Sudan University of Science and Technology
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Occurrence and Distribution of Phenotypic and Genotypic Resistant Strain of *Anopheles arabiensis* to insecticides and *Plasmodium* Sporozoites Infection in Khartoum State, Sudan

حدوث و توزيع النمط الظاهرى و الجينى للسلالات المقاومة للمبيدات و الإصابة بالبلازموديم اسبوروزويت فى الأنوفلس العربى بولاية الخرطوم، السودان

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DEDICATION

To the soul of my father

To my mother who is inspired me the meaning of patience and loyalty

To My brothers

To my beloved son

To my husband who always supported and encouraged me

My relatives and friends

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ABSTRACT

This study was conducted in Khartoum state during March 2011 to February 2014 to assess the susceptibility status of *Anopheles arabiensis* the malaria vector to different classes of insecticides in Khartoum state. Furthermore, insecticides resistance genes (*kdr*, AChE) in concomitance with infection of *Anopheles arabiensis* the vector of malaria were studied.

Anopheline mosquito larvae and pupae were collected from possible larval habitats in nine sentinel sites using standard collection methods and reared to adult in the insectary to conduct WHO-bioassays. Moreover, wild adult mosquitoes were collected from resting places in 20 sites to conduct molecular analysis for detection of knockdown resistance genes (*kdr*; L1014F and L1014S), acetylcholinesterase (ace-1^R; G119S) alleles and *Plasmodium* sporozoites infection. The collection sites of larvae and wild adults were marked using Global Positioning System (GPS). A sub-samples from emerged adult *Anopheles* mosquitoes from the colony-reared and the wild ones were identified using proper entomological keys and analyzed by PCR using species-specific primers. Using WHO procedure, susceptibility tests were conducted on adults of *An. arabiensis* from nine sentinel sites in Khartoum State using dichloro-diphenyl-trichloroethane (DDT 4%), fenitrothion 1%, malathion 5%, propoxur 0.1%, permethrin 0.75%, deltamethrin 0.05% and lambdacyhalothrin 0.05%. Mortality rates and knockdown times (KDT_{50} and KDT_{95}) of insecticides for *An. arabiensis* were calculated. Moreover, a well-designed socio-economic questionnaire was used to assess the knowledge, attitude and practice of the public health workers and farmers on the uses of pesticides in the field.

In this study, only two types of larval habitats were identified; these were habitats formed from the drinking water pipes leakage and irrigation canal.

Moreover, a total of 8325 female *An. arabiensis* were exposed to insecticides. Populations of *An. arabiensis* from Khartoum State were susceptible to only fenitrothion 1% and lambdacyhalothrin 0.05% with overall mean percentage mortalities 100±0.12 and 100±0.45 respectively. A significant differences in the mortality rates in *An. arabiensis* due to DDT 4%, permethrin 0.75% and deltamethrin 0.05% were observed between the sentinel sites. Moreover, *An. arabiensis* from Khartoum North area was resistant to most insecticides used more than populations from Khartoum and Omdurman areas. *Anopheles arabiensis* from urban areas was resistant to only malathion 5% whereas in the periurban areas, it was resistant to DDT 4%, malathion 5% and permethrin 0.75%. Although, the spatial distribution of the resistant strains of *An. arabiensis* was clear for insecticides used in different sentinel sites, the seasonal variation for the susceptibility status in this species did not follow a clear pattern. Both *kdr* (L1014F and L1014S) alleles and G119S mutation that confer resistance to pyrethroids and organochlorines, and organophosphate and carbamates insecticides respectively were detected in low frequencies *An. arabiensis* and with limited distribution in Khartoum State to periurban and urban areas respectively. Although, *P. falciparum* infection was detected in the *An. arabiensis* collected from three out of four sites, no *kdr* or G119S mutations were detected in concomitance with malaria parasites infections in this species. *Plasmodium falciparum* was detected in 6.7% of the females *An. arabiensis*. However, no *kdr* mutations was observed in concomitant with *Plasmodium* infection in these populations. The results of the KAP surveys revealed that 5 insecticides have been used in agriculture and public health practices in Khartoum State during the five last years. However, the public health workers and the farmers showed a relatively low knowledge about the proper uses of pesticides and insecticides in both practices respectively.

In conclusion, this study reports, for the first time, the identification of the L1014S and G119S mutations in *An. arabiensis* populations in Khartoum. Moreover, *An. arabiensis* the main malaria vector in different sentinel sites which categorized as urban and periurban areas in the three administrative areas in Khartoum showed multiple resistance to most insecticides used. However, the cross-resistance between DDT and permethrin, in addition to the multiple resistance in malaria vector has significant implications for the control of malaria vector populations in Sudan since pyrethroids insecticides are used in LLINs and indoor residual sprays (IRS). Therefore, more investigations are needed to determine the occurrence and frequency of resistant gene(s) in Khartoum State and other regions in Sudan to have a corrective management strategies and effective vector control programmes in the future.

المستخلص

أجريت دراسة طويلة المدي فى ولاية الخرطوم خلال الفترة مابين مارس 2011م الى فبراير 2014م للتحقق من حالة وتوزيع الأنوفلس العربى الحساس والمقاوم للفئات الأساسية من المبيدات الحشرية المستخدمة فى مجال الصحة العامة و الزراعة فى ولاية الخرطوم. بالإضافة للكشف عن الجينات المقاومة للمبيدات الحشرية ومايصاحب ذلك من إصابة الأنوفلس العربى الناقل بطفيل الملاريا.

تم جمع يرقات وعذروات بعوض الأنوفلس من تسعة محطات مختلفة للرصد الحشرى المتاحة لتوالد اليرقات بإستخدام أطقم جمع اليرقات القياسية، ومن ثم تم تربية اليرقات الى الطور البالغ فى المعمل لإجراء إختبارات الحساسية الموصى بها من قبل منظمة الصحة العالمية.وعلاوة على ذلك تم جمع إناث بعوض الأنوفلس العربى البرى من عشرون موقع لإستراحة البعوض لإجراء تحليل الاحياء الجزيئية لتحديد الجينات المقاومة للمبيدات الحشرية وانزيم الإستايل بالإضافة الى الإصابة بطفيل الملاريا. كما أخذت قراءات لنقاط تولد اليرقات ومواقع استراحة الطور البالغ بإستخدام نظام تحديد المواقع العالمى (GPS). وقد تم أخذ عينات فرعية من الطور البالغ من المستعمرة المعملية وعينات من اللأطوار البالغة البرية ومن ثم تصنيفها بإستخدام مفاتيح التصنيف الحشرية المناسبة وتحليلها بواسطة تفاعل البلمره المتسلسل (PCR) بإستخدام بادئات متخصصة. وايضا تم إجراء إختبارات الحساسية الموصى بها من قبل منظمة الصحة العالمية على الطور البالغ لأنثى الأنوفلس العربى من التسعة محطات فى ولاية الخرطوم بإستخدام المبيدات الحشرية. بالإضافة الى ذلك تم حساب معدل الوفيات وتقدير الزمن لسقوط 50% و90% من إناث الأنوفلس العربى عند التعرض للمبيد الحشرى وايضا تم تحليل الطفرات الجينيه المقاومة للمبيدات الحشرية وانزيم الإستايل وما يصاحبها من الإصابة بطفيل الملاريا في الأنوفلس العربى البرى من عشرون موقع. وعلاوة على ذلك تم تصميم إستبانة إجتماعية وإقتصادية لتقييم المعرفة والسلوك والممارسات لعمال الصحة العامة والمزارعين فى إستخدام المبيدات الحشرية.

فى هذه الدراسه، تم تحديد نوعين فقط من أنواع مواقع توالد اليرقات وهى عبارة عن تسريب فى أنابيب مياه الشرب وقنوات الري الزراعي. تم تعريض حوالى 8325 من إناث الأنوفلس العربى للمبيدات الحشرية، وقد سجلت الدراسة حساسية عالية لكل من fenitrothion 1% و lambdacyhalothrin 0.05% فقط للإناث الأنوفلس العربى فى ولاية الخرطوم مع متوسط معدلات وفيات 99±0.12 و 100±0.45 على التوالى. وجدت الدراسة أن هنالك فرق كبير بين معدلات الوفيات للإناث الأنوفلس العربى

عند التعرض لمبيد DDT و deltamethrin و permethrin بين التسعة مواقع فى ولاية الخرطوم. وعلاوة على ذلك فقد وجد أن إناث الأنوفلس العربى التى جمعت من مدينة الخرطوم بحرى مقاومه لمعظم المبيدات مقارنة بإناث الأنوفلس العربى التى جمعت من مدينتى الخرطوم وأم درمان. سجلت الدراسة مقاومة إناث الأنوفلس العربى فقط لمبيد malathion فى المناطق الحضرية بينما المناطق الشبة حضرية اعطت مقاومة لكل من مبيد DDT و malathion و permethrin. على الرغم من وضوح التوزيع المكانى لأنواع إناث الأنوفلس العربى المقاوم للمبيدات الحشرية بين التسعة مواقع الا أن حالة الحساسية لهذه الأنواع من البعوض لا يتبع نمطا واضحا خلال التفاوت الموسمى.

وقد أوضحت الدراسة أن هنالك ترددات منخفضة فى الطفرات الجينية المقاومة للمبيدات الحشرية وانزيم الإستايل التى تمنح مقاومة ضد الفئات الأساسية من المبيدات الحشرية مع توزيع محدود لهذه الطفرات الجينية فى المناطق الحضرية وشبة الحضرية فى ولاية الخرطوم. بالرغم من وجود الاصابة بطفيل الملاريا فى إناث الأنوفلس العربى التى جمعت من ثلاثة مناطق الا أنه لا وجود لطفرات جينية المقاومة للمبيدات الحشرية وانزيم الإستايل مصاحبة للإصابة بطفيل الملاريا فى هذه الأنواع من البعوض.

وقد أوضحت نتائج الإستبانة الإجتماعية والإقتصادية لتقييم المعرفة والسلوك والممارسات لعمال الصحة العامة والمزارعين فى إستخدام المبيدات الحشرية أن هنالك خمسة مبيدات حشرية تستخدم فى الزراعة والصحة العامة فى ولاية الخرطوم خلال الخمسة سنوات السابقة، ومع ذلك هنالك معرفة منخفضة وسط عمال الصحة والمزارعين بالإستخدامات والممارسات القياسية للمبيدات الحشرية فى كلا المجالين.

فى الختام، سجلت الدراسة أول ظاهرة لتواجد طفرات لجينات شرق أفريقيا المقاومة للمبيدات الحشرية وانزيم الإستايل فى إناث الأنوفلس العربى فى ولاية الخرطوم. وعلاوة على ذلك، وجدت أن هنالك مقاومة متعددة من قبل إناث الأنوفلس العربى الناقل الأساسى للملاريا فى مختلف المناطق المصنفة حضرية وشبة حضرية فى الثلاث مدن الرئيسية. و يعتبر وجود مقاومة مشتركة للDDT و permethrin و مقاومة متعددة لناقل الملاريا ذات انعكاسات هامة على مكافحة ناقل الملاريا فى السودان و ذلك لإستخدام مبيد pyrethroids فى الناموسيات المشبعة بالمبيدات طويلة المدى، ورش المنازل بمبيدات اللأثر الباقى، ولهذا توصي الدراسة لمزيد من التحقيقات لتحديد تكرار وتواجد الجينات المقاومة للمبيدات فى ولاية الخرطوم والمناطق الاخرى فى السودان و ذلك لوضع إستراجيات فعالة لمكافحة الناقل فى المستقبل.

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CHAPTER ONE

1. INTRODUCTION

Mosquito-borne diseases threaten the lives and livelihoods of millions of people worldwide (Townson *et al.*, 2005). Malaria remains the most important mosquito-borne disease. Malaria is remains the most common vector-borne diseases prevalent in tropical and subtropical areas of the world (Kamareddine, 2012). Malaria is caused by the protozoan parasites, belonging to the genus *Plasmodium*. Malaria is considered to be endemic in about 104 countries and territories (WHO, 2013a). In 2010, over 1.2 million global malaria deaths were reported in both children and adults (Murray *et al.*, 2012). Currently, an estimated 3.4 billion people are at risk of malaria with 207 million cases occurred in 2012 and 627 000 deaths worldwide (WHO, 2013a). More than 80% of the cases and 90% deaths occurred in Africa especially in children under 5 years of age (WHO, 2013a). However, worldwide, between 2000 and 2012, estimated malaria mortality rates fell by 42% and 48% in all age groups and children under 5 years of age respectively (WHO, 2013a).

In Sudan, malaria is the leading cause of morbidity and mortality. Symptomatic malaria accounts for 21% of outpatient clinic visits and approximately 30% of hospital admissions (NMCP, 2010). The entire population of Sudan is at risk of malaria with a different degrees which varied from low to moderate risk of transmission to predominantly seasonal transmission and epidemic outbreaks (NMCP, 2010). Transmission of malaria in Khartoum State has been considered low to moderate in rural areas and unstable seasonal in riverine areas especially to the north (NMCP, 2010). Khartoum state, formerly and nearly malaria-free area, increasingly suffered from malaria epidemics, with more than 700 000 cases annually between 1998

and 2001 (WHO/EMRO, 2004). However, currently this area encountered for 300,000 cases of malaria and five hundred deaths each year (Malik *et al.*, 2003; Nourein *et al.*, 2011).

Malaria is mainly transmitted by anopheline mosquitoes. Approximately, about 460 anopheline species were identified; 100 are reported as malaria vectors, and only 30-40 species of those reported vectors commonly transmit *Plasmodium* parasites (Kamareddine, 2012). In Sudan, *An. arabiensis* the member of *An. gambiae* complex, is the principle vector of malaria (Dukeen and Omer, 1986; Hamad *et al.*, 2002; Malcolm *et al.*, 2009). *Anopheles arabiensis* is most widely spread and it predominate in arid regions throughout most of the Afrotropical region, extending northwards along the River Nile to 20◦N in Sudan (Dukeen and Omer, 1986). This species is considered to be one of the most efficient malaria vector due to its ability to tolerate rapid environmental changes caused by human activities such as habitation and agricultural activities (Collins *et al.*, 1994).

Recently, a decline in malaria prevalence has attributed to efficient vector control strategies implemented in endemic areas (Nkya *et al.*, 2014). Vector control using insecticide campaigns in many countries have been mainly applied against mosquitoes and so indirectly against other insect vectors. In addition, the wide-scale use of ITNs and the increase in application of Indoor Residual Spraying (IRS) has resulted in a major reduction in disease burden in sub-Saharan Africa (WHO, 2010) including Sudan. However, improper and intensive use of insecticide for public health and in agricultural practice has led to the development of insecticide resistance in malaria vectors in tropical countries (Hemingway and Ranson, 2000; Nauen, 2006; Abdalla *et al.*, 2008; WHO, 2013b). Development of insecticide resistance in the malaria vectors threatens the effectiveness of the control measures and hence

remains as one of the major challenges facing malaria control programs (WHO, 2012). In Sudan, *An. arabiensis* the main malaria vector being resistant to several insecticide of different classes (Abdalla *et al.,* 2008; Ranson *et al.,* 2009; Himeidan *et al.,* 2011a; Seidahmed *et al.,* 2012; Abuelmaali *et al.,* 2013).

1.2. Rationale

In Khartoum state few studies were carried out on detection of insecticide susceptibility status of *An. arabiensis* (Seidahmed *et al.,* 2012; Abuelmaali *et al.,* 2013). However, no data has been recently published on the larval habitats and infection rates of malaria parasites in anopheline mosquitoes. Therefore, this study was carried out to investigate the status of resistance/susceptibility of malaria vectors, the distribution of larval habitats and the *Plasmodium* parasites infection in mosquitoes in Khartoum state.

1.3. Objectives

1.3.1. General objectives

This study was carried out during 2011 – 2014 to verify the insecticide susceptibility status, occurrence and distribution of knock down resistance gene (*kdr*) and acetylcholinesterase 1 ($ace.1^R$) in concomitance with *Plasmodium* sporozoites infection in *An. arabiensis* in selected sites spread over the three administrative areas in Khartoum state.

1.3.2. Specific objectives

1. To identify the types of larval habitats in nine sentinel sites used for *An. arabiensis* susceptibility status investigation.

2. To determine the susceptibility/resistance status of *An. arabiensis* for commonly used insecticides in Khartoum state.
3. To determine the spatial and temporal distribution of insecticide resistance strains of *An. arabiensis* in Khartoum state.
4. To determine occurrence and distribution of both knock down gene resistance (L1014F and L1014S-*kdr*) and gene encoding acetylcholinesterase 1 (*ace.1*^{R}; G119S) mutations in *An. arabiensis* in Khartoum state.
5. To determine *Plasmodium* sporozoites infection in concomitance with *kdr* and *ace.1*^{R} mutations in populations of *An. arabiensis* in Khartoum state.
6. To assess the knowledge, attitude and practice of farmers and public health workers towards the uses of insecticides.

CHAPTER TWO

2. LITEREATURE REVIEW

2.1. Malaria

Malaria is the world's most deadly parasitic disease that causes a major health public problem in the tropical areas especially in the sub-Saharan Africa. Malaria threatens the lives of 40% of the world's population. It is estimated that the disease is endemic in 104 countries and territories with approximately 3.4 billion people are at risk (WHO, 2013a). Currently, 207 million malaria cases and 627 000 deaths occur worldwide where the majority occur in Africa especially in children under 5 years of age (WHO, 2013a). The disease causes serious adverse effects in pregnant women including abortion, low birth weight and maternal anaemia (Newman *et al.,* 2003; Rogerson *et al.,* 2007). Besides, malaria has an indirect negative impacts on economic development, productivity and quality of life in endemic areas (Sachs and Malaney, 2002). The annual costs of malaria control in Africa have been estimated to be about two billion US$ (WHO, 2008).

Malaria is a parasitic disease belonging to a protozoan of the genus *Plasmodium.* There are five species of parasite which affect humans: *Plasmodium falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi*. *Plasmodium falciparum* and *P. vivax* are the most important and the most widely spread species and cause the most infections worldwide. Malaria due to *P. falciparum* is the most deadly form which is responsible for the majority of deaths that occurs in sub-Saharan Africa (Breman *et al.*, 2007). Besides, *P. knowlesi* was previously known as a malaria parasite of Old World monkeys (Cox-Singh *et al.,* 2008), it has been proposed as human malaria parasite since

it is associated with human infections in Southeast Asia countries (White, 2008; Jiang *et al.*, 2010).

Malaria is transmitted by a bite of infected female *Anopheles* mosquitoes. In addition to the female *Anopheles*, the malaria parasite requires a vertebrate host such as human to complete its lifecycle. The infected female *Anopheles* mosquito injects malaria parasites (sporozoites stage) into human during taking a blood meal which is needed to produce eggs. Contrariwise, the female *Anopheles* mosquito takes up malaria parasite (male and female gametocytes) from infected human during blood feeding. Therefore, the transmission of malaria mainly to humans in the communities is a result of periodic blood-feeding behaviour of *Anopheles* mosquitoes. In Africa, the transmission of the lethal malaria parasite *P. falciparum* predominantly occur due to the feeding behaviour of the females belonging to the members of *Anopheles gambiae* complex which are highly efficient, widespread and difficult to control (WHO, 2008).

2.2. Malaria in Sudan

In the Sudan, malaria represents a serious major health problems and it spreads all over the country. The transmission of the disease occurs as a seasonal trend in the Northern states (WHO, 2008), with level of endemicity varying in the different climatic zones (Fig. 2.1). In Sudan, malaria annually accounts for 9 million cases and more than 44 thousand (Abdalla *et al.*, 2007), with hundreds of deaths especially among infants and pregnant women (Elmahdi *et al.*, 2012). The disease causes morbidities and mortality among all age groups and was responsible for more than 95,000 hospital admissions in 2011 (Gadalla *et al.*, 2013). However, during the last few years a marked

decreases in malaria burden has been observed in Sudan as well as worldwide (WHO, 2010; Elmardi *et al.*, 2011).

In Sudan, malaria is mainly due to *P. falciparum* and transmitted by *Anopheles arabiensis*, as in other Sub-Sahara African countries, more than 95% of malaria cases in Sudan are due to *P. falciparum* although *P. malariae*, *P. ovale* and *P. vivax* have been occasionally recorded (El Sayed *et al.*, 2000). However, severe malaria due to *P. vivax* has been reported to emerge and spread over the eastern part of the country (Mahgoub *et al.*, 2012; Abdalla *et al*., 2013). The situation is further complicated by the spread of insecticide and drug resistance (Mukhtar *et al.*, 2007; Gadalla *et al.*, 2010; Himeidan *et al.*, 2004).

2.3. Life cycle and malaria transmission

Malaria is transmitted among humans by infective female mosquitoes of the genus *Anopheles.* The transmission of malaria often passive, where the female mosquitoes injects the parasite when they takes blood meals for eggs production, and hence initiate the link between the human and the mosquito hosts in the parasite life cycle. When bites an infected human, female *Anopheles* sucks gametocytes (sexual stages) along with into its gut and lead to formation of gametes (Aly *et al.*, 2009). These gametocytes continue the sexual phase of the cycle within the mosquito gut and the sporozoites that develop then fill the salivary glands of the infected mosquito. The mosquito then becomes infective and approximately, about one week later, when it takes its next blood meal, the sporozoites mixed with the mosquito's saliva are injected into the person being bitten (Prato *et al.*, 2012). The sporozoites, once in the blood stream they invade the liver and penetrate hepatocytes, where they remain for 9-16 days, multiplying within the cells. The parasites return

to the blood and penetrate red blood cells, where they produce both merozoites, which reinfect the liver, micro- and macro-gametocytes, which are the infective stage for mosquitoes.

