 50% EC, Zeolite 1% W/W) dan Agnique® MMF untuk mengawal larva nyamuk, dan status kerentanan *An. arabiensis* terhadap temephos.

Anopheles arabiensis adalah merupakan spesis yang paling dominan pada musim perubahan kering basah (54%) dan pada musim sejuk (68%). Walaupun temephos telah digunakan selama 2 dekad, *An. arabiensis* masih rentan kepada temephos LC_{50} =0.00271 (CL sebanyak 95%: 0.00138 sampai 0.00411).

Pada kajian lapangan yang pertama, penggunaan temphos 50% EC yang diulang setiap minggu pada kadar 0.5 ppm mengurangkan larva *An. arabiensis* sebanyak 99.9% (99.7 ke 100%) dan *Culex* spp sebanyak 99.1% (95.5 ke 100%). Pada dos yang lebih tinggi (1 ppm) temephos merencat perkembangan larva *An. arabiensis* 100% dan larva *Culex* spp sebanyak 99.8% (97.9 ke 100%).

Pada kajian lapangan yang ke dua 0.5ppm EC dan 1ppm ZG menghasilkan 100% pengurangan larva instar III dan IV *An. arabiensis* sehingga 12 hari, dan larva *Culex* spp sehingga 7 hari. Sementara itu, 1ppm EC memberikan 100% pengurangan instar larva III dan IV *An. arabiensis* yang lebih lama sehingga 20 hari dan untuk larva *Culex* sehingga 18 hari

Agnique® MMF bertindak lebih cepat keatas larva instar III dan IV serta pupa, dan memberi kesan ke atas populasi *Anopheles* spp lebih cepat dari populasi *Culex* spp. Namun demikian, semburan berulang setiap 6-7 hari dengan Agnique® MMF (0.25ml/m^2) menghapuskan pupa dalam masa 24 jam dan menghasilkan pengurangan larva instar III dan IV *An. arabiensis* sebanyak 79.8 - 92.6% dan larva *Culex* sebanyak 57.5 -65.6% 7 hari selepas di rawat.

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LIST OF ABBREVIATIONS

Ae.	*Aedes*
An.	*Anopheles*
°C	degree centigrade
cm.	Centimeters
Cx.	*Culex*
DDT	Dichloro-diphenyl-tri-chloroethane
EC	Emulsion concentration
EIR	Entomological inoculation rate
EMRO	Eastern Mediterranean Regional Office
ha.	Hectare
HBI	Human blood index
HCH	Hexa-chlorocyclo-hexane
hr	hour
HSNAS	Headquarter of El-Sielate North Agricultural Scheme
IVM	Integrated Vector Management
IRS	Indoor residual spraying
ITNs	Insecticide treated nets
km	kilometer
KMFI	Khartoum Malaria Free Initiative
KSMOH	Khartoum State, Ministry of Health
LC	Lethal concentration
LLINs	Long-lasting insecticidal nets
mg	milligram

ml	millimeter
MMF	Monomolecular surface film
NMCP	National Malaria Control Programme
OP	Organophosphate
P.	*Plasmodium*
pH	Hydrogen ion concentration
ppm	part per million
RBM	Roll Back Malaria
s.l.	sensu lato
s.s.	sensu stricto
SFMOH	Sudan Federal Ministry of Health
SFNCC	Sudan's First National Communications Committee
SIT	Sterile insect release technique
spp.	Species
SSA	Sub-Saharan Africa
ULV	Ultra-low volume
USM	Universiti Sains Malaysia
UTN	Untreated net
VCRC	Vector Control Research Center
VMD	Volume median diameter
WHO	World Health Organization
WP	Wettable powder
ZG	Zeolite Granules

CHAPTER 1

Introduction

1.1 Malaria in the world

Malaria is by far, the world's most prevalent vector-borne disease. It exists in more than 107 countries especially in Africa, Asia and South and Central America (WHO 2005a). These countries, inhabited by 3.2 billion people, constituting more than 40% of the world's population, are considered to be at risk to malaria infection. The latest report by WHO showed that about 350 to 650 million clinical malaria illness occur annually, of which around 1.5 to 2.7 million people die (WHO 2005a).

Malaria is a parasitic disease, caused by a protozoan of the genus Plasmodium, and is transmitted to human hosts through female Anopheles mosquitoes. Up to date four species of Plasmodium are recognized, *Plasmodium vivax, P. malariae, P. ovale* and *P. falciparum,* of which *P. falciparum* is the most pathogenic (WHO 2005a). It is thought that, malaria is principally a tropical disease as the parasites are unlikely to complete their cycle and hence to further propagate the disease, if temperature drops below 15-19°C. (Craig *et al.,* 1999).

1.2 Malaria in Africa

Malaria is one of most deadly diseases in the African continent. It is thought that 66% of the world's populations at risk to malaria are living in Africa (WHO 2005a). Approximately over 90% of global clinical malaria cases occur in Sub-Saharan Africa (SSA), where around 30% to 50% of all in-patients and 50% of outpatients are attributed to malaria. Furthermore, 80% of global malaria

deaths also take place in the African continent (WHO 2005a). The vast majority of these deaths occur among young children under 5 years old (20.2%) (Snow *et al.*, 2004). Death is not the only problem; serious long-term neurological disabilities, severe anemia and multiple organ failure are experienced in many countries, as a result of infection (Snow *et al.*, 2004).

This high burden of malaria in Africa, however, is related directly to the present of *P. falciparum,* the most deadly malaria parasite species (Snow *et al.*, 2004), co-existing with an extremely anthropophilic and endophilic vector species, namely *An. gambiae, An. arabiensis* (of the *An. gambiae* complex), and *An. funestus*, present in almost all the African countries (Coetzee 2004).

1.3 Malaria in Sudan

In Sudan, malaria is a major public health problem, where the whole country is considered endemic, with varying degrees. In the South, the wet savanna is characterized by stable malaria, while the central and northern parts of poor dry savanna is characterized by unstable malaria, with epidemic-prone areas (Sudan National Malaria Control Programme [SNMCP], Annual report, 2004).

Malaria is a leading cause of high morbidity and mortality in the country, where 75% of the population live in areas of moderate to high with seasonal and/or perennial transmission (SNMCP 2004). Malaria cases account for 20-40% of all attendance at health facilities and about 30-40% of all hospital admission deaths. Every year, an estimated 7.5 million cases and 35000 deaths are reported (SNMCP 2004). These figures represent about 50% of the total reported cases and 70% of total deaths in the Eastern Mediterranean Region of

the World Health Organization (EMRO) (Malik *et al.,* 2003). The magnitude of the malaria problem in the country far exceeds official figures, especially in remote rural areas with poor access to health services (i.e., roughly 20% of rural populations have access) and epidemiological reporting systems are very limited (Sudan National Consultant Committee Annual Report 2003).

Plasmodium falciparum is responsible for more than 90% of malaria cases in the whole country. However, other species such as *P. vivax* (8.2%) and *P. ovale* (6.2%) have been increasingly reported (El-Sayed *et al.*, 2000). In Sudan, although there are 31 species of *Anopheles*, but only 3 of them are known as malaria vectors. *Anopheles arabiensis* is the sole malaria vector, dominating the central and northern parts of the country (Omer & Cloudsley-Thompson 1970; Dukeen & Omer 1986; Himeidan *et al.,* 2004), whereas, *An. gambiae* and *An. funestus* are predominant vectors in the south (Zahar 1974; Petrarca *et al.,* 2002).

1.4 Malaria background in Khartoum

During the early 1900's Khartoum was considered as a malaria-free city (El Sayed *et al.,* 2000). From mid 1970s to early 1999, however, the incidence of malaria has increased dramatically (SNMCP, 2004). The unsustainable control efforts during this period caused some focal outbreaks. Such epidemics occurred in 1988 in Khartoum north and in 1992 and 1998 in Omdurman north and south, and at White Nile province in 1999 (KSMOH, 2006). The epidemic of Khartoum North in 1988 accounted for 25,176 cases and 500 deaths (El Sayed *et al.,* 2000).

The re-emergence of malaria in the following years is attributed to unregulated urban growth, rural-to-urban migration, and rapid expansion in agricultural activities. Such factors often lead to an increase in malaria transmission, because of increased pressure on health services, poor housing and sanitation, lack of proper storm water drainage, and increase of man-vector contact as a result of environmental changes creating more new mosquito breeding sites.

Despite the fact that Khartoum State is classified as an area of unstable malaria with epidemic-outbreak (Malik *et al.,* 2003), malaria incidence is so far a major public and political concern. It still contributes to the morbidity and mortality of the top ten communicable diseases in the state. The official health records for the period from 1999-2005 showed 28.2%, 24.1%, 23%, 21.8%, 19.4%, 15.8%, and 13.7% respectively of overall outpatients are related to malaria. Whereas, inpatient mortality due to malaria for the same period accounts for 31.3% in 1999, 16.4% in 2000, 12% in 2001,and 11.7%, 9.3%, 6.1% and 4.5% for 2002, 2003, 2004 and 2005 respectively (KSMOH, 2006). The significant reduction of malaria burden on health system since 2003 is due to the launching of the Khartoum Malaria-Free Initiative in 2002 (KMFI), as a joint venture between WHO, SFMOH, Khartoum government.

Although the registered data of malaria cases from surveillance systems did not reflect any regular and cleare trend of incidence/ month, malaria transmission is strongly associated with environmental conditions, such as rainfall, temperature, and humidity. Thus, in Khartoum State two peaks of malaria transmission have been observed. The first occurs in the rainy season (July-October) with a peak in October, while the second occurs in dry cool

winter season (December-March) with a peak in March (Malik *et al.,* 2003). During these two peaks of malaria transmission, cases may reach about 30% of total out patients.