Malaria parasites can also be transmitted in rare cases from an infected to another person through blood transfusion, organ transplant, or the shared use of needles or syringes contaminated with blood (Slinger *et al.,* 2001; Chauhan *et al.,* 2009). In addition, malaria can also be transmitted from a mother to her unborn infant before or during delivery ("congenital" malaria) (Valecha *et al.,* 2007; Sotimehin *et al.,* 2008).

2.4. The malaria vectors

Mosquitoes are two winged Nematocera insects (true flies), belong to the family Culicidae of the order Diptera. There are approximately about 3100 species of mosquitoes from 34 genera has been identified (Goma, 1966). Three subfamilies are recognized among the Culicidae: these are; Toxorhynchitinae, Anophelinae and Culicinae (Nasci and Miller, 1996). The subfamily Anophelinae comprises 3 genera however; members of the genus *Anopheles* are the exclusive vectors of human malaria (Service, 2008). Approximately, there are 460 recognised species of anopheline mosquitoes, of which over 60 species have been implicated as malaria vectors worldwide (WHO, 1997; Kamareddine, 2012). Of these species, members of the *An. gambiae* complex Giles and *An. funestus* Giles of the *An. funestus* group; are the most efficient malaria vectors in sub-Saharan Africa, (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987; Coetzee *et al.,* 2000).

2.4.1. The malaria vectors in African

In African malaria vectors, members of the *An. gambiae* Giles complex (*sensu lato*; i.e. wide sense) and *An. funestus* group Giles comprise a highly efficient and widespread vector (White, 1974; Hunt *et al.*, 1998; WHO, 2008a). These species are highly efficient because of their competence as vectors of the parasite, anthropophagic biting behaviour and longevity (Collins and Besansky, 1994: Miller and Greenwood 2002).

2.4.1.1. *Anopheles gambiae* Giles complex

Anopheles gambiae complex Giles is the most efficient vector of human malaria in the Afrotropical region (Hunt *et al.,* 1998) therefore, it is called African malaria mosquitoes. The complex comprises a group of genetically distinct species but morphologically cryptic species "sibling species" which reproductively isolated and varies in their distribution and behaviour (Coetzee *et al.*, 2000; Fanello *et al.*, 2002; Coetzee *et al.,* 2013). Until 2013, the *An. gambiae* s.l. consisted of seven species: *An. gambiae senso stricto* (s.s.) Giles, *An. arabiensis* Patton, *An. quadriannulatus* Theobald (species A; South African), *An. quadriannulatus* Hunt (species B; Ethiopian species), *An. melas* Theobald, *An. merus* Donitzand *An. bwambae* White (White, 1974; Hunt *et al.,* 1998: Fanello *et al.,* 2002). However, more recently, two new species of *An. gambiae* s.l. have been described and named; *An. amharicus* Hunt, Wilkerson & Coetzee and *An. coluzzii* Coetzee and Wikerson (Coetzee *et al.*, 2013). Therefore, to date, the *An. gambiae* s.l. comprises eight sibling species because the *An. amharicus* has been referred to the Ethiopian species *An. quadriannulatus* B.

Of these siblings, only two species; *An. gambiae* s.s. Giles and *An. arabiensis* Patton are the major malaria vector in Africa (White, 1974;

Coetzee *et al*., 2000), and that due to their competence as vectors of the parasite, anthropophagic biting behaviour and longevity (Collins and Besansky, 1994; Miller and Greenwood 2002). In addition, the two species are broadly distributed in a wide geographical region in Africa (White, 1974). The two species occur sympatrically in most areas of the Afro tropical area (White, 1974; Gillies and Coetzee, 1987). *Anopheles arabiensis* is desiccation-tolerant or "drought resistant" species (Lindsay *et al.,* 1998). It has a high tolerance to high temperatures and low humidity than *An. gambiae* s.s. (Kirby and Lindsay, 2004). Unlike, *An. gambiae* s.s., *An. arabiensis* is better adapted to severely dry environments than *An. gambiae* s.s. (Lindsy *et al.,* 1998; Petrarca *et al.,* 2000), and therefore, tends to dominate over other member of the complex during extended dry periods (Lindsay *et al.,* 1998; Coetzee, 2004; Fournet *et al.,* 2010). *Anopheles arabiensis* is also more likely to change its behaviour to avoid contacts with indoor residual spray (IRS) or insecticide treated nets (ITNs) than *An. gambiae* s.s. (Govella *et al.,* 2010; Russell *et al.,* 2011; Yohannes and Boelee, 2012), and hence it acts as a sole vector of malaria (Lindblade *et al.,* 2006). *Anopheles arabiensis* is believed to be uniform "panmictic" (i.e. freely mating) species as demonstrated by cytogenetic data from Sudan (Petrarca *et al.,* 2000). Furthermore, this species is more likely zoophilic (Killeen *et al.,* 2001; Torr *et al.,* 2008; Obala *et al.,* 2012) although, it showed some degree of anthropophagic feeding but less frequently than *An. gambiae* s.s. (Gillies and Coetzee, 1987). *Anopheles arabiensis* also showed both endophilic and exophilic resting behaviour in different African regions (Paaijmans, and Thomas, 2011; Gone *et al.,* 2014).

Anopheles gambiae s.s is the most efficient malaria vector in the complex (Gillies and De Meillon, 1968) and within the complex it has the highest vectorial capacity. In addition, it is the most widely spread species in

most African countries especially in more humid regions with high rainfall (Minakawa *et al.*, 2006; Sogoba *et al.*, 2007) and thus, its breeding is mostly restricted to the rainy seasons. Although, *An. gambiae* s.s. is highly anthropophagic (Obala *et al.*, 2012), it was found to feed readily on other animals like horses and cattle in West Africa (Diatta *et al.*, 1998; Bøgh *et al.* 2001). *Anopheles gambiae* s.s exhibits a high degree of indoor resting (endophilic) (Molina *et al.*, 1996; Cano *et al.*, 2004). Unlike *an. arabiensis*, *An. gambiae* has an extreme genetic heterogeneity where it shows five chromosomal different forms namely are; Bamako, Bissau, Forest, Mopti and Savanna (Touré *et al.*, 1998; della Torre *et al.*, 2002). These forms showed assortative mating (i.e. mating isolation) in areas where they occur in sympatry (Touré *et al.*, 1998; Wondji *et al.*, 2005). Furthermore, two distinct genotypes in the ribosomal DNA are recognized; namely molecular forms M and S (Favia *et al.*, 1997). These molecular forms are assorted independently from the chromosomal forms (Wondji *et al.*, 2005). However, more recently, based on molecular and bionomical evidence, the *An. gambiae* molecular "M form" is named *An. coluzzii* Coetzee and Wilkerson and the "S form" retains the nominotypical name *An. gambiae* Giles (Coetzee *et al.*, 2013).

Anopheles melas, *An. merus* and *An. bwambae* are of minor importance (White, 1985; Coetzee *et al*, 1993; Coetzee, 2004; Pates *et al.*, 2006) because they have a limited range of distribution. *Anopheles melas* breeds only in the brackish water of the mangrove swamps in the coastal area of West Africa from Senegal to Angola (White, 1974). This species tends to dominate during the rainy season and a short period in spring during which the larval habitats formed of rainfall-water and tidal sea-water (Gillies and DeMeillon, 1968). It also showed difference in feeding preferences where it tends to be more anthropophagic and highly zoophagic (Snow, 1983; Akogbeto, 2000). It acts

as a malaria vectors in only in areas where other members of the *An. gambiae* s.l. are absent (Gillies and De Meillon, 1968). *Anopheles merus* is also a saltwater breeder limited to brackish lagoons and swamps in coastal areas of East Africa including Kenya, Mauritius, Mozambique, Somalia and Tanzania (Gillies and DeMeillon, 1968). This species is probably more zoophilic and exophilic than *An. melas* (Coluzzi, 1984). *Anopheles bwambae* is highly restricted to humid forest foothills within a ten km radius of the geothermal springs at Mongiro in Bwamba County, Bundibugyo District, Uganda (Harbach *et al.*, 1997); therefore probably it is local importance as malaria vector (White, 1974).

Anopheles quadriannulatus is also of minor importance in transmission of malaria (Coetzee, 2004; Pates *et al.*, 2006). This species occurs in a limited geographical range of low annual rainfall like Zanzibar and southern Africa (species A) and highland in Ethiopia (Species B). *Anopheles quadriannulatus* species *An. quadriannulatus* species A and B are highly zoophilic although currently, a study in Ethiopia showed that species B anthropophilic behaviour (Pates *et al.*, 2006). More recently, *An. quadriannulatus* is retained for the southern African populations of this species, while the Ethiopian species has been named *An. amharicus* Hunt, Wilkerson & Coetzee, based on cross-mating and molecular evidence (Coetzee *et al.*, 2013).

2.4.1.2. *Anopheles funestus* Giles group

Anopheles funestus Giles group comprises of nine species that are widely distributed throughout Afrotropical region (Gillies and Coetzee, 1987; Coetzee and Fontenille, 2004). The members of *An. funestus* group showed minor or no morphological differences at adult stage (Gillies and Coetzee, 1987; Coetzee and Fontenille, 2004). Of these species, *An. funestus* s.s. is the

only species among the group that play a significant role in malaria transmission (Cohuet *et al.*, 2004). *Anopheles funestus* s.s. Giles, is highly anthropophilic and endophilic species and that due to its close association with humans and their habitations (Charlwood *et al.*, 1995; Sinka *et al.*, 2010). However, this species thrives in a wide range of habitats through the Afrotropical Region (Sinka *et al.*, 2010). Recently, a study showed that *An. funestus* s.s. can change its behaviour to evade interventions such as application of IRS or ITNs (Guelbeogo *et al.*, 2014).

2.4.1.3. Other mosquito vectors of malaria

Besides *An. gambiae* s.l. and *An. funestus* group, *An. nili* and *An. moucheti* groups are considered as malaria vectors in Africa. These mosquito species play a major role in transmission of malaria in West and Central Africa (Antonio-Nkondjio *et al.*, 2002; Fontenille and Carnevale, 2006). In addition to these main vectors, a several other *Anopheles* mosquitoes have been considered to be a secondary vectors in Africa and they have a localized importance in malaria transmission (Antonio-Nkondjio *et al.*, 2006).

2.4.2. Malaria vectors in Sudan

Up to date, approximately about 38 species of *Anopheles* were recorded in Sudan (El-Rayah, 2007). Previously, Lewis (1956) recorded about 31 *Anopheles* mosquitoes in the country. A current entomological surveys conducted in Sudan reveled 9 *Anopheles* species; these are *An. arabiensis, An. nili, An. dthali, An. squamosus, An. rufipes, An.pharoensis, An. pretoriensis, An. coustani,* and *An. multicolor* (Nugud and El Sayed, 2001). Of these species, only few species represent potential malaria vectors in different

regions in Sudan (Nugud *et al.*, 1997). However, *An. arabiensis* is the most efficient and widely spread vector in the country.

Anopheles arabiensis is only member of the *An. gambiae* s.l. complex in Sudan. It is also considered the only malaria vector in Sudan (Hamad *et al.*, 2002). This species has a wide geographical distribution in the country (Dukeen and Omer, 1986) especially in the arid regions. It is distributed over dry savannah and semi-arid parts, extending north wards along the River Nile to 20 ° N in Sudan (Dukeen and Omer, 1986; Ageep *et al.*, 2009). In Sudan, *An. arabiensis* is highly anthropophagic and have a short gonotrophic cycle (i.e. around 48 hours), a high probability of daily survival and vectorial capacity, which makes it an efficient malaria vector (Nugud and El Sayed, 2001).

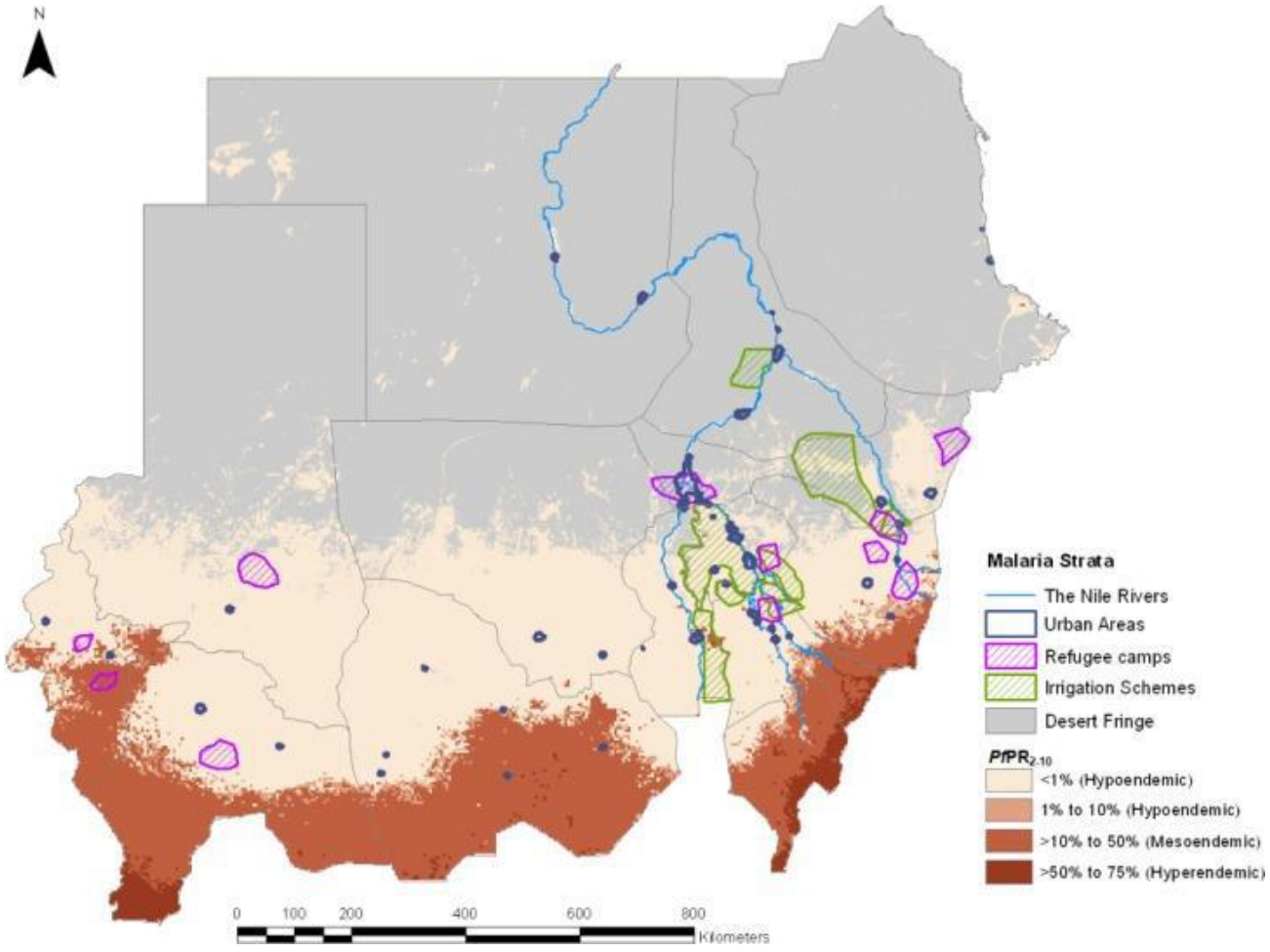

Figure 2.1: A map showing malaria strata in Sudan (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3516068/)

2.5. Ecology and biology of *Anopheles gambiae* complex

Anopheles gambiae complex species uses temporary aquatic habitats (Gillies and De Meillon, 1968) for oviposition which include small flooded depressions in the soil, hoof prints, shallow ditches, etc... Like other anopheline mosquitoes, females *An. gambiae* s.l discriminate between potential aquatic habitats (Herrera-Varela *et al.*, 2014) for oviposition which could be mediated by chemical (volatiles substances), physical and visual cues (Takken and Knols, 1999). The female when allocate suitable larval habitat, deposit their eggs singly on the water surface or on wet mud at the edge of the selected larval habitats. Often, one to two days later, the eggs hatch to give the first larval stage. The larvae are filtering surface feeders that feed on microbial food and detritus at the water surface (Service, 2008). Larvae moult four times to give the nest larval instars. Then the fully grown 4^{th} larval instar develops into a comma-shaped non-feeding pupa. The larval period lasts about 7-15 days in suitable conditions, otherwise, it takes longer time (Bayoh and Lindsay 2003; White *et al.*, 2011). The pupa lasts about 1-2 days and the adult mosquito emerges. When conditions are ideal, the development from egg to adult takes about 7-20 days (Schneider *et al.*, 2000).

The adult of *Anopheles* mosquitoes of both gender require energy for their general activities (i.e. flight, mating, etc...). Both sex obtain carbohydrates (sugar) by feeding on plant nectars or honeydew (Manda *et al.*, 2006) besides, the females require a blood meal to develop eggs. *Anopheles gambiae* complex is a nocturnal feeder with most of the blood-feeding occurring after midnight (Lindsay *et al.*, 1998). Although, the timing of blood-feeding is genetically fixed, the extensive use of insecticide-impregnated bed nets, preventing the mosquitoes from obtaining a blood meal, may select for anophelines that feed at other times (Sougoufara *et al.*, 2014). In general, for

the female one blood meal suffices to complete egg development for each gonotrophic cycle, *An. gambiae* s.s. (*An. gambiae* Giles and *An. coluzzi*), may require multiple meals (Takken *et al.*, 1998).

Often, the female *An. gambiae* s.l. is attracted to its preferred hosts by odour and chemical compounds originating from their skin and breath (i.e. fatty acids and CO_2) (Takken and Knols, 1999). *Anopheles gambiae* s.s. and *An. arabiensis* are different in their host preference and feeding behaviour. *Anopheles gambiae* s.s. is highly anthropophilic and endophagic whereas *An. arabiensis* is more zoophilic and exophagic behaviour (Gillies, 1988; Takken and Lindsay, 2003). However, *An. arabiensis* showed differences in feeding behaviour across the African continent; therefore it is considered to be an opportunistic feeder (White, 1974). For example, *An. arabiensis* tends to be more zoophilic and exophagic in East Africa region whereas, it is more anthropophilic and endophagic in West Africa (Tirados *et al.,* 2006).

Following a blood meal, females of *An. gambiae* s.l. search for a suitable resting place to digest the blood meal and develop the eggs. *Anopheles gambiae* s.s often rests indoors (endophilic) i.e. on walls and ceilings of rooms in which they acquired the blood (Gillies 1955; Lines *et al*., 1986). After development and maturation of eggs, the female leave the house in the early evening and search for a suitable oviposition site. In contrast, females of *An. arabiensis* tend to rest outdoors (exophilic) (Lines *et al*., 1986, Tirados *et al*., 2006).

Mating of *An. gambiae* s.l. takes place around dusk in swarms formed by males to which females are attracted (Takken and Knols, 1999). However, the mechanism by which the female attracted to the swarming males is unknown yet (Takken and Knols, 1999). Often, the swarms occur over a certain landmarks "swarm markers" (Takken and Knols, 1999). Swarms in

An. gambiae s.s. and *An. arabiensis* often appear at the same site, but the mechanism of this behaviour is not well understood (Takken and Knols, 1999; Hassan *et al.,* 2014).

The members of the *An. gambiae* s.l. have different ecology and range of distribution. They often occupy temporary aquatic habitats (Gillies and De Meillon, 1968) for oviposition. These are small, flooded depressions in the soil, hoof prints, tire tracks and shallow ditches. *Anopheles gambiae* s.s. and *An. arabiensis* commonly share a larval habitat in many areas in Africa (White and Rosen 1973). Both species prefer small, temporary habitats with algae and little or no aquatic vegetation (Fillinger *et al.,* 2004; Ageep *et al.,* 2009) such as shallow ponds, borrow-pits, brick-pits, ditches, human foot, animal hoof prints (Mutuku *et al.,* 2006). However, *An. gambiae* s.s. usually outcompetes *An. arabiensis* when they share a certain habitat (Koenraadt and Takken, 2003).

Both *An. gambiae* s.s. and *An. arabiensis* share a continent-wide distribution in Africa (Lindsay *et al.,* 1998; Coetzee *et al.,* 2000), and they occur sympatrically in much the range. Unlike *An. gambiae* s.s., *An. arabiensis* is better adapted to severely dry environments (Lindsay *et al.,* 1998; Petrarca *et al.,* 2000) and therefore, during extended dry periods this species tends to be the most dominant member of the complex. Thus, at the beginning of the rainy season the population of *An. arabiensis* increase rapidly and outcompetes *An. gambiae* s.s. (White, 1972; White and Rosen, 1973). In generally, *An. arabiensis* tends to dominate during the dry season in arid as well as humid regions of Africa and act as the major vector of malaria (White, 1972; White and Rosen, 1973).