Plasmodium falciparum is the predominant malaria parasite, while *An. arabiensis* is the main malaria vector as reported by several investigators in the state (Babekir *et al.,* 2000; Petrarca *et al.,* 2002; Elfadol 2006).

1.5 The rationale of the study

Presently the most effective method available to control malaria is vector control (WHO 1992). This can be achieved either by adulticiding (adult control) or larviciding (larval control). Adult control is the most valuable (e.g. IRS), but the widespread resistance to nearly all insecticide group and/or high insecticide avoidance behaviors in addition to other technical, financial and social factors made this method unpractical and less effective.

Control of malaria problem primarily through larval control is the most logical approach of reducing adult mosquito densities. This is the only time in the insect's life cycle when it is truly limited in mobility, concentrated and most readily controlled (Killeen *et al.,* 2002). These measures include: environmental (source reduction), biological and chemical control (WHO 1997).

Chemical control with temephos (non-systemic organophosphorus insecticide) is worldwide the most preferable agent to control mosquito larvae at a very minimum dose of 56-112ml/ha (WHO 1997).

Khartoum State in the last three years after the launching of the Khartoum Malaria Free Initiative (KMFI) has intensified all methods of control activities, with much emphasis on larviciding. All potential larval habitats,

positive or negative, are treated at six day's interval. In a month about 375-400 liters of temephos were used (KS. Malaria control unit unpublished data).

Most malaria-related studies in Sudan focused either on seasonal transmission /parasite species (Babiker *et al.,* 1998; El Sayed *et al.,* 2000; Hamad *et al.,* 2002; Malik *et al.,* 2003; Elghazali *et al.,* 2003), investigation on the biology, ecology, behaviour and seasonal abundances of malaria vector (Omer & Cloudsley-Thompson 1970; Harridi, 1972a; Duckeen & Omer 1986; Himeidan *et al.,* 2004; Elfadol 2006), adult control (El Gadal *et al.,* 1985; Petrarca *et al.,* 2002), ITNs (Onwujekwe *et al.,* 2005), or insecticide resistance (adult) (Harridi 1972b; Himeidan *et al.,* 2004).

Except for the few works (Balfour 1904, Nugud & White 1982, El Safi & Haridi 1986), field studies on larval control have rarely been reported. It is for that reason the present study is carried out.

1.6 Study objectives

1.6.1 General objective

The overall goal of this study was to evaluate the efficacy and residual activity of various larvicides in controlling *Anopheles arabiensis* larvae and pupae; to elucidate alternative choices of larvicides with new mode of action ready to use at state level, and to update *An. arabiensis* larval susceptibility status to temephos.

1.6.2 Specific objectives

- To assess the susceptibility status of *An. Arabiensis* larvae to temephos.
- To evaluate the efficacy of temephos (Terminate® 50% EC) at an application of 0.5 ppm, when compared to the routinely applied rate of 1ppm on a weekly basis.
- To evaluate the persistence (effective control duration) of temephos (Terminate® 50% EC) at 0.5 & 1ppm, AZAI®-SS (temephos 1% w/w) at 1g/m² and Agnique® MMF at 0.25 ml/m² against the mosquito larvae in breeding habitats.
- To determine the re-application frequency of control materials in the natural breeding habitats of targeted species.

CHAPTER 2

LITERATURE REVIEW

2.1 Malaria vectors in Africa

2.1.1 Distribution

The susceptibility of anopheline mosquitoes to malaria infection is considered as an inherited characteristic, which is not necessarily common to all the species. For an anopheline mosquito to be a malaria vector, it not only has to be susceptible to infection, but also must feed on human blood. Moreover, the longevity of the *Anopheles* has to be long enough as to permit the completion of the sporogonic cycle and their survival for a certain time during infective life (Service & Towson 2002). All the above restrictions limit the possibility of being malaria vectors to just a few species. Up to date there are some 430 *Anopheles* species, of which 70 are known to be malaria vectors worldwide (Phillips 2001).

In Africa there are about 10 anopheline species responsible for malaria transmission (Robert *et al.,* 2003). Mosquitoes within the *An. gambiae* complex (senso lato), in addition to *An. funestus,* are the most important vectors of malaria in Sub-Saharan Africa (SSA) (White *et al.,* 1972; White & Rosen 1973). In relation to the *An. gambiae* complex, there are today at least seven sibling species known, of which two have the larval cycle in salt water (and as a consequence their distribution is limited to the coastal area) and the other develop their larval cycle in fresh water (Hunt *et al.,* 1998).

The salt-water species, *An. merus* and *An. melas* are well adapted to the coast of East and West Africa respectively (Zahar, 1974; Coetzee *et al.,* 2000; Levine *et al.,* 2004). These species do not have a very high vectorial capacity

and therefore are localized vectors (Gillies & Coettzee 1987). In contrast three are fresh water species, notably An. *quadriannulatus* spp. (C & B) and *An. bwambae* (Hunt *et al.,* 1998), which show zoophylic, exophagic and exophylic behavouior, and consequently, play a very minor role in malaria transmission whereas *An. bwambae* is a local vector in a small area in Uganda (White 1974; Coetzee *et al.,* 2000).

The other two fresh water species known respectively as *An. gambiae* s.s. (senso stricto) formerly named species A and *An. arabiensis*, species B, are the most powerful vector in the world (White 1974; Gillies & Coettzee, 1987; Hunt *et al.,* 1998). These two species are responsible for approximately about 80% of malaria transmission in SSA (Levine *et al.,* 2004). They are widespread in nearly all African countries, south of the Saharan; *Anopheles arabiensis* is associated more with dry Savanna and desert parts of Africa (Omer & Cloudsley-Thompson 1970; Dukeen & Omer 1986; Gillies & Coettzee, 1987; Shililu *et al.,* 2003a) whereas *An. gambiae* and *An. funestus* are most abundant in the wet humid areas (White & Rosen 1973; Coluzzi *et al.,* 1979; Coetzee *et al.,* 2000; Fontenille *et al.,* 2004). With regards to *An. funestus*, many varieties are known, but only the typical form proved to be a vector next in importance to *An. gambiae* (White 1974; Gillies & Coettzee 1987).

2.1.2 Seasonal abundance

The relative seasonal abundance and density of *An. gambiae* s.s and *An. arabiensis* appear to be strongly influenced by climatic factors, such as seasonal pattern of rainfall, temperature and relative humidity (Patz *et al.,* 1998). Thus in arid and semi-arid areas of Africa, with a single rainy season, in

the former, densities start to rise sharply soon after the first main falls, reaching a peak in the middle of the rains and declining thereafter, whereas the later lagged for at least two months after rain cessation. Many workers have noted these seasonal variations, for example, in the Sudan, Duckeen & Omer (1986); in Ghana, Chinery (1984); in Senegal, Dia *et al.* (2003); and in Ethiopia Shililu *et al.,* (2003b). A similar pattern can occur on a local scale elsewhere.

In equatorial belts with two rainy seasons, such as Kenya, Cameron and Tanzania (White *et al.,* 1972; Minakawa *et al.,* 2002; Antonio-Nhondjio *et al.,* 2005), the populations of the two species respond with two annual peaks, the highest peak of production following the short rains (October-December) rather than the long rains (March-June).

2.1.3 Feeding and resting habits

There is growing evidence that *An. gambiae* and *An. arabiensis* show different behaviour pattern of host preference and feeding habits throughout Africa. The first is largely anthropophagic and endophilic (Habtewold *et al.,* 2001; Antonio-Nhondjio *et al.,* 2005), whilst *An. arabiensis* tend to show a higher level of zoophily and exophily (Haridi 1972a; Shiliu *et al.,* 2003a).

In Tanzania, precipitin tests of blood-meals among indoor resting mosquitoes caught revealed that out of 1121 *An. gambiae* tested, 91.2% were human-fed and only 8.5% were cattle-fed, whereas, 60.9% and 38.1% out of 1277 *An. arabiensis* caught fed on human and cattle, respectively (White *et al.,* 1972). The opposite was true among the outdoor resting population. Meanwhile, Service *et al.* (1978) observed no significant difference in human blood index

(HBI) between *An. gambiae* and *An. arabiensis* captured indoors in the region of Kisumu in Kenya.

The biting cycle of *An. gambiae* and *An. arabiensis* have been studied on a broad aspect to cover host seeking as well as the act of feeding in many parts of Africa. Its essential features at least in mainland Africa such as Ethiopia and eastern Sudan, are an initial period of very low activity up to 19:00–20:00 h, with a peak occurring between 23:00 and 03:00h (Himeidan *et al.,* 2004; Taye *et al.,* 2006). Similar observations were recorded by Marrama et al. (2004) in Southern Madagascar. However, in most situations these two sibling species show little tendency to bite in the daytime.

2.1.4 Flight pattern

As with many mosquitoes, the prevalence and dispersal rates of *An. gambiae* and *An. arabiensis* from breeding-places vary substantially by location, often by a factor of 1-2 km (Gillies & Coettzee 1987). However, most observations in the African continent documented that dispersal of these species tend to be no more than a few 100 meters from the breeding sites (Coetzee *et al.,* 2000; Levine *et al.,* 2004). While in some situations female anopheline mosquitoes could fly passively (e.g. on airplanes, ships etc.) for some thousands of kilometers, such as the invasion of *An. gambiae* in to the Upper Nile of Egypt and Brazil (Shusha 1948; Killeen *et al.,* 2002).

2.1.5 Breeding places of *An. arabiensis*

All mosquitoes require water to complete their life cycle. Development is of the complete type, consisting of four stages: egg, larva, pupa, and the adult.