2.6. Identification of sibling species in *Anopheles* mosquitoes

2.6.1. Morphological identification

The morphological identification of anopheline mosquitoes are based on certain features common for species. Morphological identification keys of *Anopheles* mosquitoes have been provided by Gillies and De Meillon (1968) and Gillies and Coetzee (1987).Adult females of the *An. gambiae* s.l can be differentiated from others anopheline mosquitoes by their smooth palps with 3 pale bands on the 3rd, 4th and 5th segments; the pale wings with yellowish or creamy markings and pale fairly long costal spots. The femora, tibia and 1st tarsal segments are speckled to a variable degree. The abdomen is pale brown and hairy with scales on the 8th tergite and on the cerci. For *An. gambiae* s.l., there are no reliable morphological features that can be used to distinguish between the siblings species (Gillies and Coetzee, 1987; Coetzee, 2004). However, morphological features such as the number of antennal sensillae and the palpal index can be used to differentiate between the two salt-water and the fresh-water species (Coluzzi, 1964).

2.6.2. Cross-matting experiments

Species complexes are often morphologically similar but they are reproductively isolated (Mayr, 1942). Identification of the sibling species of the *An. gambiae* s.l. first was due to results from crossing mating experiments (Davidson *et al.*, 1967; Hunt *et al.*, 1998). Muirhead-Thomson (1948) conducted the first cross mating experiment between fresh water species *An. gambiae* s.l. populations which resulted in sterile males in first progeny. Later, in 1962, Paterson indicated that *An. merus* a salt water species is a distinct biological species. This method is tedious, time consuming and difficult to be used for a large-scale. More recently, this method has been used to rename

An. quadriannulatus species B to *An. amharicus* Hunt, Wilkerson and Coetzee, based on cross-mating and molecular evidence (Coetzee *et al.*, 2013).

2.6.3. Cytogenetic analysis

The cytogenetic analysis involve the classification of mosquitoes "or any organism" based on the detection of differential banding patterns of the polytene chromosomes. These chromosomes often exist in the fourth instars larvae and in the ovaries nurse cells of semi or half gravid females. This method has been used to identify all sibling species of the *An. gambiae* s.l. (Coluzzi *et al*., 1979). This method showed genetic polymorphism between sibling species of *An. gambiae* s.l. due to paracentric inversions; where in the fresh water species the inversions occur on the X chromosome whereas the salt water species on the autosomes (Coluzzi, 1993). This technique, however, is limited by the need of well skilled persons for its wide application and routine field analysis. Besides, the polytene chromosomes must be prepared from early fourth instars larvae or semi-gravid females which cannot be used to identify large numbers of samples (Gale and Crampton, 1987).

2.6.4. Cuticular hydrocarbon analysis

Insect cuticle consists of fatty acids, sterols, esters and hydrocarbons therefore, the cuticular hydrocarbons has been used in taxonomy especially for the closely related species. This technique is based on presence of the variation in composition of carbon content of cuticles waxes among different species. Carlson and Service (1980) used cuticular hydrocarbon analysis to identify members of the *An. gambiae* s.l. using discriminant analysis Anyanwu *et al.* (2000), recorded differences in hydrocarbon content in four

strains of *An. gambiae* s.s. larvae. Although, this method has been used successfully to distinguish geographic variants in many insect species, it needs highly skilled workers and sophisticated equipment; therefore it is not practical for routine fieldwork. In addition, it also the specimens that should be identified using another method before analysis.

2.6.5. Allozyme "Isoenzyme analysis"

This method has been used for several years for the identification of sibling and cryptic species in mosquitoes (Pasteur *et al*., 1988). The technique is a biochemical method which is based on electrophoresis separation of the protein molecules "enzyme variant" (Pasteur *et al*., 1988). The method was described by Mahon *et al.* (1976) to distinguish between four members of the *An. gambiae* complex and it led to the discovery of diagnostic allozyme foci for wild sympatric populations of *An. quadriannulatus* A and B. Thus, a dichotomous electrophoretic taxonomic keys for the members of the complex (Miles, 1978) and for three species within the *An. quadriannulatus* complex (Lanzaro *et al*., 1990) have been developed. Unlike, cytogenetic analysis, this method does not require a specific sex or larval stage (Miles, 1978). However, the problem is that the technique is time consuming, tedious, and requires large and fresh specimens (or to be stored in liquid nitrogen) (Norris, 2002) besides, the cost of the enzymes needed for the analysis.

2.6.6. Polymerase chain reaction (PCR)

The PCR method is a very helpful tool in the identification of mosquito vectors, especially the closely related or sibling species that are morphologically indistinguishable. The PCR is an in-vitro method which mainly amplifies a certain region of the target DNA by using two

oligonucleotide primers that hybridized to opposite strands of the DNA (Saiki *et al*., 1985). The first PCR method to distinguish between the members of the *An. gambiae* complex was developed by Paskewitz and Collins (1990). The identification of the *An. gambiae* s.l. species has been carried out based on specific DNA nucleotide differences in the intergenic spacer (IGS) of the ribosomal DNA (rDNA) on the X chromosome (Scott *et al*., 1993). In addition, PCR-based techniques; PCR- restriction fragment length polymorphism (PCR-RFLP) has been used for identification of *An. gambiae* s.l. species as well as further identification of *An. gambiae* s.s. to M and S forms (della Torre *et al*., 2001; Gentile *et al*., 2001; Fanello *et al*., 2002). This PCR-based method is a combination of the protocols established by Scott *et al.* (1993) and Favia *et al.* (1997). This method is based on the restriction site for *Hha*1 enzyme (Favia *et al.*, 1997) lays within the *An. gambiae* s.l. specific fragment (Scott *et al.*, 1993). PCR-RFLP has been also used to verify the distribution of other molecular markers, such as the pyrethroid resistance gene (*kdr*) among chromosomal forms (Chandre *et al*., 1999).

The PCR method is very sensitive and can be species- specific however, the technique should be validated before being used. Other advantages of this method; samples of different life stage and sex either extracted DNA or fragments of a specimen, and the samples can be stored dry (on silica gel) or in ethanol. Moreover, large samples can be easily processed using this method and the results obtained can be easily interpreted (Paskewitz and Collins, 1990; Scott *et al*., 1993). However, this method is relatively inexpensive.

2.7. Malaria control

Malaria control is mainly to prevent mortality and morbidity due the infection as well as to reduce its social and economic negative impacts (Gupta and Guin, 2010). It advocates two major approaches, these are; 1. Control of parasites through the use of antimalarial drugs and 2. Vector control for reducing human-vector contact. Based on these approaches, a global malaria control strategy has been laid by the World Health Organization in partnership with the Roll Back Malaria (RBM) programme to reduce world's burden of malaria to 50% by 2010, 75% by 2015. Therefore, the Global Malaria Action Plan (GMAP) was developed with a global framework for action to coordinate partnership's efforts for a substantial and sustained reduction of malaria burden in near, and eradication in a longer-term. To achieve these goals, the RBM has outlined three-part global strategies that; 1. Malaria control to reduce the current burden and sustain as long as possible, 2. To eliminate malaria over time country by country and, 3. Developing new tools and approaches to support the ongoing control for elimination of malaria (WHO, 2008).

2.7.1. Parasite control

Malaria control is aimed at parasite clearance in addition to reducing illness and pains, and infected people. However, the main problems facing the control of the disease are the early and proper diagnosis infected persons i.e. rapid diagnostic test (Msellem *et al.,* 2009). Since early diagnosis and prompt treatment is an appropriate method in treating infected people besides, it is a basic technical element of the global malaria control strategy (WHO, 1993, 2001). Moreover, the major problem facing malaria control efforts in Africa

is the emergence of drug resistance in the most deadly malaria parasite *P. falciparum* to the affordable drugs (i.e. chloroquine, and sulphadoxine-pyrimethamine; SP (Fansidar®)) (Hastings *et al.*, 2002; Marks *et al.*, 2005). Therefore, the World Health Organization has recommended an alternative malaria treatment advocated for the uses of a combination therapies (i.e. artemisinin-based combination therapies; ACTs) to slow down the emergence of drug resistant parasite strains (Mutabingwa, 2005; Sutherland *et al.*, 2005). The use of Artemisinin Combination Therapy has resulted in reductions in malaria morbidity and mortality (Barnes, 2005; Bhattarai *et al.*, 2007). However, ACTs is more expensive than the conventional monotherapies (WHO, 2010) besides resistant strains of *P. falciparum* from elsewhere has been reported (White, 2008b; WHO, 2010; Ferreira *et al.*, 2013; Sharma *et al.*, 2014).

2.7.2. Vector control

Human malaria can be reduced or eradicated from endemic areas by reducing anopheline vector populations so as to decrease vector-man contact as well as reducing the vector population. Vector control represents one of the four basic technical elements of the Global Malaria Control Strategy (GMCS). It's strategy relay mainly on the selection of the most appropriate control measures that fits with the local circumstances and degree of malaria risk in the endemic areas. However, malaria transmission intensity is almost defined as the number of infective bites that a person receives per given unit of time and it is mainly measured by entomological inoculation rate (EIR).Therefore, malaria transmission in a given endemic area can be reduced by reducing the EIR through vector control (adult or larval control) (Killeen *et al.*, 2002). This can be achieved through the effective implementation of existing vector

control interventions i.e. indoor residual spraying (IRS), insecticide- treated bed nets (ITNs) and larval control (Barnes, 2005; Bhattarai, 2007; Noor *et al.*, 2009; Fillinger *et al.*, 2003).

2.7.2.1. Conventional approaches

Historically, malaria vector control using insecticides directed at indoor resting mosquitoes began in the 1930's with the use of organic chemicals extracted from plants and flowers such as nicotine, rotenone and pyrethrum (De-Meillon, 1936). Then after, pyrethrum insecticides were replaced with the organochlorine dichlorodiphenyltrichloroethane (DDT) in the 1940's. Since, the World Health Organization assembly launched the malaria eradication initiative with the use of DDT as the primary tool (reviewed in: Hemingway and Ranson, 2000), the DDT has historically been the most commonly and wide spread indoor residual insecticide for control of mosquito vector, given its high effectiveness, durability and low costs (Mandavilli, 2006). Although, the use of DDT was banned in the 1970's, however in year 2006 the WHO announced the re-application of this insecticide but in a limited and controlled areas for better control of malaria vectors (Sadasivaiah *et al.*, 2007). The re-introduction of the DDT in these limited areas, has resulted in a significant reduction in densities of malaria vectors (Curtis, 2002), even in areas of high pyrethroid resistance (Maharaj *et al.*, 2005). However, the impact of DDT on the environment and human health remains a major concern. Therefore the use of DDT for malaria control needs to be limited to avoid misuse and the development of resistance in major malaria vectors (Hargreaves *et al.*, 2003).

Recently, the use of ITNs becomes the most important intervention for vector control in many African countries. This method aims to prevent malaria in areas where the infection is common. The ITNs are widely promoted by

international agencies and governments to reduce the malaria burden (WHO, 2005). ITNs have resulted in substantial reductions in malaria mortality and morbidity (Noor *et al.*, 2009) by providing 15-20% protective efficacy compared to no nets and up to 23% protective efficacy compared to untreated nets (Mathanga *et al.,* 2005). Now days, most of malaria endemic countries are advocating the use of long-lasting impregnated bed nets (LLINs) as they have a long life span than the conventional ITNs. Unlike the conventional ITNs which require re-impregnation every 6-12 months, LLINs are capable of retaining lethal concentrations of insecticide for 4-5 years. The WHO is strongly recommending the use of these LLINs for the prevention of malaria in Africa (WHO, 2008). Therefore, during the year 2013, malaria vector control interventions using LLINs has been scaled up, where the coverage was estimated at 44% of the population at risk (WHO, 2014). However, the cost of an LLIN remains a major constraint to ownership for a large proportion of Africans who are poor and are also the most affected by malaria (Magesa *et al.,* 2005).

Larval control or larviciding is another vector control approach which mainly rely on the use of chemicals, usually Temephos 50% EC (albeit). However, albeit is commonly applied in urban areas in the big cities. Nevertheless, this method has a long history in areas where some vectors breed in specific habitats such as water reservoirs. It has a potential application especially in areas with plenty of larval habitats such as water reservoirs, flowing or pooled streams and other water-ways especially in agricultural development projects and irrigated schemes (WHO, 2006). This method control mosquitoes before they reach the adult stage, thus preventing disease transmission (Killeen *et al.*, 2002). In addition, larviciding is more efficient to reduce malaria vectors than adult control because larvae have a

lower mobility than adults which have ability to avoid insecticides (Darriet *et al.*, 2005). Although some studies have shown promising results (Fillinger and Lindsay, 2006; Fillinger *et al.*, 2009), the large-scale application of larvicides in Africa is problematic due to the heterogeneity and extensive number of larval habitats (Killeen *et al.*, 2002; Majambere *et al.*, 2010). However, other larvicides such as *Bacillus thuringiensis israelensis* (Bti), a bacterium that produces toxins that is effective in killing mosquito larvae (Fillinger *et al.*, 2003).

Historically, environmental management was the most effective method for reducing malaria in some regions especially during the early 1900s. Environmental management for vector control is mainly relaying on the modification and/or manipulation of environmental factors or their interaction with humans to prevent or reduce vector propagation and hence reducing human-vector-pathogen contact (WHO, 1982). This intervention can be achieved specifically by manipulations larval habitats such as removal of obstructions in the waterway, swamp drainage, control of water levels, stream flushing, changes of water salinity, shading of stream banks, use of larvivorous fish, etc. However, for adult can be achieved using house screening. Moreover, the efficiency of this intervention to control malaria depends basically on how well it is matched to the ecological requirements (climate conditions and habitat) and behaviour of the target malaria vectors in an area (Lindsay *et al.*, 2004).

Biological control is an alternative approach which depends on non-chemical materials (living organisms). The use of this method has increased over the last decades. This intervention depends on the uses of biological agents such as predatory fish (Legner, 1995), bacteria (Becker and Ascher, 1998), protozoa (Legner, 1995), nematodes (Kaya and Gaugler, 1993) and

entomopathogenic fungi (Scholte *et al.*, 2004; Farenhorst *et al.*, 2008). Although this method have shown a significant result in malaria vector control, large-scale application of any of these biological agents is still not available (Farenhorst *et al.*, 2008).

2.7.2.2. Modern approaches

Genetic control is new modern innovative control approaches which has been developed in order to control malaria (Feachem and Sabot, 2008; Greenwood *et al.*, 2008). Mainly two genetic control approaches are underway for future application, these are; Sterile Insect Techniques (SIT) and Genetically-Modified mosquitoes (GM). The SIT (Dyck *et al.*, 2005), a well-established method relies on the sterilization and release of male mosquitoes into the field to compete against wild conspecifics for mating with virgin females suppressing the population in the targeted area (Dyck *et al.*, 2005). Male sterility can be induced by ionizing radiation or chemosterilisation, hybridization or by chromosomal rearrangement (Knipling *et al.*, 1968).

The GM insect is an approach that depends on rendering wild vector populations refractory to parasite infection by releasing large number of transgenic laboratory-reared males into the field to drive the refractoriness genes into natural populations. Currently, GM insects that are refractory to infection by malaria parasites and dengue fever virus have been developed to control these diseases (Catteruccia, 2009; Franz *et al.*, 2006).

2.7.2.3. Integrated vector management (IVM)

The current malaria vector control efforts have suggested the delivery of multi-intervention packages for vector control to reduce the disease transmission. The WHO has therefore recommended integrated vector

management (IVM) to combat neglected tropical diseases (WHO, 2008). The IVM is a systematic approach for planning and implementing 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. It aims at the integration of different sustainable vector control interventions that reduce the use of pesticides to the lowest level possible. Although, IVM is successful in many areas (Chanda *et al.*, 2008), it is facing some difficulties such as a lack of stable funding for mosquito control operations and the lack of well-coordinated malaria entomological information. However, IVM is a promising approach for an effective, environmentally benign and long-lasting malaria control.

2.8. Malaria vector control in Sudan

Malaria Control Programme in Sudan has a long history and it is the oldest in Africa (Malik and Khalafalla, 2006). However, the most noticeable control programme in the country was during the Blue Nile Health Project (BNHP) which was conducted during 1980-1990s (El Gadal *et al.*, 1985). This project was carried out as partnership between the government of the Sudan, World Health Organization (WHO), World Bank, Kuwait, Japan, USA and other collaborators (El Gadal *et al.*, 1985). During that period, BNHP succeeded to decrease the malaria prevalence from over 20% to less than 1% and could sustain that for more than 10 years (El Gadal *et al.*, 1985). Recently, the strategic interventions adopted by Sudan NMCP for vector control includes ITN/LLINs, IRS and Larval Source Management (LSM) in big cities (NMCP, 2014).

2.8.1. Indoor residual spraying

In Sudan, the first insecticide used for indoor residual spraying was BHC (benzene hexachloride) which resulted in a significant reduction in malaria cases (to 1.9% malaria) in 1961 (El Gadal *et al.*, 1985). Later, DDT was introduced for IRS due to resistance in mosquito vectors to BHC where it had also resulted in a remarkable reduction in malaria cases (El Gadal *et al.*, 1985). However, in 1970s, DDT was banded due to resistance that had become widespread in the country (Haridi, 1972a). Malathion then was used in 1975 for IRS and also due to resistance it was replaced by fenitrothion during the BNHP in 1979 as recommended by the WHO (El Gadal *et al.*, 1985). During 1990s, pyrethroids have been introduced for IRS, however, recently bendiocarb is the main insecticides that used for this intervention in Sudan (NMCP, 2007). Now the IRS using bendiocarb includes several regions in Sudan including Gezira, Gedaref, Kassala Darfur, White Nile, Nile River States (NMCP, 2007).

2.8.2. Insecticide-treated nets (ITNs)

The uses of insecticide treated nets for personal as well as community-wide protection has stated in Sudan with conventional ITNs. Late, Sudan has developed a national strategic plan of ITNs coverage in malaria endemic areas especially seasonal malaria transmission areas and irrigated schemes (WHO, 2001). Currently, malaria control programme has shifted the vector control strategy using treated nets towards free distribution and wide-scale coverage of LLINs in different region of Sudan (NMCP, 2014)

2.8.3. Larval source management (LSM)

Historically, larval control had started with the treatment of larval habitats with paris green and diesel oil, water management and, intermittent irrigation (El Gadal *et al.*, 1985). Currently, larval control mainly relies on the use of chemicals (i.e. Temephos® EC50) and LSM. Larval Source Management has successfully been used in Khartoum State by Khartoum Malaria Free Project (KMFP) (Elkhalifa *et al.*, 2008). The KMFP has applied LSM through intermittent irrigation, rehabilitation and immediate repair of leaking water pipes in urban areas and in the irrigated agricultural areas (periurban areas) (Elkhalifa *et al.*, 2008).

2.8.4. Space spraying

The uses of space spraying is not a priority method for malaria control and hence its uses is very limited. In Sudan, space spraying method is used only in complex emergency situations (Elkhalifa *et al.*, 2008). It may not necessarily impact on transmission control but may be useful to advocate for political commitment and for addressing urban biting nuisance mosquitoes (WHO, 2001).

2.8.5. Sterile insect technique (SIT) in Sudan

Currently, a SIT feasibility study has been initiated in 2003 by the Republic of Sudan and IAEA jointly (El Sayed *et al.*, 2009). The Project aims at developing and evaluating all relevant components needed for a wide-area integrated Pest Management (AW-IPM) to control African malaria vectors using the SIT (El Sayed *et al.*, 2009). The field site of the feasibility project is situated in Northern state, Sudan and it extends from Dongola in the north to Merowe in the south (about 350 km long following the Nile).

2.8.6. Entomological surveillance

In Sudan, the malaria vector control interventions mainly relies on the uses of insecticides, therefore entomological surveys for monitoring of insecticide resistance in malaria vector is important. Previously, several studies were conducted to elucidate the insecticide status where evidence of resistance to organophosphates, DDT and recently to pyrethroids in *An. arabiensis* was recorded in different states in the country (Abdalla *et al.,* 2008; Himeidan *et al.,* 2011a). However, more studies and continuous monitoring of status of insecticides commonly used in agriculture and public health practices as well as occurrence of resistant genes are needed.

2.9. Malaria vector control in Khartoum state

Malaria control in Khartoum dates back to 1904 when retained oil was used as the main vector control tool leading to the eradication of the disease in the state. (Nourein *et al*., 2011). The reduction in control efforts and increasing migration from malaria endemic states into Khartoum were thought to have contributed to this resurgence (Elkhalifa *et al*., 2008). By the1990s, malaria was a leading cause of morbidity and mortality recorded at public health facilities in the state. The federal system of governance was introduced in 1993 providing state ministries of health the power to define and implement their priority health activities. In January 1994, the Khartoum state Ministry of Health outlined plans to decrease malaria outpatient attendances by 5% every year and malaria deaths to the minimum level. In 2002, the Khartoum Malaria Free Initiative (KMFI) was set up with support from the WHO and the Japanese government which named Khartoum Malaria Free Project (KMFP) (Nourein *et al*., 2011). The main theme of KMFP was vector control

through different interventions targeting the malaria vector *An. arabiensis* in urban and periurban setting of the state. Mainly control against *An. arabiensis* has been adopted by weekly treatment of larval habitats by Temephos® EC50, environmental management (Elkhalifa *et al.*, 2008) and insecticide space spraying during the emergency situation and larval (Himeidan *et al.*, 2011a).