The immature stages of both *An. gambiae* s.s. and *An. arabiensis* occur in a great variety of water bodies, of which the most important are small, shallow, completely or partially exposed to sunlight (Charlwood & Edoh 1996; Minakawa *et al.,* 1999 & 2002; Gimnig *et al.,* 2001).

The breeding-places that come within this definition are numerous, and the roles of human and animal activities are implicit in many of these habitats, either directly or indirectly, particularly in Africa. This, of course, is of great epidemiological importance since it brings the vector close to its host. It is a common experience that when the breeding-places are of a temporary nature, such as provided by rain, it appear to be more productive (Service 1971). Because small and sunlit habitats have higher water temperatures, mosquito larval and pupal developmental time may be shortened if the warmer habitats produce more algal food (Giming *et al.,* 2002). Also, the larval predation of mosquitoes is less prevalent in temporary habitats than in large and permanent habitats (Service 1977; Sunahara *et al.,* 2002; Carlson *et al.,* 2004).

In Natal, South Africa, Le Sueur and Sharp (1988), West Kenya, Minakawa *et al.* (1999), Giming *et al.* (2001), and Carlson *et al.* (2004), and in Western Nigeria, White and Rosen (1973), and Okogun *et al.* (2005), detected the larvae of both *An. gambiae* and *An. arabiensis* in small sun-lit pools, shallow grassy marshes, slow moving edges of the stream, large ponds, brick-making pits, tree holes, rock holes, hoof-prints, and artificial habitats such as bore holes and catch basins. Whereas in the central and northern part of Africa, it has been shown to breed in pools left by Receeding Rivers, broken water pipes, open water tanks, ponds, drainage channels, irrigation ditches, car tracks and rainwater collected in natural depressions (Duckeen & Omer 1986; Petrarca *et*

al., 2002; Himeidan *et al.,* 2004; ElFadol 2006). However, consistent differences in habitats used by *An. gambiae* or *An. arabiensis* larvae have not been observed, and both species often have been found occupying the same habitats (White & Rosen 1973; Charlwood & Edoh 1996; Miankawa *et al.,* 1999; Gimnig *et al.,* 2001).

Larvae of *An. gambiae* s.l. have been found breeding in uncommon places. In Sudan, for instance Elfadol (2006), found that *An. gambiae* species (B) breed in domestic clay pots, and in Accra, Ghana, Chinery (1984) found the larvae in domestic water containers, such as barrels, drums, organic polluted ponds, and even in completely darken wells. Whilst, in Malindi, Kenya, Keating *et al.* (2005), noted that water bodies with pollution or floating debris were 14 times more likely to have anopheline larvae, as compared to water bodies identified with no pollution or floating debris.

Large scale irrigation, particularly rice fields, constitutes another prolific source of mosquito-borne diseases in association with human population (Mutero *et al.,* 2004). A flooded or partly flooded field presents a variety of breeding habitats that are often difficult to describe the breeding conditions with any precision. In areas such as Mali and Kenya, (Chandler & Highton 1975; Klinkenberg *et al.,* 2003), the rice field plots are most productive of *An. gambiae, An. arabiensis* and *An. pharoensis* in the first 6 to 8 weeks of the rice cycle. Later on, when the rice is fully-grown, breeding is at a lower level and confined mainly to the margins of the field. However, pockets of breeding may occur even within the rice plots (Briet *et al.,* 2003). Afrane *et al.* (2004) and ElFadol (2006) reported that vegetable irrigated areas in and around cities can provide suitable breeding sites for *An. gambiae* and *An. arabiensis.*

2.1.6 Physio-chemical characteristics

Several workers have tried to define the physio-chemical characteristics of *Anopheles gambiae* complex breeding-places (Le Sueur & Sharp 1988; Minakawa *et al.,* 1999, 2002, & 2005; Gimnig *et al.* 2001; Carlson *et al.,* 2004; Huang 2005; ElFadol 2006). The physico-chemical parameters studied include water temperature, sunlight, water surface area and depth, presence/absence of vegetation and algae, substrate types, pH, dissolved oxygen, conductivity, total dissolved solids, alkalinity, ammonium nitrogen, nitrate nitrogen, calcium, magnesium, carbon dioxide, ferrous iron, phosphate, colour and turbidity. In general, these studies concluded that there are no precise physico-chemical properties that attract the gravid female mosquitoes for ovipositaion. The attractiveness largely depends on the interactions between these parameters and also on the availability of suitable water bodies.

For example, in Kenya, Gimnig *et al.* (2001), recorded larvae in ponds at water temperatures of 25.7 to 27.1°C, while Tuno *et al.* (2005), gave a range of 31.6 to 34 ° C as optimal for larval development. Most observations noticed that the thermal death-point is in the range 41 to 42° C. A range in water pH value of 7.2 to 9.3 with a mean value of 8.2 has been recorded in harboring immatures *An. gambiae* and *An. arabiensis* (Le Sueur & Sharp 1988), but larvae have been found in natural water with pH reading as low as 4.0 (De Meillon 1951). Some studies showed that *An. gambiae* and *An. arabiensis* larvae were associated with highly turbid water, with algae and little or no aquatic vegetation (Gimnig *et al.,* 2001; Ye-Ebiyo *et al.,* 2003), while other studies recorded presence of algae and vegetation as limiting factors (Mutero *et al.,* 2004; Fillinger *et al.,* 2004).

The question of the chemical content of potential waters has been investigated by several authors (Le Sueur & Sharp 1988; Gimnig *et al.,* 2002; Piyaratne *et al.,* 2005; Sanford *et al.,* 2005), but no precise conclusions were drawn beyond the fact that it can be highly variable.

The size and persistence of the breeding sites in addition to substrate colors are of critical important in attracting gravid mosquitoes to lay their eggs. In an oviposition experiment, *An. gambiae* laid significantly fewer eggs in the rainwater with backswimmers and tadpoles than in rainwater without these predators (Munga *et al.,* 2005). The occurrence of such predators is an indication of the persistence of such larval habitat. Moreover, female *An. gambiae* laid more eggs in dark than white substrates against a light background (Huang *et al.,* 2005).

2.1.7 Larval seasonal abundance

Studies that have investigated the seasonal fluctuation in larval abundance and species composition in more longitudinal surveys have related the changes in the two species to rainfall patterns. A long-term survey of one to three years period (Le Sueur & Sharp 1988; Gimnig *et al.,* 2001; Marrama *et al.,* 2004) showed that the proportion of larvae of *An. gambiae* relative to that of *An. arabiensis* was much greater during and immediately after the rains compared with abundance of the latter with ceasetion of rains in the drier periods.

2.2 Malaria vector control

Since the discovery of the relationship between *Anopheles* vectors and malarial transmission in 1897, vector control strategies have been the most widely used as the malarial control measures (Mabaso *et al.,* 2004), and so far it is one of the four basic technical elements of the Global Malaria Control Strategy (WHO 1992). Furthermore, it can add sustainability to strategies of preventive chemotherapy, and may be the most cost-effective option when unit costs of individual case detection and treatment become progressively greater as case numbers drop (WHO 2004).

However, effective vector control strategies are based on four elements; incrimination of vector species, knowledge of vector biology and ecology, surveillance, and implementation of cost-effective control measures (Killeen *et al.,* 2002).

More recently, WHO introduced a more holistic concept known as integrated vector management (IVM) (WHO 2004). IVM is a systematic approach to planning and implementing disease vector control in an inter-sectoral context. It entails the use of a range of interventions of proven efficacy, separately or in combination for the implementation of locally cost-effective control. Generally there are two basic kinds of mosquito vector control. Those directed against the adult and those against the aquatic stages (Curtis 1989).

2.2.1 Adult vector control

Malaria prevention by suppressing adult mosquitoes is generally favoured because moderately reducing adult longevity can radically suppress transmission at community-level (Killeen *et al.,* 2002). This can be achieved

either by: Indoor residual spraying (IRS) with insecticides; use of personnel protection methods including insecticide-impregnated mosquito nets (ITNs); ultra-low volume space spraying (fogging); and genetical control. These vector control methods vary considerably in their applicability and cost, as well as the sustainability of their results (WHO 1973).

Currently, indoor residual spraying (IRS) and impregnated mosquito nets (ITNs) are the main option in malaria prevention. As vector control interventions, both are effective and offer the greatest potential for large-scale reduction of the burden of disease in a range of epidemiological settings, through reducing entomological inoculation rate (EIR) and infectivity of malaria vectors, and hence reduce overall transmission and protect all individuals within a community (Townson *et al.,* 2005).

2.2.1.1 Indoor residual spraying (IRS)

Residual spraying is still the most powerful and feasible method for the chemical control of mosquito vectors of malaria. For the control of other mosquito-borne diseases, the use of this method is rather limited (Mabaso *et al.,* 2004; Conteh *et al.,* 2004).

The technique consists of indoor residual spraying which normally refers to the spraying of insecticides that have a persistent effect on all surfaces where mosquitoes are likely to rest. These include inside walls and ceilings of houses, stables, and the undersides of roof eaves and other structural projections, under sides of beds, tables and other furniture. The attack is mainly directed to endophilic mosquito vectors which frequent human habitations, and bite and rest indoors. These vectors, while resting on the sprayed surfaces, come into

contact with the insecticide and should die before they become infective and able to transmit the disease (WHO 2000). The efficacy and residual effect of an indoor residual spray normally depends on a multiple set of factors (WHO 2000), which include:

i) The properties of the insecticide such as its formulation and stability, the dosage, its mode of action and its effect on the vector.

ii) The nature and types of the sprayable surface which plays a major role in the duration of the residual effect of the insecticide through the absorption and/or adsorption phenomena and,

iii) The spraying cycles in which the time of spraying is most critical when it is necessary to protect an area during transmission season with a single round of spraying.