2.10. Classification of insecticides

Insecticides used for malaria control can be classified into four groups: organochlorines, organophosphates, carbamates and pyrethroids (Table 2.1).

2.11. Insecticides and mode of action in mosquito vectors

Insecticides often target the nervous system of insects (Fig. 2.2). On contact, first it usually passes through the integument to the target sites in an altered form and/or as an active derivative (Narahashi, 1992). The organochlorines DDT as well as the pyrethroids insecticides have the same mode of action. They act on the same biomolecule but in different receptor sites (Miller and Saldago, 1985). The DDT prevents normal nerve impulses in insects by destroying or causing a leakage of the sodium and potassium ions within the axons of the neurons (Whiteacre and Ware, 2004). Finally, the affected nerves fire impulses suddenly, which causes spontaneous contraction and convulsions of muscles leading to insect death (Busvine, 1951). In contrast, pyrethroids cause axonic poisons by binding to protein in the nerve cells known as voltage-gated sodium channel. As a result, it prevent normal closing of voltage-gated sodium channel that leads to continuous nerve stimulation and tremors that leads the insects to lose control of their nervous system and produce coordinated movement (Narahashi, 1992; Vijverberg *et al.*, 1982).

Organophosphates (Ops) and Carbamates (CM) usually target the cholinergic nerve junctions at nervous system. Upon stimulation, the normal motor nerve releases the neurotransmitter acetylcholine to transmit the impulse to a muscle or organ. Then after, the enzyme acetylcholinesterase immediately breakdown the acetylcholine and thus lead the muscle or organ to the relax state. Eventually, the mode of action of both OPs and CM insecticide occur due to the disruption of the nervous system by to formation a covalent bond through either carbamylation or phosphorylation with the site of the enzyme where acetylcholine normally undergoes hydrolysis (breakdown). As a result, the acetylcholine builds up and continues to act so that nerve impulses are continually transmitted and muscle contraction continues (Corbett, 1974) leading to insect death.

2.12. Insecticides resistance

The WHO defined insecticide resistance as the ability of an insect to withstand the effects of an insecticide by becoming resistant to its toxic effects by means of natural selection and mutations (WHO, 2001). It was defined as inherited characteristic that imparts an increased tolerance to a pesticide (WHO, 1992). Pesticide resistance has appeared in every major vectors of diseases (WHO, 1976). Resistance also could occur in insects as multiple resistances which are identified as the simultaneous resistance to several insecticides of different categories, which is normally acquired by separate exposure to the insecticides concerned (Najera and Zaim, 2003). Likewise, cross-resistance also can occur between different classes of insecticides that share the same mode of action such as organochlorines and pyrethroids and organophosphates and carbamates insecticides.

Table 2.1: Insecticides used for IRS in malaria control, their classes, dosage and effective action as recommended by the WHO pesticide Evaluation Scheme (Prato *et al.*, 2012).

Product	Class group*	Dosage (g/m2)	Duration of effective action (months)
DDT	OC	1-2	>6
Fenitrothion	OP	2	3-6
Malathion	OP	2	2-3
Pirimiphosmethyl	OP	1-2	2-3
Bendiocarb	C	0.1-0.4	2-6
Propoxur	C	1-2	3-6
Permethrin	PY	0.5	
Deltamethrin	PY	0.01-0.025	2-3
Lambdacyhalothrin	PY	0.02-0.03	3-6
Alphacypermethrin	PY	0.02-0.03	4-6
Cyfluthrin	PY	0.01-0.05	3-6
Etofenprox	PY	0.1-0.3	3-6

*OC= Organochlorines; OP= Organophosphates; C= Carbamates; PY= Pyrethroids.

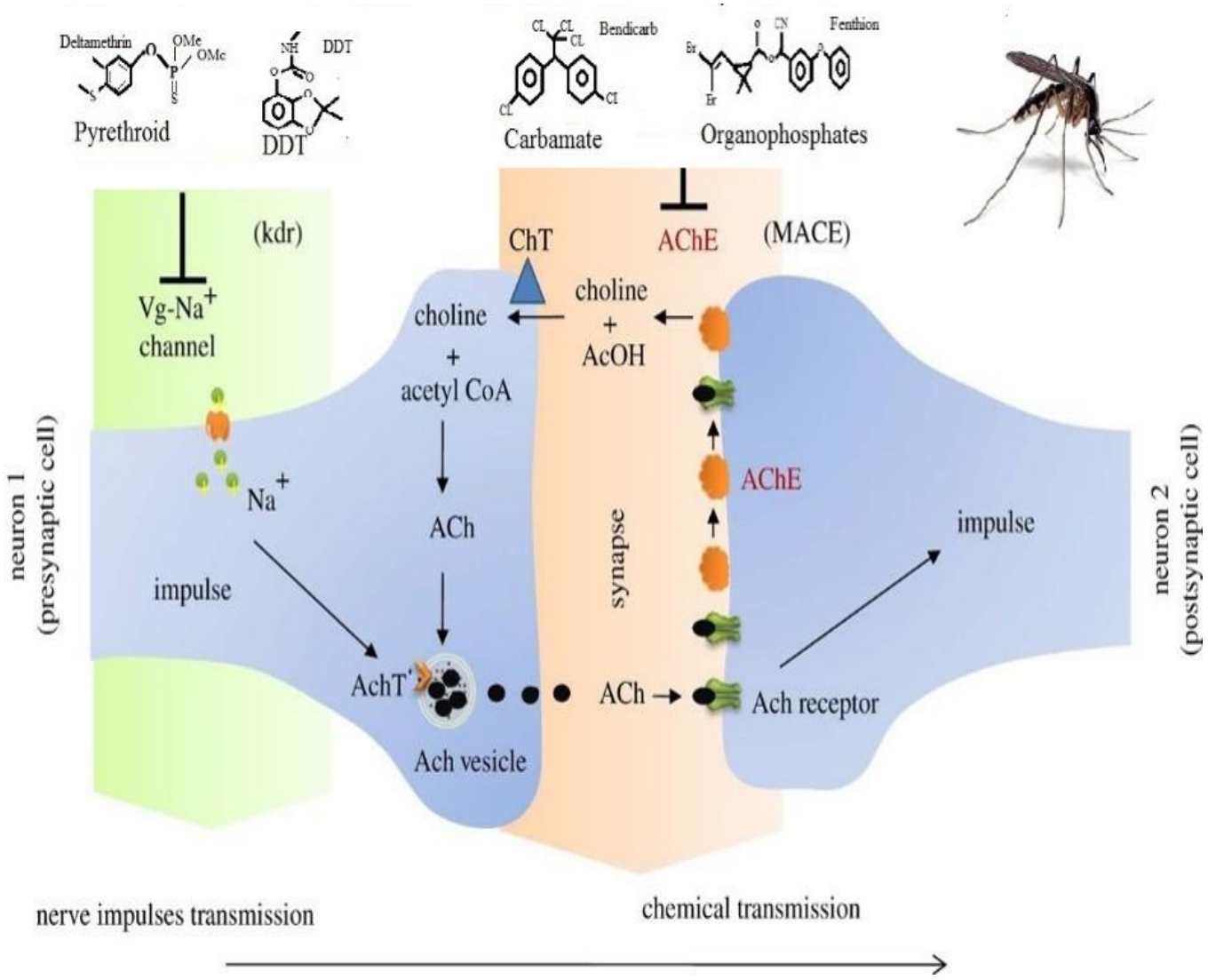

Figure 2.2: Biochemical target sites of synthetic insecticide in insects (after Nauen, 2006).

Insecticides used for malaria control have included organochlorine, organophosphorus, carbamate, and pyrethroid insecticides, with the latter now taking increasing market share for both indoor residual spraying and Long Lasting Insecticidal mosquito Nets (LLINs) (WHO, 2013b). A major concern on the use of currently available insecticides for malaria control is represented by increasing insecticide resistance (Enayati and Hemingway, 2010). Resistance is being likely to follow the use and switches of these insecticides (Hemingway and Ranson, 2000). Currently, insecticide resistance has been observed in more than 500 insect species worldwide including many vectors of malaria (Hemingway and Ranson, 2000). Resistance in malaria vectors to insecticide is growing worldwide due to the increasing selection pressure on mosquito populations that caused by extensive uses of chemical insecticides in urban, domestic and/or agricultural area (Nkya *et al.,* 2013). For example, DDT was first introduced for mosquito control in 1946; however, in 1947 the first cases of DDT resistance occurred, and up to now DDT resistance at various levels has been reported in several malaria vectors (Hemingway and Ranson, 2000; Prato *et al.*, 2012). More recently, several malaria vectors in different African countries have been reported resistant to all major classes of insecticides used in public health practice (Ndjemai *et al.,* 2009; Verhaeghen *et al.,* 2010; Balkew *et al.,* 2010; Yewhalaw *et al.,* 2011; WHO, 2013b; Toé *et al.,* 2015; Gnanguenon *et al.,* 2015).

The first recorded insecticide resistance to DDT in East Africa was from Sudan in El Guneid sugar estate (Haridi, 1972a). In central Sudan, *A. arabiensis* has been found to be resistant to several insecticides: BHC and DDT (Haridi, 1972b), malathion (Hemingway, 1983; Zahar, 1985). Furthermore, in Gezira irrigated area and Sinnar state, recent surveys showed multiple resistance of *An. arabiensis* to permethrin, DDT and malathion,

representing three of the four classes of insecticides approved by WHO for use in malaria vector control (Abdalla *et al.*, 2008). More recently, resistance in populations of *An. arabiensis* to major insecticide classes has been reported from different states in Sudan including Gizera, Sennar, Kassala, Gedaref, White Nile and Khartoum (Abdalla *et al.*, 2014; Himeidan *et al.*, 2011a; Yagoop *et al.*, 2013; Ismail *et al.*, 2012; Seidahmed *et al.*, 2012; Abuelmaali *et al.*, 2013; Mohammed *et al.*, 2015).

Insecticide resistance, especially to pyrethroids, is a serious threat to sustained use of LLINs and IRS programmes. The nets are impregnated with this class of insecticides, to which vectors are already resistant in some areas of the world. Although 12 insecticides are currently recommended for indoor residual spraying, they belong to only four chemical classes, and cross-resistance among insecticides is frequent (WHO, 2012).As a result multiple or cross-resistance between the insecticides in populations of major malaria vectors in Africa has become common (Ranson *et al.*, 2011).Cross-resistance can be due either to detoxification of insecticide by enzymes or by mutation on its target site due to substitution in amino acids: sodium channels for DDT and Pyrethroids, and acetyl-cholinesterase for organophosphates and Carbamates (WHO, 2006; Ranson *et al.*, 2011).

2.13. Mechanism of insecticide resistance in malaria vectors

Two insecticide resistance mechanisms have been identified in mosquitoes: increased metabolic detoxification and reduced target site sensitivity (Qin *et al.*, 2014).

2.13.1. Target site resistance

Each insecticide targets a specific molecule in the nervous system of the mosquito (Walsh *et al.,* 2001). Single base point mutations are the most common cause of target-site resistance changing the properties of these target sites and reducing their susceptibility to insecticide binding (Enayati and Hemingway, 2010).

2.13.1.1. Insensitive acetylcholinesterase (AChE)

The neurotransmitter acetylcholine plays an important role in the transmission of the external stimuli which enables the nervous system to translate it into effective action. When the appropriate message is successfully passed, the acetylcholinesterase (AChE) terminates the nerve impulses by catalyzing the hydrolysis of the acetylcholine (Walsh *et al.,* 2001).

Organophosphate and carbamate insecticides target AChE by phosphorylating or carbamoylating the active-site serine and preventing it from hydrolyzing the acetylcholine. Therefore, this causes the continuous action of the acetylcholine and finally the death of the insect (Vontas *et al.*, 2002).

2.13.1.2. Gamma-amino butyric acid receptor mutation (GABA)

The GABA receptor is a wide spread inhibitory neurotransmission channel in the central nervous system and neuromuscular junctions of insects (Prato *et al.,* 2012) and contain five subunits found around the central ion channel (Hemingway, 2000). Each subunit has an extracellular cysteine loop and four transmembrane domains (M1 – M4). The transmembrane domain two (M2) is the most important one because it forms the ion channel and contains the conserved alanine residue 302 (Hemingway *et al.,* 2004). Binding

to its ligand GABA, the receptor increases the flow of chloride through the membrane. Cyclodiene insecticides (e.g. dieldrin and BHC) block the GABA receptor thus preventing the inhibition of neural activity, leading to the death of the insect. Amino acid substitutions from alanine to serine or glycine within the second transmembrane region of the RDL subunit at position 302 are found associated with resistance to dieldrin in many insects (Hemingway *et al.*, 2004). In *An. arabiensis*, the resistance was found to be due to an alanine-glysine substitution at the same position (Du *et al.*, 2005).

2.13.1.3. Mutations in the voltage-gated sodium channel

The voltage-gated sodium channels play an integral role in the transmission of nerve impulses. (Hemingway, 2000). PYs and OCs target the voltage-gated sodium channel in insect neurons (Davies *et al.*, 2007). Insecticide binding delays closure of the sodium channel prolonging action potential and causing repetitive neuron firing, paralysis and eventual death of the insect (Ranson *et al.*, 2011). Molecular characterizations have revealed that various mutations in the S1-S6 transmembrane segments of domain II of the sodium ion channel give rise to DDT and pyrethroid resistance in malaria vectors (Diabaté *et al.*, 2004).

Mutations in the sodium channel conferred by DDT and pyrethroid resistance are known as knockdown resistance (*kdr*), so-called because insects with these alleles can withstand prolonged exposure to insecticides without being 'knocked-down'(Hemingway *et al.*, 2004). In West African *An. gambiae* a mutation resulting in an amino acid change from leucine to phenylalanine (Leu → Phe) within the S6 hydrophobic transmembrane segment has been associated with DDT/pyrethroid resistance (Kulkarni *et al.*, 2006). A resistance associated mutation in the same codon resulting in an

amino acid change from leucine to serine (Leu →Ser) was found in an East African population of *An. gambiae* (Verhaeghen *et al.*, 2006).

2.13.2. Metabolic resistance

Metabolic resistance occurs when elevated activity of one or more enzymes results in a sufficient sequesters or detoxification of the insecticide before it reaches the target site (Ranson *et al.*, 2011). Increased expression of the genes encoding the major xenobiotic metabolizing enzymes is the most common cause of insecticide resistance in mosquitoes. (Hemingway and Ranson, 2000).

Three major enzyme groups are responsible for metabolically based resistance to OCs, OPs, Cs, and PYs: a) glutathione S-transferase (GST), like DDT dehydrochlorinase, which was first recognized as a GST in the house fly, Muscadomestica; b) esterases, often involved in OP, C, and to a lesser extent, PY resistance; and c) monooxygenases, involved in PY metabolism, OP activation and/or detoxication and, to a lesser extent, C resistance (Prato *et al.*, 2012).

2.13.2.1. Glutathione S-transferase (GSTs)

The Glutathione S-transferases (GSTs) are a large family of detoxification enzymes found in almost all living organisms. They are cytosolic dimeric proteins with two subunits, consists of two domains, each containing two binding sites, the G site and the H site (Ding *et al.*, 2003). The GSTs are classified according to their location in the cell i.e. microsomal or cytosolic (Enayati *et al.*, 2005). Six classes of the insect GSTs have been identified, Delta, Epsilon, Omega, Sigma, Theta and Zeta (Hemingway *et al.*, 2004). The two classes, Delta and Epsilon are the most important because of

their role in insecticide resistance to the major classes of insecticides (Ding *et al.*, 2003).

Resistance to organophosphates is due to increases in GST detoxification rates by Odealkylation or O-dearylation reaction (Hemingway *et al.*, 2004). GSTs also protect the insect against the toxicity of pyrethroids either by detoxification of the lipid peroxidation products induced by the insecticide or by sequestering the insecticide (Vontas *et al.*, 2002).

2.13.2.2. Carboxylestarase (esterases)

Carboxylesterases are a large group of enzymes with different substrate specificity. According to Aldridge (1953), they are classified as A or B esterases according to their preference for the substrates α or β-naphthyl acetate. Esterases produce resistance either by rapid-binding and slow turnover of the insecticide (elevated esterase) i.e. sequestration, or metabolism of the insecticide by catalyzing the hydrolysis of carboxylic and phosphotrieste bonds in a wide range of insecticides such as organophosphate, carbamates and pyrethroids (Hemingway and Ranson, 2000).

Esterases which produce resistance by metabolism of the insecticide are associated with a single amino acid substitution in the structural genes (Hemingway *et al.*, 2004).

2.13.2.3. Cytochrome P450 monooxygenase (P450s)

Cytochrome P450 monooxygenases are hydrophobic, heme containing enzymes. The P450 family is one of the largest gene super-families and is found in all the living organisms. The insect P450s are involved in insect growth, development, reproduction and insecticide resistance (Rongnoparut *et al.*, 2003). The P450s play an integral role in the metabolism of endogenous

and exogenous compounds such as steroids, fatty acids and xenobiotics (Hemingway *et al.*, 2004).

Monooxygenase enzymes are named as CYP followed by a number, a letter and a number respectively for example CYP6D1 (Scott and Wen, 2001).

2.13.3. Behavioural resistance

Behavioural resistance or insecticide avoidance is the ability of some vectors to avoid contact with an insecticide. This type of resistance is not based on biochemical mechanisms but conferred by behavioural changes in response to prolonged exposure to an insecticide. This is triggered from actions evolved in response to selective pressure excreted by the toxicant. This type of response can be further divided into direct contact excitation (stimulus-dependent) and non-contact spatial repellency (stimulus-independent). The first type of response involve the detection and avoidance of insecticide treated areas whereas the second one the insects move away from the insecticide-treated area before making direct contact (Roberts *et al.*, 1997; Chareonviriyaphap *et al.*, 1997). The stimulus-dependent response particularly important for indoor residual spraying and for the use of LLINs (Najera and Zaim, 2003). This type of response has been reported previously, where a change in vector composition from *An. minimus* to *An. harrisoni* has been observed following implementation of ITNs in Vietnam (Garros *et al.*, 2005). Moreover, in Tanzania it has been observed that *An. funestus* changed its biting behaviour from indoor to outdoor due to large-scale coverage of pyrethroid-impregnated net (Russell *et al.*, 2011).

2.14. Methods of detecting resistance

Different approaches to detect the emergence of insecticides resistance are now possible. This is part of resistance management techniques to counteract against resistance. The idea is to have baseline susceptibility data to detect resistance in their early stages and monitor resistance levels over time (WHO, 1992).

2.14.1. The WHO standard protocol for insecticide susceptibility

Resistance monitoring relies on bioassays that are based on fixed insecticide diagnostic concentrations to detect the susceptibility status (percentage mortality) among specific populations and/or knockdown (KD) effect. A diagnostic concentration, as defined by the WHO, is the concentration of a given insecticide which results in 100% mortality after 1hour exposure. The method can only detect the overall levels of resistance. Therefore, when more than 5% of the samples survive the test after the 24 hours recovery period, resistance is said to be suspected and requires further investigation. When >20% survive, resistance is confirmed. Molecular and biochemical assay can be used to detect the mechanism/s involved (WHO, 2013b).Furthermore, it is important that the individual mosquitoes used for this assay are standardized for age, sex and physiological status because these factors can affect the outcome of the tests. To obtain reliable information, adults raised from female lines or F1 progeny reared from field collected larvae and pupae should be used.

Although, detection of resistance in malaria vector is highly dependent on susceptibility tests, these assays have some of the limitations. Limitation of the tests includes a single concentration of insecticide used in these assays which do not provide information about the level of resistance in a population.

Therefore, dose response assays and/or alternative as well as complementary method to WHO-susceptibility test would be needed to compare the levels of resistance in different populations (Skovmand *et al.*, 2008), these may include median knockdown time (MKDT), because, the results obtained from only WHO-susceptibility test cannot be used to compare the levels of resistance to two different insecticides.

Currently, an alternative and or a complementary method known as Centre for Disease Control and Prevention (CDC) bottle bioassay also has been developed (Brogdon and McAllister, 1998a). This method is used for detecting insecticide resistance in malaria vector populations and it is being adopted for routine monitoring of mosquito populations elsewhere as recommended by Brogdon and Chan (2010). This method has been developed to avoid the limitations of the diagnostic dose for as well as sometime the difficulties in obtaining a regular supply of the insecticide impregnated papers from WHO. It uses glass bottles coated with a known concentration of insecticide that can be used to detect and characterize resistance to an active ingredient of an insecticide in a selected mosquito species. Furthermore, this method measures the time for mosquito mortality due to the insecticide effect (a time versus mortality curve) to state the proportion of the resistant strain. Although, this assay is reliable and follows simple protocols that are inexpensive and effective, it shows resistance trends regardless of mechanism. In addition, this bioassay needs a uses of a proven susceptible population as a reference strain (i.e., lab colony) for comparison to the field population.