For the eradication of malaria, which implies the interruption of transmission for a sufficient number of years, the spraying coverage of the structures (mentioned above) should aim at being total, **complete**, **sufficient** and **regular** (WHO 2000). The actual practice in many situations has shown that these requirements are difficult to fulfill, but nevertheless malaria transmission has been successfully interrupted in most situations (Najera 1989). Where such transmission has not been interrupted, it is considerably reduced and with the addition of other measures helped to further reduce or even interrupt malaria transmission (WHO 2000).

Up to date, the following four groups of pesticides have been used in indoor spraying in malaria control programmes.

2.2.1.1(a) Organochlorine insecticides

The major insecticide belonging to this group is DDT (dichloro-diphenyl-tri-chloroethane), a highly effective chemical in controlling insect vectors of human diseases and many crop pests. For many years, DDT played a key role in malaria eradication era of 1950-1960s and early 1970s (WHO 2000). Malaria transmission has been successfully interrupted in U.S.A., former Soviet Union and European countries by means of IRS with DDT. Disease incidence was also significantly reduced in many countries in the tropical region of South-East Asia, India and South America (Najera 1989). According to a recent report, every year an average of 845,036 tonnes of the active ingredient of DDT is used for IRS, accounting for 71% of the total DDT use at global level, of which 63% is used in South-east Asia, 36% in Africa and 1% in American regions (Zaim & Jumblingam 2004).

Other chemicals in this group are benzene hexachloride and the cyclodienes (dieldrin, aldrin and edrin) which are chemically similar to DDT. Previously, dieldrin and aldrin (HCH) had been used effectively for IRS, but their use has been discontinued because of their high acute toxicity to humans. Eventually the use of whole subgroup was discontinued due to the rapid development of resistance to all chlorinated hydrocarbons (WHO 2000).

In sub-Saharan Africa early pilot projects for malarial eradication with DDT also showed highly effective vector control by IRS but transmission could not be interrupted in the endemic tropical and lowland areas. As a result, IRS was not taken to scale in most endemic areas of the continent with the exception of southern Africa and some island countries such as Reunion, Mayotte, Zanzibar, Cape Verde and Sao Tome (Mabaso *et al.,* 2004). The time-

limited eradication policy was eventually abandoned in 1969 and replaced by a long-term Global Malaria Control Strategy in 1992 (WHO 1992).

2.2.1.1(b) Organophosphate insecticides

The development of vector resistance to organochlorine compounds led to the use of the organophosphorus and carbamate insecticides as substitutes for mosquito control. The latter insecticides are more expensive, and least toxic to human, and have a shorter residual effect (2-3 months) than the organochlorine compounds. These three factors contribute to higher operational costs, more frequent cycles of application, greater bulk to be transported, and requiring more safety measures (WHO 2000).

Among organophosphate, Malathion was most widely used for IRS during the period 1970-1980 and early 1990s. Malathion has been used successfully in several anti-malarial programmes, for example in parts of India, Sri Lanka, in Gezera irrigated areas of the Sudan (El Gadal *et al.,* 1985). Zaim & Jumblingam (2004) reported that during 2000 and 2002, about 4,360,502, 308,119 and 255,409 kg, respectively of active ingredient of Malathion was used for IRS by all WHO regions. Fenitrothion is another organophosphorus compound which has longer residual effect than Malathion, but is more costly, and its toxicity limits its use in residual spraying.

2.2.1.1(c) Carbamate insecticides

These chemicals break down readily and leave no harmful residues, and their mammalian toxicity varies from low to high. Among this group, bendiocarb and propoxur are used most extensively for IRS in malaria vector control

programmes. Due to their highly toxic and airborne effect, they have been used since 1979 in large-scale anti-malaria programmes in Central America, India and Iran. However, the high cost of insecticides belonging to this group limits their use and since late 1995 to the present their use is restricted to Central and Latin Americas (Zaim & Jumblingam 2004).

2.2.1.1(d) Pyrethroid insecticides

Pyrethrum is the oldest effective insecticide that has been used for large-scale malaria control by killing the adult *Anopheles*. Currently, pyrethroid insecticides represent important weapon against insect pests of both economic and medical importance. These products show remarkably high toxicity and rapid action against insects and have low mammalian toxicity. It is usually available as solution, emulsion concentrate formulations and even as water-dispersible powders (Zaim & Jumblingam 2004).

Among this group Alpha-cypermethrin, cypermethrin, deltamethrin, lambda-cyhaloyhrin, permethrin and others have been developed and used for indoor residual spraying (IRS), ULV space spraying, and impergenation of mosquito-nets and other materials (Zaim & Jumblingam 2004).

Deltamethrin has been used effectively and still in use in many parts of the world (e.g. Botsawana, Zimbabwe, South Africa and Southern Mozambique) as IRS for controlling malaria at a dosage of 0.05mg/m^2 with residual effect lasting at least 2 months (Mabaso *et al.,* 2004). In contrast, permethrin is a safer pyrethroid (LD50=500g/kg) when applied at a rate of 0.5g/ m^2 and was fairly effective as IRS for about 3 months.

The latest synthetic pyrethroid, discovered in the early 1980s, and introduced as insecticide for agricultural and public health applications, is lambda-cyhalothrin. It is available as 2.5% EC and 10% wettable powder (WP). Its recommended doses for IRS range from 10-25g/ m^2 for prolonged action of 6 months (Zaim & Jumblingam 2004).

Since the 1970s the tendency to use IRS for malaria control has decreased (Beales & Gillies 1988). For example, in Mexico of Latin America, the number of houses sprayed each year for malaria control decreased from 15 million in 1964 to 1.6 million in 1997. Also, there has been a trend to replace DDT by other insecticides such as Malathion, Fenitrothion and Pyrethriods (Zaim & Jumblingam 2004). The use of the Pyrethriods group such as deltamethrine, permethrin, and alpha-cypermethrin are mostly in common use for indoor residual spraying.

2.2.1.2 Insecticide-treated bed nets (ITNs)

Before the development of insecticide-treated nets (ITNs) as a new technology in the mid-1980s, people in many countries were already using nets, mainly to protect themselves against biting insects and for cultural reasons (Mac Cormack & Snow 1986). In response to the increase in malaria morbidity and mortality in the last two decades in sub-Saharan Africa due to civil unrest, resistance to available drugs and insecticides, human migration, deteriorating health system and HIV/AIDS epidemic which consumed much of the resources for malaria prevention, international agencies together with WHO launched the Roll Back Malaria (RBM) initiative, a global partnership for prevention and control malaria (WHO 2000).

The primary goal of RBM is to achieve a 50% reduction of global malaria burden by year 2010. RBM has adopted the use of insecticide treated bednets as a major tool for the achievement of its malaria control objectives (WHO 2000).

However, treatment of mosquito nets with insecticide was probably introduced for the first time during World War II, when nearly half a million cases of American army were infected with malaria. Wider use of insecticide- treated nets began in the 1980s following the development of synthetic pyrethroids which are fast-acting, effective in small quantities, relatively stable, adhere to fabric, and relatively safe to humans (WHO 1992).

Generally bed nets and other materials usually are made of nylon, polyester, or cotton; these coverings are most often treated with permethrin, deltamethrin and lambda-cyhalothrin (Hossain *et al.,* 1989). In treated nets the pyrethroids work in three ways: first, they act as killing agent when the insect makes contact with the insecticide by landing on the net; secondly, pyrethroids have an irritating (excito-repellent) effect and the insect rests only briefly on the treated fabric and thirdly, the formulation in which the pyrethroid is presented contains volatiles that cause deterrence, leading to fewer mosquitoes entering a room where an ITNs is present (Lindsay *et al.,* 1991; Mathenge *et. al.,* 2001). Thus treated nets can also prevent malaria in unprotected individuals by suppressing vector numbers, survival, human blood indices and feeding frequency in local populations (Bogh *et al.,* 1998).

Many field trials around the world have shown that ITNs can produce a significant reduction in malaria morbidity and mortality through substantial reductions of vector density, survival and sporozoite load; others have found

little or no effect on the vector population as a whole (Lindsay *et al.,* 1993; Quinones *et al.,* 1998; Habtewold *et al.,* 2004). For example, in Burundi; deltamethrin-impregnated bed nets have been evaluated in a trial as tool for malaria control in hyper-endemic regions. After introduction of impregnated bednets (intervention area) the proportions of children under 5 years of age with high parasitaemia were reduced by 42 and 53% in the 2 parasitological survey areas, where the average bed net coverage were 55 and 44%, respectively. Whereas, in the control areas of the survey, no significant change occurred during the same period (Van Bortel *et al.,* 1996).

In Tanzania, Abdualla *et al.* (2001) evaluated the protective effect of ITNs in the Kilombero insecticide-treated nets project, and reported that parasitaemia and anaemia in ITNs users (children less than 5 years old) were significantly reduced by 63% and 62% respectively. In the Gambia, the National ITNs Programme achieved an 83% net treatment rate and reported that 77% of children under-5 years old and 78% of women of childbearing age slept under ITNs. Overall mortality in children under-5 year's fell by 25%, and case control studies suggested that there were 59% fewer episodes of uncomplicated malaria in ITNs users (D'Alessandro *et al.,* 1997).

A study in a community based malaria control intervention programme using ITNs in Yombo Division, Bagamoyo District in Tanzania (holoendemic area), revealed that children using nets showed marked improvement in several malariometric indices. Following an initial clearance of parasitaemia with sulphadoxine/pyrimethamine, when compared with unprotected children, those with nets were slower to become re-infected (Relative Risk 0.45), had lower parasitaemia and showed marked improvement in anaemia (RR 0.47). Use of