Both WHO diagnostic doses and CDC bottle bioassays can be modified to incorporate synergists. The synergists which are non-insecticidal compounds (e.g. piperonylbutoxide) that often blocks or weaken the activity of two major detoxification enzyme families can be used to judge the extent

to which detoxifying enzymes contribute toward the production of resistant phenotypes (WHO, 2013b). If resistance is due to increased metabolism, exposure to an appropriate synergist prior to insecticide bioassays should increase the level of mortality observed.

2.14.2. Biochemical (microplate) assays

Biochemical or microplate assays that are designed to detect alterations in activities of enzyme families associated with insecticide resistance mechanisms in individual mosquitoes (Hemingway *et al.*, 1997; Brogdon and McAllister, 1998b). This assay is based on that insecticide resistance in insect vectors, is due to over expression of specific enzymes that are involved in detoxification of allelochemicals. It has been developed over two decades and are sometimes used in combination with insecticide bioassays (WHO, 2013b).These assays typically need substrates to record the activity of glutathione transferases, carboxylesterases or cytochrome P450s in individual insects as well as it can be to detect target site resistance to organophosphate and carbamate insecticides caused by insensitive acetylcholinesterase (AChE) (WHO, 2013b). Although, this method has the ability to detect low resistance in an individual insect (Brown and Brogdon, 1987), specimen mosquitoes used should be fresh or kept on ice from the point of collection to the performance of the assay.

2.14.3. Molecular methods

Molecular methods have been developed for the detection of resistance at molecular level i.e. resistance associated with mutations in the target-sites. These methods include PCR-based methods for the detection of the gene coding for a subunit of the γ-aminobutyric acid (GABA) receptor, a chloride

channel (Stilwell *et al.*, 1995; Du *et al.*, 2005) and mutations within the voltage-gated sodium ion channel (VGSC) (knockdown gene; *kdr*) in mosquito vectors (Martinez-Torres *et al.*, 1998; Bass *et al.*, 2007). Currently, new techniques have been developed for detection of the resistance *kdr* mutations because of the important and wide use of pyrethroid insecticides in malaria control. These methods include the hot ligation oligonucleotide assay (HOLA) (Lynd *et al.*, 2005), Fluorescence Resonance Energy Transfer/Melt Curve analysis (FRET/MCA) (Verhaeghen *et al.*, 2006) and the sequence-specific oligonucleotide probes/ELISA (SSOP-ELISA) (Kulkarni *et al.*, 2006).

These assays are mainly used in research laboratories; however they are gradually being incorporated into some national malaria control programmes for resistance monitoring. These methods are very sensitive and can detect genetic mutation(s) responsible for the resistance phenotype in individual insects that provide an early warning of the emergence of resistance which may not have been detectable by bioassays. However, detection of these genes is currently dependent on RNA based techniques using relatively sophisticated equipment e.g. RTqPCR.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Study design

A longitudinal case study was conducted during March 2011 – February 2014 in Khartoum state to investigate the status and distribution of susceptibility/resistance *An. Arabiensis* to major classes of insecticides used in public health and agriculture practices. In addition, to detection of insecticide resistance genes (*kdr*, AChE) in concomitance with infection of *An. arabiensis* the vector of malaria.

3.2. Study area

The present study was carried out in Khartoum state which is considered the most populated among others. The area is located in central Sudan (15°.10'-16°.30' N and 31°.35'-34°.20' E) (Figure 3.1). The state occupies approximately about 28 000 km^2. The confluence of the Blue and the White Niles divided the state into three administrative areas; Khartoum, Khartoum North and Omdurman areas.

In general, the land in the state is flat but interrupted in some areas by seasonal khors and small hills. Most of the state lies within the semi-desert region whereas the northern part is mostly desert climate.

Vegetation in Khartoum state consists of dry desert scrub and riverine systems. The state is characterised by a dry cold winter between December to February, a dry hot summer between April to June and a short rainy season between July to September. The average annual rainfall is 160 mm and the temperature in the state is high, reaching 46°C in the summer and decreased to less than 20°C during the winter. The average humidity varied between

36%-64%. Table 3.2 shows the monthly mean of the climatic conditions data of Khartoum state, Sudan during 2011-2012.

Most of the population in the state, are workers and officers at the public and private sectors. The main activity of the people in the per-urban areas is farming. In this state, the farming activities are mainly along the riverbanks. The agricultural and farming systems mainly concentrated in Khartoum North area. In addition, to farming activities, water pipe leakage and rain water are the main sources of *An. arabiensis* larval habitats in the state. Malaria transmission in the state is considered as urban with low incidence, unstable and highly seasonal where *P. falciparum* parasite is the main causative agent (El Sayed *et al.,* 2000; Malik *et al.,* 2003).

3.3. Selection criteria and study sites

Nine sentinels were selected to collect immature stages of *An. arabiensis* according to their environmental settings; urban and peri-urban areas. These selected areas are the main sentinel sites set by the Khartoum Malaria Free Project (KMFP), Khartoum state and Ministry of Health to conduct the routine monitoring of the insecticides susceptibility status for control of *An. arabiensis* in the state. The urban area are; Arkaweet (15° 32' 52.7964" N, 32° 33' 58.7298" E), Shambat (15° 39' 39.4446" N, 32° 31' 25.683" E), Abuseid (15° 34' 20.7942" N, 32° 30' 32.6154" E), and the peri-urban: Soba West (15° 31' 12.954" N, 32° 40' 51.5028" E), Edekheinat (15° 26' 9.042" N, 32° 28' 41.4768"), Elmaygoma (15° 18' 12.654" N, 32° 35' 43.7496" E), Eltumanyat (15° 57' 41.8392" N, 32° 33' 55.9908" E), Elsalamania West (15° 18' 12.654" N, 32° 28' 13.5294" E) and Gizera Islang (15° 53' 2.9544" N, 32° 32' 6.3738") (Figure 3.1).

Furthermore, 20 sites set by the KMFP for mosquito adult surveys also categorized as urban and periurban areas were selected to collect wild adult anopheline mosquitoes. These sites spread throughout the three administrative areas of Khartoum state. The study sites cover almost about 20140 km^2 and occupied the area between 15°.10'-16°.30' N and (31°.35'-34°.20' E (Fig. 3.1). The collection sites were also categorized into urban and periurban. The latter includes agricultural schemes and farming systems (Table 3.1).

3.4. Study population

Populations of *An. arabiensis* from nine sentinel sites were tested against seven insecticides that commonly used in public health and agriculture practices in Khartoum state (Fig. 3.1). In addition, populations of *An. arabiensis* from 20 sites across Khartoum state were analyzed for knockdown resistance gene (*kdr*), acetylcholinesterase gene (ace.1^R) and *Plasmodium* sporozoites infection (Fig. 3.1).

3.5. Collection of mosquito specimens

3.5.1. Sampling of immature stages

Anopheline mosquito larvae and pupae were collected during March 2011 - February 2013 from nine sentinel sites in Khartoum state (Section 3.2.). In each sentinel site, a number of 10 to 15 aquatic habitats were randomly surveyed. Larvae and pupae were collected from possible larval habitats of different types (Fig. 3.3.) during two years period from March 2011 to February 2013. Larvae and pupae of anopheline mosquitoes were collected using standard collection methods including scoops, pipettes and collection nets. Larvae and pupae were then kept in plastic bottles and bowl covered with mosquito mesh and transported to the KMFP insectary in Khartoum.

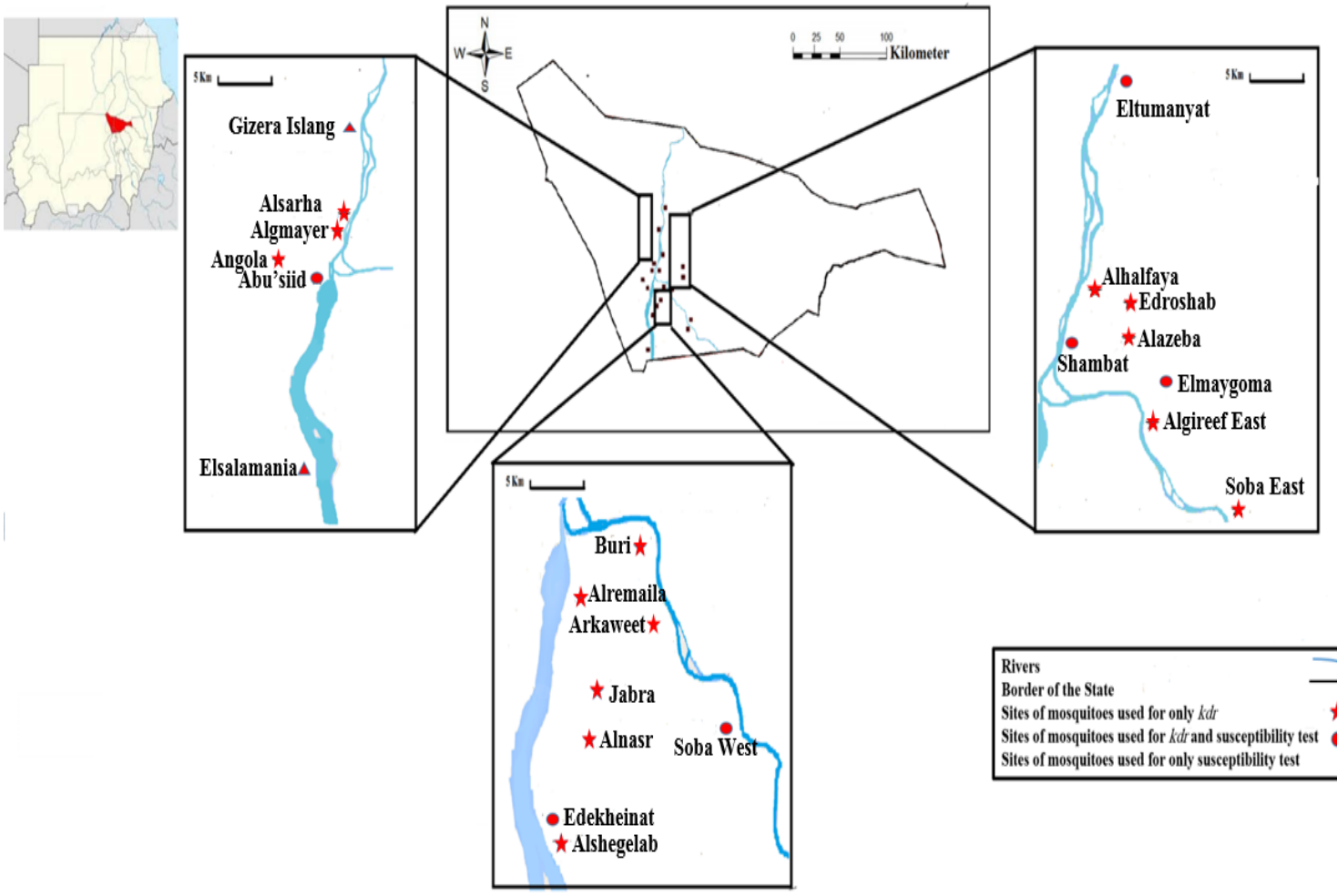

Figure 3.1: A map of Khartoum state showing the adult and immature stages collection sites.

Table 3.1: Location, description of the *Anopheles arabiensis* collection sites and the numbers of sampled mosquito specimens from the three administrative areas in Khartoum state, Sudan.

Location				Social environment	Number of mosquitoes collected
Administrative area	**Sites**	**Coordinates**			
Khartoum	Soba West	15° 29' 37.4166" N	32° 39' 32.7126" E	Peri-urban	30
	Arkaweet	15° 32' 52.7964" N	32° 33' 58.7298" E	Urban	29
	Edekheinat	15° 26' 9.042" N	32° 28' 41.4768" E	Peri-urban	30
	Alshegelab	15° 53' 2.9544" N	32° 32' 6.3738" E	Peri-urban	33
	Alnasr	15° 29' 7.7922" N	32° 31' 17.8818" E	Urban	29
	Alremaila	15° 18' 12.654" N	32° 28' 13.5294" E	Urban	30
	Jabra	15° 30' 58.95" N	32° 31' 44.3382" E	Urban	33
	Buri	15° 36' 23.8926" N	32° 34' 43.302" E	Urban	36
Khartoum North	Shambat	15° 39' 39.4446" N	32° 31' 25.683" E	Urban	34
	Elmaygoma	15° 18' 12.654" N	32° 35' 43.7496" E	Peri-urban	31
	Eltumanyat	15° 57' 41.8392" N	32° 33' 55.9908" E	Peri-urban	34
	Edroshab	15° 41' 41.2074" N	32° 34' 43.8636" E	Peri-urban	30
	Alhalfaya	15° 42' 12.261" N	32° 32' 40.2468" E	Urban	33
	Alazeba	15° 39' 52.2282" N	32° 34' 35.868" E	Urban	36
	Algireef East	15° 35' 33.5652" N	32° 35' 51.396" E	Urban	38
	Soba East	15° 31' 12.954" N	32° 40' 51.5028" E	Peri-urban	38
Omdurman	Abuseid	15° 34' 20.7942" N	32° 30' 32.6154" E	Urban	30
	Alsarha	15° 41' 13.8402" N	32° 30' 45.5142" E	Urban	31
	Algmayer	15° 40' 8.6442" N	32° 30' 27.3816" E	Urban	38
	Angola	15° 37' 14.0838" N	32° 25' 41.6814" E	Peri-urban	38

Table 3.2: The monthly mean of the climatic conditions data (temperature, relative humidity and wind direction and speed) of Khartoum state, Sudan during 2007-2014.

Month	Temperature (Max-Min)	Evaporation	Relative humidity	Total rainfall	Wind (Direction + speed)	
January	31.1-16.9	13.2	28.4	0.0	N	9.4
February	34.5-19.0	13.9	20.0	0.0	N	9.6
March	36.6-21.5	15.4	15.6	0.0	N	9.2
April	41.2-25.6	16.8	15.0	8.7	N	11.0
May	42.3-28.0	15.1	15.4	1.2	NNW	8.6
June	41.9-28.8	15.1	25.2	2.0	SW	8.6
July	38.6-26.7	12.8	46.6	20.6	SW	9.8
August	37.7-26.0	12.3	56.0	70.5	SW	9.8
September	39.5-27.1	12.5	50.4	6.1	SW	7.8
October	40.1-27.0	13.9	28.2	2.5	N	6.4
November	36.1-22.5	14.5	24.4	0.0	N	8.4
December	33.0-18.8	14.1	27.8	0.0	N	8.6

3.5.2. Sampling of adult stages

Collections of adult mosquitoes were done regularly during April and June 2013 from the 20 sites mentioned above. Moreover, female anopheline mosquitoes were also collected in November - December 2014 from only four out of the mentioned sites (Soba West, Shambat, Elmaygoma and Alazeba sites). The wild female anopheline mosquitoes were captured by active search at the resting places using a hand light torch and mouth aspirators. Adult mosquitoes were collected from resting sites at outdoor (cracks, between vegetation and wet holes close to riverbanks and irrigation canals) and indoor sites (bedrooms, cow shelters) (Fig. 3.4). The sampled specimens from each site were kept in paper cups covered at the top with mosquito fixed with a plastic rubber.

3.6. Preservation of adult mosquitoes

The sampled alive adult female from each site during the two different periods were killed using a cotton wool soaked with chloroform. Then the mosquito specimens kept individually in eppendorff tubes containing silica gel for subsequent PCR analysis to detect *kdr*, AChE mutation and *Plasmodium* parasites (sporozoites) infection. The tubes containing the preserved specimens were well coded with a given number, site and date of collection.

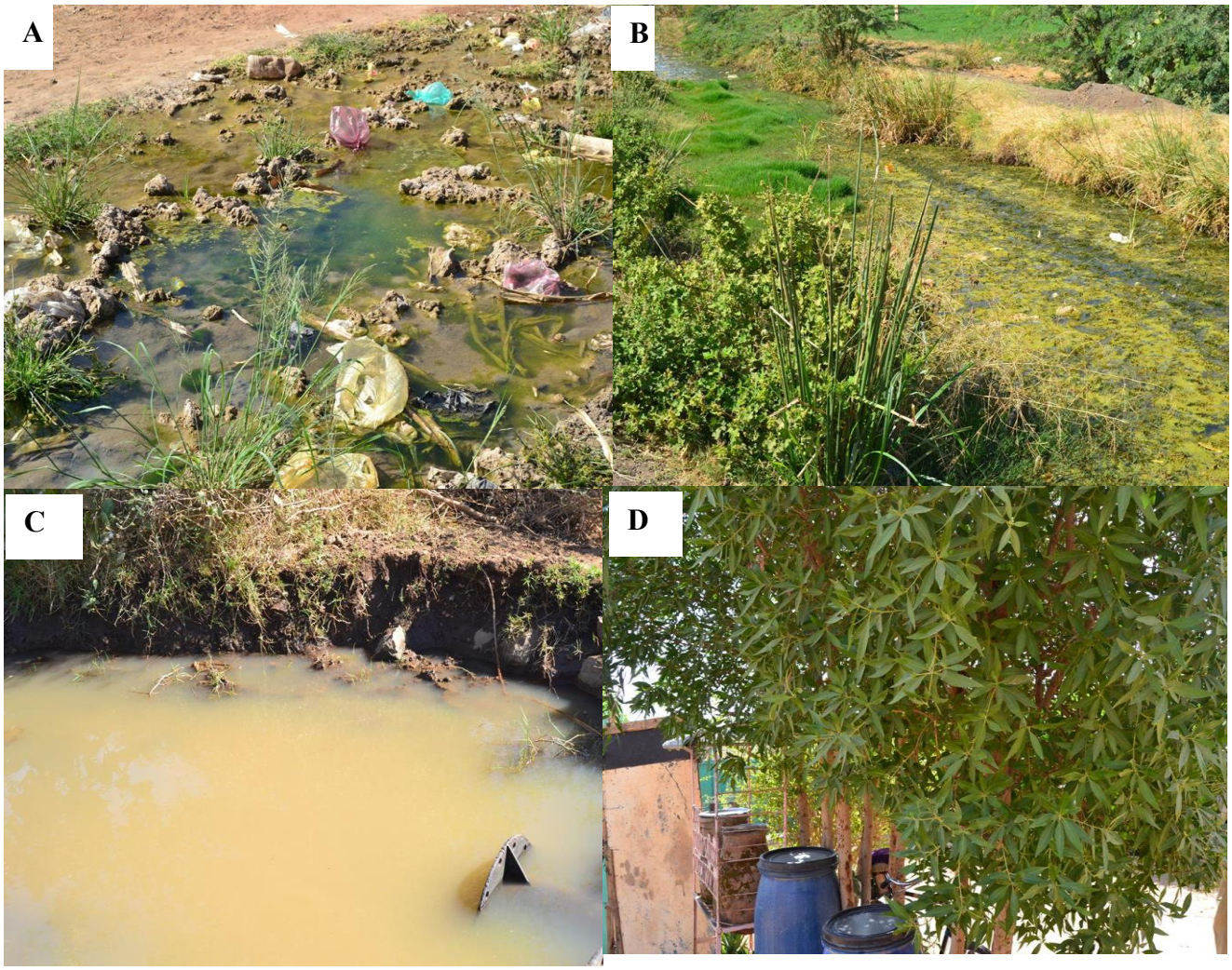

Figure 3.3. Representative types of larval habitats from where larvae and pupae were collected in different sites in Khartoum state.

A: Water pipe leakage

B: Irrigation canal

C: Water pool

D. Water pots (zeers) and barrels

Figure 3.4. Representative types of adult anopheline mosquitoes resting places from where wild specimens were sampled in different sites in Khartoum state.

A: Bed room

B: Animal shelter

3.7. Rearing of mosquitoes under laboratory conditions

Immature stages of the anopheline mosquito collected from the nine sentinel sites were transported to KMFP insectary in Khartoum. In the insectary, the immature stages were transferred into larval trays and sorted out from other organisms such as predators and culicines. The anopheline larvae and pupae were then maintained and reared using standard method for mosquito rearing (Helinski *et al.*, 2006).The larvae in the trays were reared provided with Tetramin1 fish and the pupae were placed in plastic cups and then transferred to mosquito cages (30 x 30 cm^3) for emergence. The adults when emerged, they were maintained on a 10% glucose solution on filter-paper and/or a piece of cotton wool until they subsequently used for WHO insecticide susceptibility tests.

3.8. Identification of mosquitoes

3.8.1. Larvae

Anopheline mosquito larvae were randomly selected from the collection immature stages from each site and examined under the dissecting microscope. For this purpose, the 4^{th} larval instars were selected and then identified morphologically to species level using proper anopheline mosquito entomological keys (Mattingly and Kinght, 1956; Harbach, 1988).

3.8.2. Adult

Representative specimen of the emerged adult *Anopheles* from the colony-reared mosquitoes as well as the wild collected ones from different sites were identified based on morphological features using proper

entomological keys (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987).

3.9. Protocol of the study

3.9.1. Descriptive data on larval habitats in nine sentinel sites used for susceptibility tests

Larval habitats surveyed for collection of immature stages used for susceptibility tests in this study were fully described. During the larvae and pupae collection, information on larval habitats were recorded using a well-designed format. Information on the larval habitats collected were type (source of water), presence of vegetation, land cover and land uses were observed and recorded in each sentinel sites.

3.9.2. WHO-insecticide susceptibility test

In this study, seven insecticides used in public health sector for control of malaria vector recommended by WHO were selected (Table 3.3) to determine the susceptibility/resistance status and the knockdown time for 50% and 95% ($KDT_{50\%}$and $KDT_{95\%}$) for *An. arabiensis*. The selected insecticides belongs to the classes which are widely used to control public health and agricultural pests in different African countries including Sudan.

Insecticide susceptibility tests were conducted according to WHO standard procedures (WHO, 2013b) using impregnated papers provided by WHO in March 2010. *Anopheles arabiensis* mosquitoes were tested against insecticides impregnated papers with discriminating doses shown in table 3.3. For each insecticide, different numbers of batches of 25 sugar fed females *An. arabiensis* of 2–3 days old were exposed to impregnated paper. Furthermore, controls included batches of mosquitoes from each sentinel site exposed to

untreated papers. In each sentinel site, different numbers of females were tested against different insecticides. A standard exposure time of 1 hour was used for all the tested insecticides except for fenitrothion 1.0% for which the exposure was 80 minutes. The numbers of knockdown and dead flies were recorded after 10, 20, 30, 40, 50 and 60 minutes of exposure. After one-hour (or 80 min for fenitrothion) exposure time, the knockdown and the surviving mosquitoes were transferred into clean holding tubes, provided with 10% sucrose solution on cotton. Final mortalities were recorded 24 hours post-exposure.

The insecticide susceptibility tests were conducted under optimum conditions (temperature 26°C and 70 - 80% relative humidity) at KMFP insectary.

3.9.3. Seasonal variation in *Anopheles arabiensis* susceptibility status

To investigate the temporal variation in susceptibility status for *An. arabiensis* in Khartoum state and in different sentinel sites, larvae and pupae were collected during three different seasons in the year. These were dry cold (November – February), dry hot (Late March – June) and wet (July – October). WHO-susceptibility tests were then conducted on emerged adult from collected larvae during three different seasons in each sentinel sites using the seven mentioned insecticides. Mortality rates and knockdown times (50% and 95%) for each population of *An. arabiensis* in different seasons were recorded and analyzed.

Table 3.3: Insecticides used to elucidate the susceptibility/resistance status in *Anopheles arabiensis* in nine sentinel sites in Khartoum state, Sudan.

Classes	Insecticides	Concentration %	Manufacture-Expiry date
Organochlorines	DDT	4	July 2010- July 2015
Organophosphates	Fenitrothion	1	March 2010- March 2013
	Malathion	5	July 2010- July 2013
Carbamates	Propoxur	0.1	June 2010- July 2013
Pyrethroids	Permethrin	0.75	July 2010- July 2012
	Deltamethrin	0.05	July 2010- July 2012
	Lambdacyhalothrin	0.05	July 2010- July 2012

3.9.4. Molecular assays

3.9.4.1. DNA extraction

Genomic DNA was extracted from individual wild female mosquitoes using Livak laysis buffer (Livak, 1984). The buffer was heated to 65ºC in a heating block for 15 min and mixed before used to re-dissolve precipitate. Each mosquito specimen was grinded in 200 ml LIVAK buffer in 1.5 ml eppendorff tubes. Then the tubes were incubating at 70ºC for 30 min. After incubation, the tubes were centrifuged at 13, 000 rpm for 5 min to collect condensation. Then 28 ml of 8M of K-acetate was added to each sample in the tubes and mixed well. The samples were then incubated on ice for 30 min. After incubation, the samples were centrifuged at 13,000 rpm for 10 min. The supernatant were transferred to a new 1.5 ml eppendorff tubes and centrifuged for 10 min at 13,000 rpm. Then after, the supernatants were then transferred to a new tubes and 400 ml of ice cold absolute alcohol was added and mixed gently by inversion. The samples were incubated overnight at -20 ºC. The samples were centrifuged at 13,000 rpm for 15 min, the supernatant were discarded and the pellet from each sample was rinsed in 200 ml of ice cold 70% alcohol. Finally, the samples were centrifuged at 13,000 rpm for 15 min and the pellets were left to dry on the bench and re-suspended in 200 m of PCR water. The DNA samples were kept at -20ºC for subsequent PCR analysis.

3.9.4.2. Identification of *Anopheles arabiensis* using species – specific primers

A sub-samples selected from both emerged adults collected as immature stages and wild ones were analyzed using PCR. The genomic DNA samples from individual females *An. arabiensis* were analyzed by PCR using *An. arabiensis* species-specific primers (A^o: ATGCCTGAACGCCTCTAAGG and A^{05}: CAAGATGGTTAGTTACGCCAA). PCR conditions used in this study were similar to that described by Scott *et al.* (1993). These species specific primers gave PCR amplicons of 500 bp band sizes characteristic for *An. arabiensis*.

3.9.4.3. PCR detection of *kdr* mutation

Samples from 20 localities were assayed for *kdr* mutation. The *kdr* genotype for each mosquito was determined using polymerase chain reaction (PCR) procedure and primer sequences from (Martinez-Torres *et al.*, 1998; Ranson *et al.*, 2000). In the reaction, primers Agd1 (5'-ATAGATTCCCCGACCATG-3') and Agd3 (5'-AATTTGCATTACTTACGACA-3') were used to amplify the West African resistance allele, whereas primers Agd1 (5'-ATAGATTCCCCGACCATG-3') and Agd5 (5'-TTTGCATTACTTA CGACTG -3') were used to detect the East African resistance allele. Both of these reactions amplify a 195-bp fragment. In each of the above multiplex PCR reaction, the susceptible allele was assayed using primers Agd2 (5'-AGACAAGGATGAT GA ACC-3') and Agd4 (5'-CTGTAGTGATAGGAAATTTA-3') which yields a 137-bp fragment.

DNA amplification followed the protocol: 5µl of 2 × Taq Master Mix, 0.3µl of Agd1 and Agd2 primers,1.5µl of Agd3 and Agd4 (West African resistance allele) and Agd5 (East African resistance allele) primers, 5µl of template DNA mixed in 25µl final volume with 11.4µl of PCR water. Amplification was performed under the following conditions: 5 minutes initial denaturation at 95 °C, 40 cycles of: 1 minutes denaturation at 94 °C, 1 minutes annealing at 48 °C, and 1 minute extension at 72 °C, and a 10 minutes final extension at 72°C. (Matambo *et al.*, 2007).

3.9.4.4. PCR assay for detection of ace-1^R mutation

Attempt was done to identify the ace-1^R (ace.1 G119S) resistant gene in populations of *An. arabiensis* in Khartoum state using PCR method. Genomic DNA were assayed for the same samples by an Intentional Mismatched Primer-PCR (IMP-PCR) according to the protocol of Wilkins *et al.* (2006) using specific primers CDCWT (5'TGTGGATCTTCGGCGTCG3'), CDCG119SR 5'CGGTGCCGGAGTAGAATCT3'), CDCACEF (5'GGTGGACGTGTGTGGCTC3') and CDCACER (5'CTACCGTAGCGCAAGGTTC-3'). These primers gave amplicons a 456-bp fragment as universal band, 288bp fragment for resistance allele and 196-pb fragment for susceptible allele.

The DNA amplification was done using a final volume of 20 µl. The PCR mix included 5µl of GoTaq Master Mix, 0.5µl of each of the above mentioned primers, 5µl of template DNA and 9.5µl of PCR water. Amplification was performed under the following conditions: 5 minutes initial denaturation at 95 °C, 35 cycles of: denaturation at 94 °C for 30 sec, annealing

at 61 °C for 1 min, extension at 72 °C for 7 min, and a 10 minute final extension at 72 °C.

3.9.4.5. Sporozoites detection by nested PCR

A nested PCR was performed for all samples of *An. arabiensis* collected in the two years to detect infection with *Plasmodium* sporozoites. In this assay, two amplification reactions were carried out as described by Snounou *et al.* (1993). *Plasmodium* genus specific SSUr DNA primers rPLU5 (5′-CCTGTTGTTGCCTTAAACTTC-3′) and rPLU6 (5′-TTAAAATTGTTGCAGTTAAAACG-3′) were used in the first amplification reaction (Nest1). In the second amplification reaction (Nest2), species-specific primers rFAL1 (5′TTAAACTGGTTTGGGAAAACCAAATATATT-3′) and rFAL2 (5′ACACAATGAACTCAATCATGACTACCCGTC3′), and rVIV1 (5′CGCTTCTAGCTTAATCCACATAACTGATAC3′) and rVIV2 (5′ACTTCCAAGCCGAAGCAAAGAAAGTCCTTA-3′) were used for detection of *P .falciparum*, and *P. vivax* respectively.

In the first round, DNA amplification was performed using 5µl of 2 × Taq Master Mix, 1µl of rPLU5 and rPLU6 primers, 5µl of template DNA and 13µl of PCR with 25µl final volume. For the Nest2, 1µl of Nest1 PCR products were used as the template for the second amplification where 1µl of two pairs of species specific primers (rFAL1, rFAL2, rVIV1 and rVIV2) was added to 5µl of 2 × Taq Master Mix and 15µl of PCR water with 25µl final volume. The PCR condition for each amplification reaction was 95°C for 5 min, 25 cycles of: 94°C for 30 sec, 58°C for 2 min, 72°C for 2 min, final extension at 72°C for 5 min.

A reference strain of *P. falciparum* and *P. vivax* were used as positive control whereas PCR water was used as a negative control in these experiments. Amplified DNA samples were regarded as positive for *P. falciparum* and *P. vivax* if any fragment of 120 bp and 205 bp was obtained in the Nest 2.

3.9.4.6. Agarose gel electrophoresis for detection of resistance genes (*kdr* and ace.1^{R}) and *Plasmodium* sporozoite DNA

PCR products obtained from all assays were visualized using 1.5% agarose gel electrophoresis. The 1.5% gel was prepared by melting 1.5g agarose in 1X TBE (Tris, Boric acid, EDTA) and then 2µl of ethidium bromide was added. The agarose gel mixture was then poured in a horizontal apparatus (electrophoresis tank). Then after, the gel was covered with 1X TBE buffer. Five-µl of each PCR product was then mixed with 2µl of loading dye (bromophenol blue and water). A DNA ladder (100 pb molecular weight; vivantis, UK) was also loaded. The gel electrophoresis apparatus was connected to the power supply of a voltage 80V for 1 hour. The gel was then observed under ultraviolet light to determine if PCR products have been successfully amplified. The gel with DNA amplicons was photographed by gel documentation system (UVP-91786, USA).

Mosquito specimens were identified as *An. arabiensis* when amplicons of 500 bp were detected (Scott *et al.*, 1993). According to Martinez-Torres *et al.* (1998) and Ranson *et al.* (2000), 137 bp, 195 bp DNA fragments or both together (i.e 137 and 195 bp) when observed with 293 bp internal control, the samples were homogeneous susceptible, homogenous *kdr* resistance (mutation at L1014 position) and heterogeneous suspected resistance (RS) respectively. When the primers used were Agd1 and Agd3, the homogenous

kdr resistance were L1014F-*kdr* (West African *kdr*), and if the used ones were Agd1 and Agd5, the amplicon would be identified as L1014S-*kdr* (East African *kdr*). For ace.1 G119S resistance gene primers create a 456 bp universal band, 288 bp for resistant individuals, and 196 bp for susceptible individuals. In addition, amplicons of 205 bp and 120 bp created by *Plasmodium* species specific primers were considered as a positive for *P. falciparum* and *P. vivax* **(**Snounou *et al.* 1993**)** respectively.

3.10. Spatial distribution and mapping of insecticide resistance *Anopheles arabiensis* in Khartoum state

To determine the spatial distribution and mapping of resistant strains of *An. arabiensis*, collection sites of immature stages used for WHO-susceptibility tests and wild adult used for target site resistance genes (L1014F and L1014S-*kdr*, and ace.1 G119S) were geo-referenced using Global Position System (GPS; 12 XL; German, U.S.A) with accuracy of 1-5 meters. The results of WHO-susceptibility tests for the seven insecticide used, *kdr* resistance gene and ace.1 G119S mutation in population of *An. arabiensis* in from sites surveyed in were overlaid on maps of Khartoum state (www.google.earth.com).

3.11. Socio-economic investigation of the uses of pesticides in public health and agricultural practices in Khartoum state

To assess the knowledge, attitude and practice of the public health workers and farmers on the uses of pesticides in the field, a socio-economic questionnaire was designed. The questionnaire was designed to collect demographic data and information on the types and numbers of insecticides

used during the last five years, their doses and method of preparation, knowledge about mosquitoes and the method of protection (for farmers) The questionnaire was written in English and the workers and farmers were asked in Arabic (Appendix 1). A sample of 60 health workers in two urban areas (30 in Shambat and 30 in Arkaweet) and 60 farmers (30 in Elmaygoma and 30 in Elsalamania West) were recruited to answer the questionnaire. The questionnaire was pre-tested in other areas not used as a study site.

The selection of the areas to recruit health workers was based on urban residential (Arkaweet) and urban agricultural area (Shambat) where differences in the selection pressure for *An. arabiensis* could be possible. In addition, the selection of farmers were due to the areas of high (Elmaygoma; Khartoum North) and low farming activities (Elsalamania West; Omdurman) which also might cause differences in selection pressure due to differences in numbers of insecticides used. Furthermore, more than 50% of the health workers and farmers in the surveyed areas were recruited.

3.12. Data collection and data analysis

Data were collected using standard WHO susceptibility tests for format (Appendix 2). The mortality rates after 24 exposures to each insecticide was calculated as the number of dead mosquitoes/total tested for each test replicate using Excel Software. Furthermore, the resistance/susceptibility status of the tested *An. arabiensis* due to each insecticide was determined according WHO criteria (WHO, 2013b): mortality rate ≥98% = susceptible, 90-97% = suspected/potential resistance, and <90% = resistant. Kruskal–Wallis tests and Mann–Whitney tests were used to assess the differences in the mortality rates in populations of *An. arabiensis* between sentinel sites, and between urban and periurban areas respectively using SPSS software version 20. The

knockdown times (minutes) of 50% and 95% (KDT50 and KDT_{95}) exposed populations of *An. arabiensis* collected from urban and periurban areas were Probit analysis were estimated by Probit analysis (logtimeprobit model) using SPSS software version 20. The knock down resistance ratio (KRR) was calculated by dividing KDT_{50} of the tested population/ KDT_{50} of the area with the shortest time.

The frequencies of *kdr* genotypes in *An. arabiensis* populations from different sites were compared by Chi^2 tests using SPSS software version 20. The genotypic frequencies of L1014F and L1014S in mosquito populations were compared to Hardy-Weinberg expectations using the online calculator (http://www.tufts.edu/~mcourt01/Documents/Court%20lab%20%20HW%20calculator.xls). In the analysis, *kdr* mutations L1014F and L1014S mutations were considered as independent bi-allelic loci because these alleles do not target the same base at the DNA level in codon 1014.

Moreover, descriptive statistics and *t*- test were used to analyze the data obtained by socio-economic questionnaire. All P value < 0.05 was considered significantly different.

3.13. Ethical considerations

In this study no ethical approval was required because the study was a part of routine surveys of the Khartoum Malaria Free Project (KMFP) and Integrated Vector Management Unit (IVM), Federal Ministry of Health, Sudan. In addition, the surveyed sites are the main areas included in the routine surveys of the KMFP.

CHAPTER FOUR

RESULTS

4.1. Descriptive data on the larval habitats in the sentinel sites in Khartoum state

Table 4.1 shows descriptions and types of larval habitats and their numbers from where mosquito larvae and pupae were collected for WHO-susceptibility tests in each sentinel sites in Khartoum state during March 2011 to February 2013. A total of 113 sites were surveyed during this study for collection of larvae and pupae. Of these, 32.7% were in sentinel sites in Khartoum, 38.1% in Khartoum North and 29.2% in Omdurman area. In this study, two types of larval habitats were recorded; these were drinking water pipes leakage and irrigation canals. The most dominant types were those formed by the drinking water pipes leakage (54.4%). With except of Elsalamania West site, larval habitats formed of drinking water pipes were recorded in all sentinel sites investigated. Moreover, larval habitats formed of irrigation canals were found in 5 sentinel sites, these were; Shambat, Elmaygoma, Eltumanyat, Elsalamania West and Gizera Islang. The majority of the larval habitats (72; 63.7%) were observed in settlement areas (Fig. 4.1). Most of these habitats were formed of fresh clean water (46.90%) followed by habitat with presence of grasses (46.9%) (Fig. 4.2).

Table 4.1: Descriptions, types of larval habitats and their numbers in each sentinel sites in Khartoum state during March 2011 to February 2013.

Sites	Number (%) of larval habitat types		Description of larval habitats (No; %)			Presence of other organisms	Total
	Pipe leakage	Irrigation canals	Clean	Presence of grasses	Presence of algae		
Soba West	12 (10.6)	0 (0)	6 (5.3)	3 (2.6)	3 (2.6)	6(50)	**12**
Arkaweet	15 (13.3.7)	0 (0)	9 (7.9)	5 (4.4)	1 (0.9)	6(40)	**15**
Edekheinat	10 (8.8)	0 (0)	8 (7.1)	1 (0.9)	1 (0.9)	2(20)	**10**
Shambat	9 (7.3)	4 (3.5)	3 (2.6)	4 (3.5)	6 (5.3)	10(76.9)	**13**
Elmaygoma	7 (6.1)	8 (7.1)	2 (1.8)	5 (4.4)	8 (7.1)	13(86.7)	**15**
Eltumanyat	2 (1.8)	13 (11.5)	3 (2.9)	2 (1.8)	10 (8.8)	13(86.7)	**15**
Abuseid	10 (8.8)	0 (0)	4 (3.5)	3 (2.6)	3 (2.6)	6(60)	**10**
Elsalamania West	0 (0)	10 (8.8)	10 (8.8)	0 (0)	0 (0)	0(0)	**10**
Gizera Islang	1 (0.9)	12 (10.6)	8 (7.1)	5 (4.4)	0 (0)	4(30.8)	**13**
Total	**66 (58.4)**	**47 (41.6)**	**53 (46.9)**	**28 (24.8)**	**32 (28.3)**	**60(53.10)**	**113**

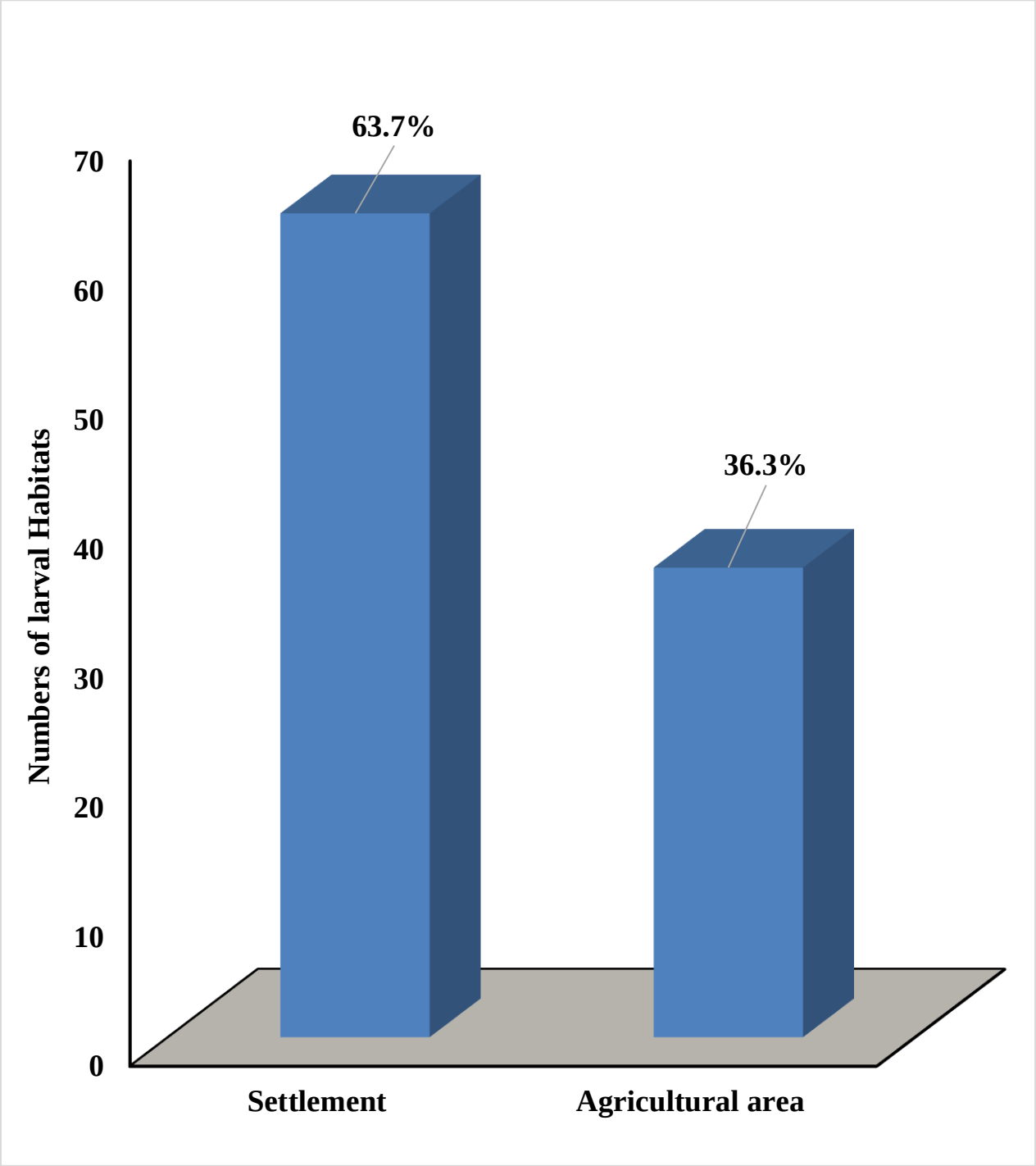

Figure 4.1: Percentages of larval habitats in two different areas land cover surveyed in Khartoum state during 2011 -2013.

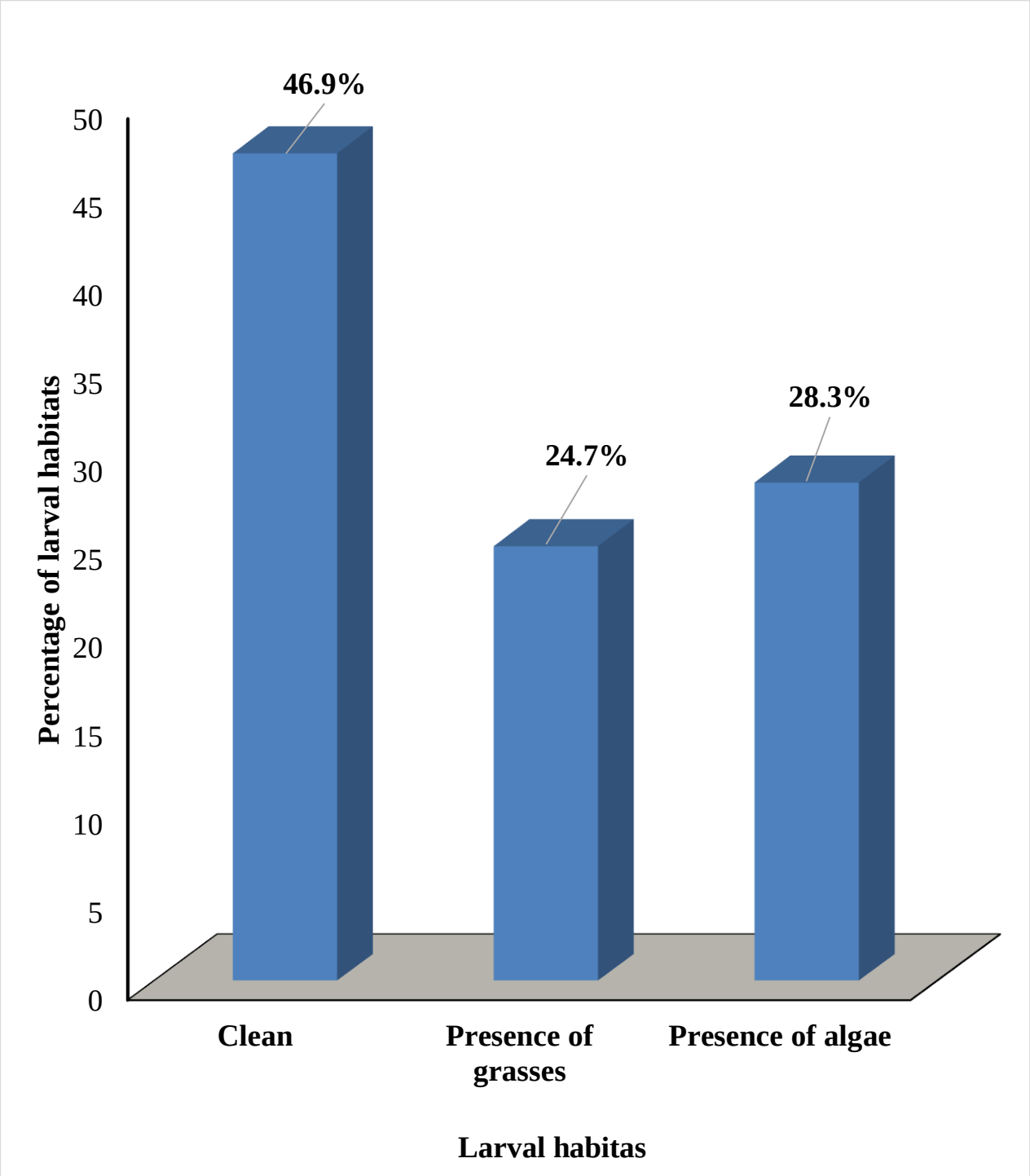

Figure 4.2: Percentages of larval habitats with different characteristics surveyed in Khartoum state during 2011 -2013.

4.2. WHO susceptibility tests for populations of *Anopheles arabiensis* from nine sentinel sites in Khartoum state

4.2.1. Anopheline mosquitoes

A total of 8325 adult anopheline mosquitoes were obtained from larval collection from the nine sentinel sites and reared in the insectary. All mosquito specimens were identified morphologically as *An. gambiae* s.l. Furthermore, all the 500 randomly selected sub-samples analyzed by PCR were *An. arabiensis* (Fig. 4.3).

4.2.2. Descriptive data

In this study, seven insecticides were used to test populations of *An. arabiensis* from nine sentinel sites in Khartoum state. The insecticides with diagnostic dosages used were 4% DDT, 1% fenitrothion, 5% malathion, 0.1% propoxur, 0.75% permethrin, 0.05% deltamethrin and 0.05% lambdacyhalothrin. These insecticides were used to determine susceptibility/resistance status of *An. arabiensis* from Soba West, Arkaweet, Edekheinat, Shambat, Elmaygoma, Eltumanyat, Abuseid, Elsalamania West and Gizera Islang sites.

All the seven insecticides were used to test populations of *An. arabiensis* from three sentinel sites; Soba West, Edekheinat and Elsalamania West. DDT 4% and fenitrothion 1% used to test mosquitoes from seven sentinel sites. However, deltamethrin 0.05% was the only insecticide that was used to test mosquitoes from the all sentinel sites investigated. Furthermore, 0.05% lambdacyhalothrin insecticide was used to test mosquitoes from only three sentinel sites; Soba West, Edekheinat and Elsalamania West.

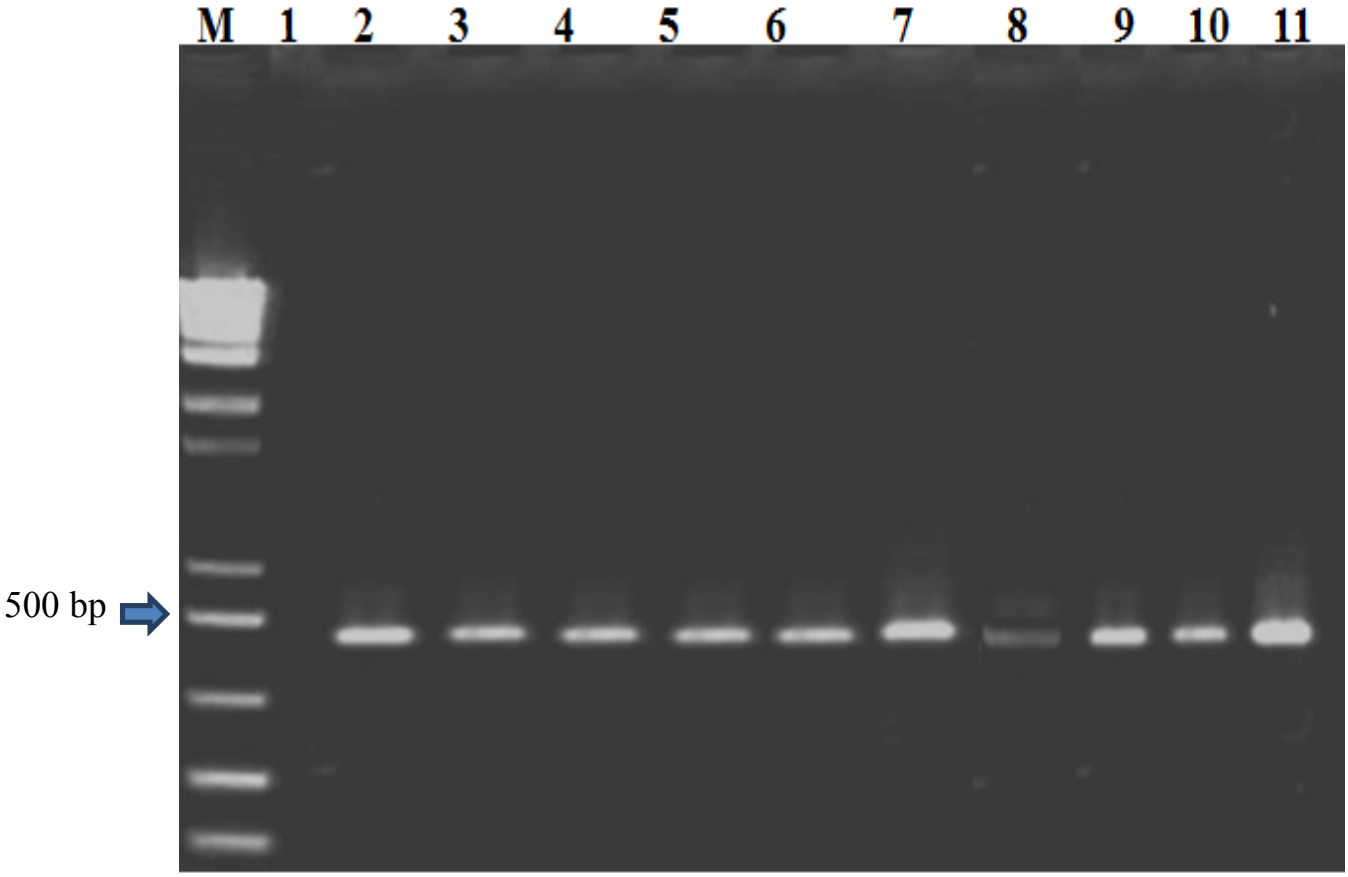

Figure 4.3: Polymerase Chain Reaction (PCR) product showing specific DNA amplicons for identification of *Anopheles arabiensis* **collected from nine sentinel sites in Khartoum state.**

Lane M: 100 kb DNA molecular markers; lane 1: negative control (PCR water); lane 2 -4: samples from Soba West, Arkaweet and Edekheinat respectively; lane 5-7: specimens from Shambat, Elmaygoma and Eltumanyat respectively; lane 8-10: mosquitoes from Abuseid, Elsalamania West and Gizera Islang respectively, and lane 11: reference strains from Dongola colony-reared *An. arabiensis*.

4.2.3. Mortality rates of *Anopheles arabiensis* in Khartoum state

The overall mean percentage of mortalities in populations of *An. arabiensis* from Khartoum state after 24 hour exposures to seven WHO different insecticide impregnated papers are shown in table 4.2 and figure 4.4. A total of 8325 females *An. arabiensis* arranged in 331 patches of 25 individual mosquitoes in each replicate were exposed to the above mentioned insecticides. Abbott's formula was not required for correction of mortality results because percentage of mortality in control groups was less than 5% to all diagnostic concentrations. The results showed that populations of *An. arabiensis* in Khartoum state were fully susceptible to fenitrothion 1% and lambdacyhalothrin 0.05% and were highly resistance to DDT 4%, malathion 5%, propoxur 0.1%, permethrin 0.75% and deltamethrin 0.05% (Fig. 4.4).

The results on the susceptibility/resistance status of populations of *An. arabiensis* from all sentinel sites are shown in tables 4.3. With exception to propoxur 0.1%, significant differences were observed in mortality rates in *An. arabiensis* between the sentinel sites due to insecticides (for DDT 4%χ^2 = 41.108, degree of freedom [df] = 7, P = 0.00; fenitrothion 1%χ^2= 70.97, df = 8, P = 0.00; Malathion 5% χ^2= 29.651, df = 6, P = 0.00 for permethrin 0.75%χ^2 = 14.566, df = 4, P = 0.006 and for deltamethrin 0.05% χ^2 = 20.897, df = 8, P = 0.007). Populations of *An. arabiensis* were resistant to DDT 4% in most of the sentinel sites investigated. However, specimens of *An. arabiensis* from Soba West and Elsalamania West sites were susceptible (99±0.53 and 98±2.0 respectively) and those from Eltumanyat site were suspected/potential resistance (96±1.71) to DDT. Moreover, populations of *An. arabiensis* from all sentinels sites were susceptible to fenitrothion. Resistance to malathion 5% was observed in *An. arabiensis* from five sentinel sites; Soba West (73±9.36), Shambat (80±2.67), Elmaygoma

(81±2.47), Eltumanyat (78±7.39) and Elsalamania West sties (89±1.0). Nevertheless, suspected/potential resistance was observed in specimens from Edekheinat (92±0.57) and Abuseid (94±1.25) sites. Of the seven populations of *An. arabiensis* tested using propoxur 0.1% two showed resistance and five were suspected/potential resistance to this insecticide (Table 4.3). Resistance to permethrin insecticide was observed in three out of five tested populations of *An. arabiensis*; these were from Edekheinat (88±5.42), Elmaygoma (70±6.4) and Elsalamania West sites (89±1.91). However, *An. arabiensis* from Arkaweet site was fully susceptible (100%) whereas, specimens from Soba West were suspected/potential resistance (95±2.52) to permethrin. Specimens of *An. arabiensis* from all sentinel sites were tested using deltamethrin. Resistance to this insecticide was observed in specimens from Eltumanyat and Gizera Islang sites, suspected/potential resistance in Soba West, Edekheinat, Shambat, Elmaygoma and Abuseid sites and highly susceptible in Arkaweet and Elsalamania West sites. All the three populations of *An. arabiensis* tested using lambdacyhalothrin were fully susceptible (100% for all).

Mortality rates in populations of *An. arabiensis* from the three administrative areas in Khartoum state exposed to seven insecticides are depicted in table 4.4. A significant difference in mortality rates were observed in *An. arabiensis* between the three administrative areas due to exposure to DDT 4% (χ^2=14.35, df = 2, P = 0.001), malathion 5% (χ^2=19.25, df = 2, P = 0.00), propoxur 0.1, permthrin 0.75%(χ^2=10.308, df = 2, P = 0.006) and deltamethrin 0.05% (χ^2=6.89, df = 2, P = 0.032). *Anopheles arabiensis* from the three areas were fully susceptible to fenitrothion 1% (100 ± 0.0 for each). In addition, high mortality rates were observed in the two tested populations against lambdacyhalothrin 0.05%, these were specimens from Khartoum (100 ± 0.5) and Omdurman (99 ±

1.00) areas. Resistance to DDT 4% was observed in *An. arabiensis* in Khartoum North (86 ± 1.93) whereas the two other populations were suspected resistance to this insecticide (Table 4.4). *Anopheles arabiensis* from Khartoum (95 ± 1.22) and Khartoum North (87 ± 4.56) were highly resistant to malathion 5% with those from Omdurman area were suspected/resistance (95 ± 1.22). In contrast, population of this species from only Khartoum North was resistant to propoxur 0.1% (85± 4.23). Resistances to permethrin 0.75% were observed in *An. arabiensis* from the three areas (Table 4.4). Resistance to deltamethrin 0.05% was observed in *An. arabiensis* form Khartoum North (89 ± 3.5) whereas those from Khartoum and Omdurman areas were fully susceptible (100 ± 0.0) and suspected/resistance (95 ± 1.82) respectively.

Table 4.5 and figure 4.5 illustrate the mean percentage mortality rates of *Anopheles arabiensis* from two different land scape areas in Khartoum state during 2011 -2013. Using Mann-Whitney test, a significant differences in the mortality rates were observed between the populations of *An. arabiensis* from urban and periurban areas due to DDT 4%, malathion 5% and permethrin 0.75% (P = 0.042, , 0.005 and 0.003 respectively). *Anopheles arabiensis* from both urban and periurban areas were susceptible to fenitrothion (100 ± 0.0 for each) and lambdacyhalothrin (urban, 99± 0.50 and periurban, 99 ± 1.0). In contrast, this species was resistant to malathion 5% and propoxur 0.1% (Table 4.5 and fig. 4.5). In the urban area *An. arabiensis* was suspected resistance to DDT (92 ± 1.74), permethrin (94 ± 2.33) and deltamethrin (95± 1.66).Whereas in the periurban areas, it was suspected resistance to deltamethrin (92 ± 2.28).

Table 4.2: Overall results of insecticides mean percentage and stander error of mean of mortality rates after 24h exposure for the *Anopheles arabiensis* from Khartoum state during 2011-2013.

Insecticide used	No. of females tested (replicates)	Mean % mortality After 24 hours	(±SE) % mortality After 24 hours
DDT 4%	1700 (68)	88	1.23
Fenitrothion 1%	1800 (70)	100	0.12
Malathion 5%	1900 (76)	84	1.75
Propoxur 0.1%	1050 (42)	88	4.11
Permethrin 0.75%	550 (22)	88	1.76
Deltamethrin 0.05%	1025 (41)	89	1.48
Lambdacyhalothrin 0.05%	300 (12)	100	0.45

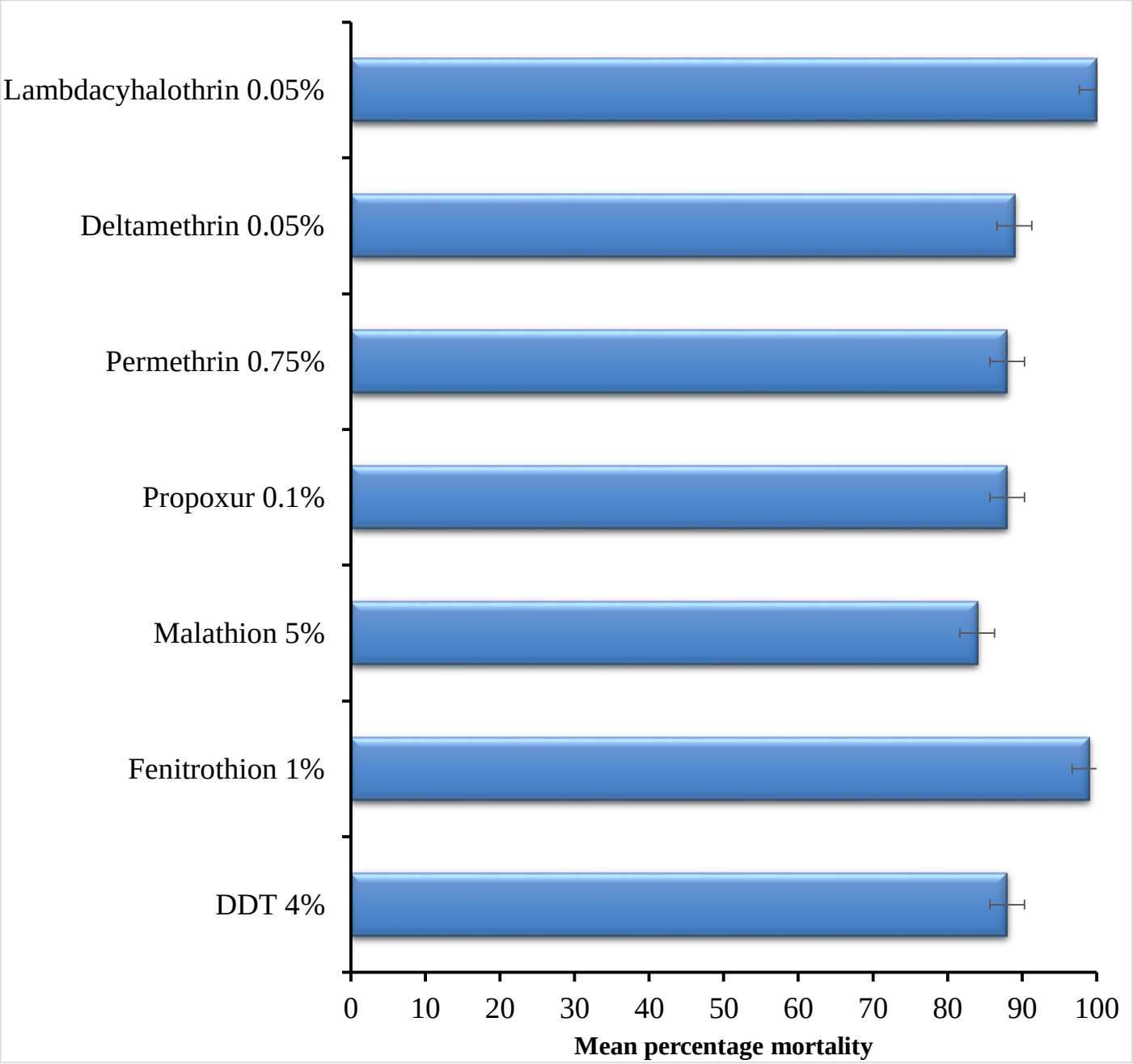

Figure 4.4: Overall mean mortality rates after 24 hours exposure to insecticides tested for *Anopheles arabiensis* populations from different sentinel sites in Khartoum state

Table 4.3:

Table 4.4: Mortality rates of *Anopheles arabiensis* from different areas in Khartoum state exposed to seven insecticides during 2011-2013.

Administrative areas	Insecticide used	No. of females tested (replicates)	Mean ± SE
Khartoum	DDT 4%	475 (19)	93 ± 1.96
	Fenitrothion 1%	800 (32)	100 ± 0.0
	Malathion 5%	700 (28)	87 ± 4.56
	Propoxur 0.1%	400 (16)	93 ± 1.42
	Permethrin 0.75%	300 (12)	94 ± 2.33
	Deltamethrin 0.05%	350 (14)	96 ± 2.03
	Lambdacyhalothrin 0.05%	200 (8)	100 ± 0.50
Khartoum North	DDT 4%	825 (33)	86 ± 1.93
	Fenitrothion 1%	650 (26)	100 ± 0.34
	Malathion 5%	900 (36)	80. ± 1.77
	Propoxur 0.1%	350 (14)	85.11± 4.29
	Permethrin 0.75%	100 (4)	71 ± 6.40
	Deltamethrin 0.05%	275 (11)	88.7 ± 3.510
	Lambdacyhalothrin 0.05%	**ND**	**ND**
Omdurman	DDT 4%	400 (16)	95 ± 1.82
	Fenitrothion 1%	350 (14)	100 ± 0.0
	Malathion 5%	300 (12)	95 ± 1.22
	Propoxur 0.1%	300 (12)	93 ± 1.42
	Permethrin 0.75%	150 (6)	89 ± 1.91
	Deltamethrin 0.05%	400 (16)	95 ± 1.93
	Lambdacyhalothrin 0.05%	100 (4)	99 ± 1.00

ND: Not done

Table 4.5: Mean (±SE) mortality rates of *Anopheles arabiensis* from urban and periurban areas in Khartoum state exposed to seven insecticides during 2011 -2013.

Land scape	Insecticide used	No. of females tested (replicates)	Mean ± SE
Urban	DDT 4%	1300(52)	92 ± 1.74
	Fenitrothion 1%	1600(64)	100 ± 0.0
	Malathion 5%	1700(68)	88 ± 3.31
	Propoxur 0.1%	850(34)	72 ± 9.81
	Permethrin0.75%	400(16)	94 ± 2.33
	Deltamethrin0.05%	650(26)	95± 1.66
	Lambdacyhalothrin 0.05%	200(8)	99± 0.50
Periurban	DDT 4%	400(16)	88 ± 1.70
	Fenitrothion 1%	200(8)	100 ± 0.28
	Malathion 5%	200(8)	82 ± 1.83
	Propoxur 0.1%	200(8)	88 ± 2.88
	Permethrin0.75%	150(6)	80 ± 4.60
	Deltamethrin0.05%	375(15)	92 ± 2.28
	Lambdacyhalothrin 0.05%	100(4)	99 ± 1.00

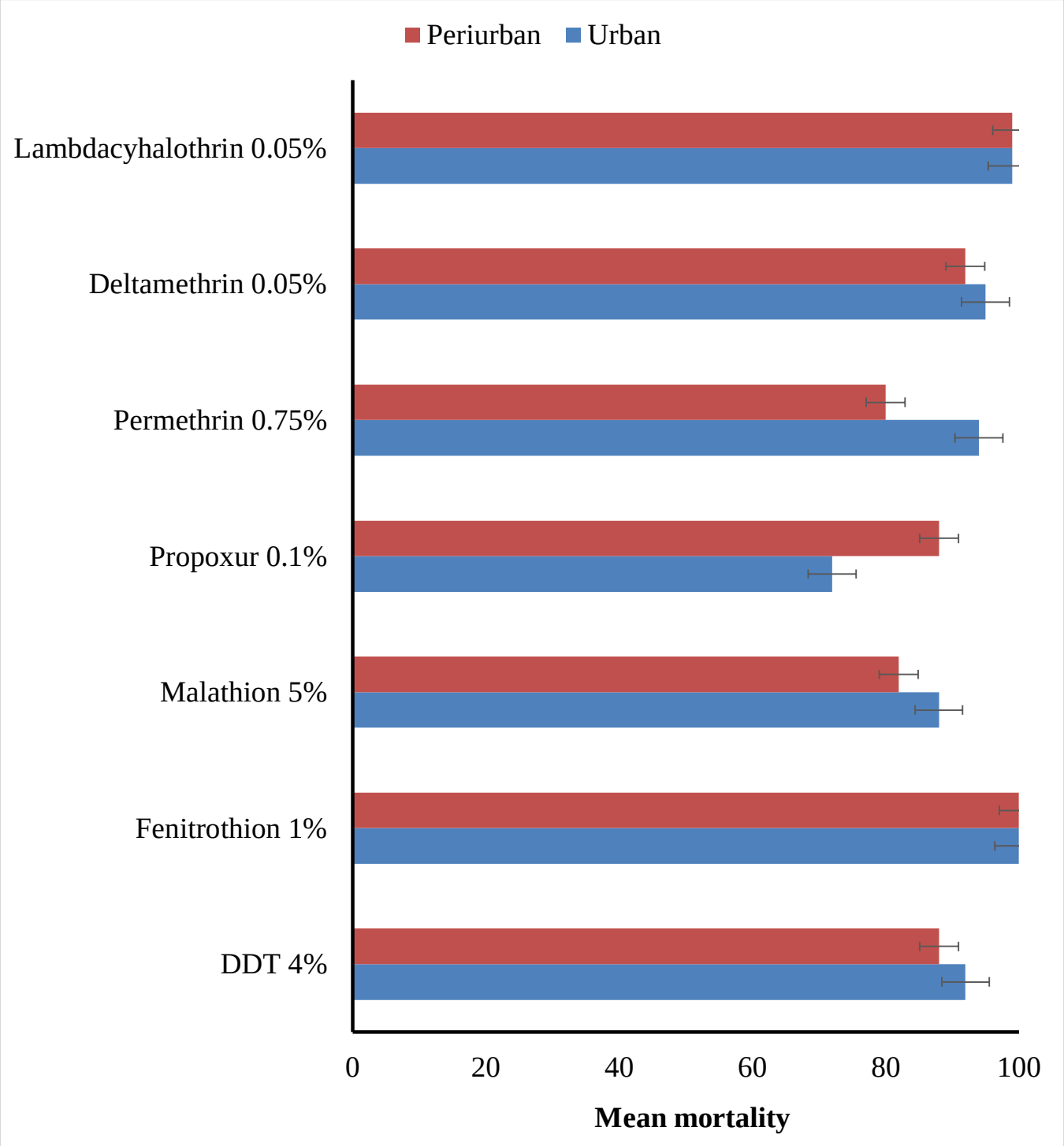

Figure 4.5: Mean percentage mortality in populations of *Anopheles arabiensis* **from urban and periurban areas in Khartoum state exposed to seven insecticides during 2011 -2013.**

4.2.3. Knockdown time for 50% and 95% *Anopheles arabiensis* due to insecticides tested in Khartoum state

The knockdown effects of the tested insecticides to the *An. arabiensis* population collected from nine sentinel sites in Khartoum state are presented in table 4.6 to 4.11. Using Probit analysis, the knockdown time for *An. arabiensis* from Abuseid site showed the lowest KDT_{50} (32.5 min) and KDT_{95} (74.81 min) for DDT 4% compared to other sentinel sites (χ^2 = 2454.36, df = 576, P = 0.00). Similarly, the knockdown time for *An. arabiensis* for fenitrothion was significantly lowest in Shambat area (52.72 and 146.75 minutes for KDT_{50} and KDT_{95} respectively) than in other sites (χ^2 = 2157, df = 495, P = 0.00). Moreover, the population of the vector from Edekheinat site showed the lowest KDT_{50} and KDT_{95} for malathion 4% (36.08 and 92.77 minutes respectively; (χ^2= 1868.41, df = 468, P = 0.00) and propoxur 0.1% (44.79 and 94.15 minutes respectively; (χ^2= 1169.34, df = 300, P = 0.00). In contrast, populations of *An. arabiensis* from Arkaweet site showed the lowest KDT_{50} and KDT_{95} for permthrin 0.75% (21.20 and 47.49 minutes respectively; (χ^2= 289.42, df = 133, P = 0.00) and deltamethrin 0.05% (21.67 and 45.19 minutes respectively; (χ^2= 658.88, df = 276, P = 0.00) than other sites. Lambdacyhalothrin 0.05% was tested against only three population of *An. arabiensis* with its highest and lowest knockdown ($KDT_{50}$and KDT_{95}) effect in populations of Soba West (44.0 and 77.17 minutes respectively) and Elsalamania West sites (35.17 and 61.68 minutes respectively) respectively (χ^2= 118.22, df = 79, P = 0.003).

Table 4.6: Mean mortality rates and knockdown time of *Anopheles arabiensis* **from different sentinel sites in Khartoum state exposed to DDT 4% for a period of 60 min.**

Site	Number exposed (N) (replicates)	Mean mortality (%) ±SE	KDT_{50} (in min) (95% Cl)	KDT_{95} (in min) (95% Cl)	KDT_{50} ratio (RR)
Soba West	175 (7)	99 ± 0.53	35.60 (33.66 -37.67)	81.0 (75.02 -88.15)	1.1
Edekheinat	300 (12)	84 ± 3.61	38.70 (36.10 - 41.52)	88.03 (80.54 - 97.04)	1.2
Shambat	175 (7)	83 ± 4.68	56.32 (51.93 - 61.24)	128.13 (115.39-143.74)	1.7
Elmaygoma	500 (20)	82 ± 2.18	55.18 (51.92 - 58.78)	125.54 (114.79 - 138.66)	1.7
Eltumanyat	150 (6)	96 ± 1.71	45.10 (40.75 - 49.97)	102.60 (91.33 - 116.28)	1.4
Abuseid	150 (6)	57 ± 3.64	32.88 (29.82 - 36.26)	74.81 (67.11 - 84.05)	1.0
Elsalamania West	100 (4)	98 ± 2.0	34.90 (30.89 - 39-44)	83.707 (69.63 - 91.25)	1.1
Gizera Islang	150 (6)	99 ± 0.89	36.82 (33.33 - 40.70)	83.76 (74.89 - 94.44)	1.1

Table 4.7: Mean mortality rates and knockdown time of *Anopheles arabiensis* **from different sentinel sites in Khartoum state exposed to fenitrothion1% for a period of 80 min.**

Site	Number exposed (N) (replicates)	Mean mortality (%) ±SE	KDT_{50} (in min) (95% Cl)	KDT_{95} (in min) (95% Cl)	KDT_{50} ratio (RR)
Soba West	400 (16)	100 ± 0	64.24 (58.54-71.37)	178.82 (150.28-221.88)	1.2
Arkaweet	100 (4)	100 ± 0	73.82 (61.28-90.25)	205.50 (161.83-272.77)	1.4
Edekheinat	300 (12)	100 ± 0	98.97 (85.55-117.02)	275.51 (220.154-362.97)	1.9
Shambat	300 (12)	99 ± 0	52.72 (47.9-58.52)	146.75 (124.14-180.34)	1.0
Elmaygoma	300 (12)	100 ± 0	75.68 (67.42-86.27)	210.67 (137.67-267.301)	1.4
Abuseid	200 (8)	100 ± 0	78.83 (68.37-92.40)	219.43 (177.50-284.08)	1.5
Elsalamania West	150 (6)	100 ± 0	106.06 (86.58-132.92)	295.23 (226.43-405.65)	2.0

Table 4.8: Mean mortality rates and knockdown time of *Anopheles arabiensis* from different sentinel sites in Khartoum state exposed to malathion1% for a period of 60 min.

Site	Number exposed (N) (replicates)	Mean mortality (%) ±SE	KDT_{50} (in min) (95% Cl)	KDT_{95} (in min) (95% Cl)	KDT_{50} ratio (RR)
Soba West	300 (12)	73 ± 9.36	59.48 (53.60-66.29)	152.86 (133.36-178.00)	1.7
Edekheinat	400 (16)	92 ± .57	36.08 (33.51-38.92)	92.77 (83.73-104.06)	1.0
Shambat	300 (12)	80 ± 2.67	42.51 (39.41-45.94)	109.25 (98.10-123.28)	1.2
Elmaygoma	500 (20)	81 ± 2.47	45.96 (43.27-48.94)	118.13 (106.86-132.40)	1.3
Eltumanyat	100 (4)	78 ± 7.39	120.44 (93.68-156.48)	309.53 (235.31-416.26)	3.3
Abuseid	200 (8)	94 ± 1.25	43.43 (39.47-47.88)	111.63 (98.84-127.75)	1.2
Elsalamania West	100 (4)	89 ± 1.0	42.71 (37.35-49)	109.78 (94.28-129.51)	1.2

Table 4.9: Mean mortality rates and knockdown time of *Anopheles arabiensis* from different sentinel sites in Khartoum state exposed to propoxur 0.1% for a period of 60 min.

Site	Number exposed (N) (replicates)	Mean mortality (%) ±SE	KDT_{50} (in min) (95% Cl)	KDT_{95} (in min) (95% Cl)	KDT_{50} ratio (RR)
Soba West	300 (12)	58 ± 17.63	51.26 (47.29-55.82)	107.74 (95.55-124.35)	1.1
Edekheinat	100 (4)	87 ± 1.91	44.79 (39.99-50.16)	94.15 (82.04-110.36)	1.0
Shambat	200 (8)	95 ± 1.3	46.17 (42.61-50.16)	97.04 (86.53-111.19)	1.0
Elmaygoma	150 (6)	92 ±6.88	59.36 (57.86-64.75)	124.76 (110.145.05)	1.3
Abuseid	100 (4)	94 ± 2.58	52.32 (46.43-59.23)	109.97 (94.95-130.42)	1.2
Elsalamania West	100 (4)	94 ± 2.58	54.53 (48.31-61.87)	114.620 (98.66-136.375)	1.2
Gizera Islang	100 (4)	95 ± 253	49.91 (45.45-55.03)	104.90 (92.38-121.88)	1.1

Table 4.10: Mean mortality rates and knockdown time of *Anopheles arabiensis* from different sentinel sites in Khartoum state exposed to permthrin (permth) 0.75% and lambdacyhalothrin (Lambda) 0.05% for a period of 60 min.

Insecticide used	Site	Number exposed (N) (replicates)	Mean mortality (%) ±SE	KDT_{50} (in min) (95% Cl)	KDT_{95} (in min) (95% Cl)	KDT_{50} ratio (RR)
Permeth	Soba West	100 (4)	95 ± 2.52	41.46 (37.95-45.38)	93.07 (82.74-106.60)	2.0
	Arkaweet	100 (4)	100 ± 0	21.20 (19.36-23.16)	47.59 (42.82-53.63)	1.0
	Edekheinat	100 (4)	88 ± 5.42	57.47 (51.93-63.99)	129.00 (112.27-151.60)	2.7
	Elmaygoma	100 (4)	70 ± 6.4	45.15 (41.36-49.43)	101.34 (89.85-116.53)	2.1
	Elsalamania West	100 (4)	89 ± 1.91	44.48 (40.75-48.71)	99.84 (88.56-114.70)	2.1
Lambda	Soba West	100 (4)	100 ± 0	35.17 (33.14-37.29)	61.68 (57.22-67.28)	1.0
	Edekheinat	100 (4)	100 ± 0	42.04 (39.67 - 44.55)	73.73 (68.21-80.73)	1.2
	Elsalamania West	100 (4)	99 ± 1.0	44.00 (41.65-46.54)	77.17 (71.38-84.59)	13

Table 4.11: Mean mortality rates and knockdown time of *Anopheles arabiensis* from different sentinel sites in Khartoum state exposed to deltamethrin 0.05% for a period of 60 min.

Site	Number exposed (N) (replicates)	Mean mortality (%) ±SE	KDT_{50} (in min) (95% Cl)	KDT_{95} (in min) (95% Cl)	KDT_{50} ratio (RR)
Soba West	100 (4)	97 ± 1.91	29.09 (26.69-31.70)	60.68 (55.07-66.89)	1.3
Arkaweet	100 (4)	100 ± 0	21.67 (19.85-23.64)	45.19 (41.22-49.76)	1.0
Edekheinat	150 (6)	91 ± 4.46	32.34 (30.20-34.63)	67.44 (62.40-73.26)	1.5
Shambat	100 (4)	90 ± 3.46	31.87 (29.35-34.60)	66.46 (60.76-73.05)	1.5
Elmaygoma	100 (4)	97 ± 1.91	28.87 (26.49-31.46)	60.20 (54.89-66.36)	1.3
Eltumanyat	75 (3)	43 ± 10.58	34.63 (31.36 – 38.24)	72.20 (64.96 – 80.68)	1.6
Abuseid	100 (4)	92 ± 1.63	28.50 (26.22-30.97)	59.42 (54.26-65.41)	1.3
Elsalamania West	200 (8)	100 ± 0.65	33.70 (31.75-35.76)	70.27 (65.47-75.81)	1.6
Gizera Islang	100 (4)	89 ± 5.89	32.52 (29.82-35.46)	67.81 (61.74-74.86)	1.5

Variations in the knockdown resistance ratio (KRR) for *An. arabiensis* tested against the seven insecticides were observed. For DDT 4%, higher KRR by 1.7 fold for both Shambat and Elmaygoma and by 1.4 fold for Eltumanyat than in Abuseid site. Likewise, fenitrothion 1% showed the highest KRR among the other sentinel sites which represent 2.0 fold of that in Shambat site. The KRR for *An. arabiensis* due to malathion 5% in Elsalamania West was higher as 3.3 fold of that of Edekheinat whereas, for propoxur 0.1% it was higher by 1.3 fold than that in Shambat. For permethrin, KRR in *An. arabiensis* from Edekheinat, Elmaygoma and Elsalamania West was higher by 2.7, 2.1 and 2.1 folds respectively compared to those from Arkaweet site. Relatively similar KRR due to deltamethrin 0.05% were observed in *An. arabiensis* from Edekheinat (1.2) and Elsalamania West (1.3) compared to those from Soba West. With exception of *An. arabiensis* from Arkaweet, the KRR due to lambdacyhalothrin 0.05% varied from 1.3 to 1.6 in other sentinel sites.

Knockdown times for populations of *An. arabiensis* from the three administrative areas in Khartoum state are depicted in table 4.12. A significant differences in knockdown times in *An. arabiensis* were observed between the three administrative areas for all insecticides tested; DDT 4% ($\chi^2 = 244.42$, df =58, P = 0.00), fenitrothion 1% ($\chi^2 = 9307.84$, df= 500. P= 0.00), malathion 5% ($\chi^2 = 2413.78$, df = 472, P= 0.00), propoxur 0.1% ($\chi^2 = 1355.12$, df = 304, P= 0.00), permthrin 0.75% ($\chi^2 = 814.11$, df = 135, P = 0.00), deltamethrin 0.05% ($\chi^2 = 802.65$, df = 282, P = 0.00) and lambdacyhalothrin 0.05% ($\chi^2 = 146.57$, df = 80, P= 0.00).

Table 4.12: Mean mortality rates and knockdown time of *Anopheles arabiensis* from the three administrative areas in Khartoum state exposed to insecticides during 2011 - 2013.

Insecticide used	Site	Number exposed (replicates)	KDT_{50} (in min) (95% Cl)	KDT_{95} (in min) (95% Cl)	KDT_{50} ratio (RR)
DDT 4%	Khartoum	475 (19)	36.81 (35.24 ± 38.46)	84.10 (78.45 ± 90.89)	1.1
	Khartoum North	825 (33)	53.65 (51.29 ± 56.23)	122.56 (113.23 ± 133.97)	1.5
	Omdurman	400 (16)	34.80 (32.76 ± 36.97)	79.50 (73.42 ± 86.76)	1.0
Fenitrothion 1%	Khartoum	800 (32)	69.40 (61.02 ± 83.04)	156.30 (120.8 ± 235.5)	1.1
	Khartoum North	650 (26)	60.94 (53.88 ± 71.75)	137.24 (107.58 ± 201.88)	1.0
	Omdurman	350 (14)	80.72 (66.0 ± 105.27)	181.80 (133.45 ± 292.42)	1.3
Malathion 5%	Khartoum	700 (28)	43.87 (40.88 ± 47.21)	119.18 (105.97 ± 136.58)	1.0
	Khartoum North	900 (36)	48.40 (45.78 ± 51.37)	131.50 (117.40 ± 150.23)	1.1
	Omdurman	300 (12)	43.55 (39.70 ± 47.89)	118.31 (103.89 ± 137.25)	1.0

Table 4.12 continued

Propoxur 0.1%	Khartoum	650 (26)	73.26 (64.69 ± 85.89)	293.99 (219.19 ± 438.22)	1.4
	Khartoum North	350 (14)	52.07 (49.48 ± 55.00)	95.56 (49.48 ± 107.03)	1.0
	Omdurman	300 (12)	51.35 (48.47 ± 54.60)	94.25 (85.69 ± 105.90)	1.0
Permethrin 0.75%	Khartoum	400 (16)	48.62 (45.83 ± 51.73)	89.23 (81.23 ± 100.06)	1.1
	Khartoum North	100 (4)	46.72 (39.32 ± 56.15)	125.33 (99.24 ± 169.02)	1.0
	Omdurman	150 (6)	45.86 (38.57 ± 55.15)	123.03 (97.53 ± 165.56)	1.0
Deltamethrin 0.05%	Khartoum	300 (12)	36.45 (33.14 ± 40.31)	97.79 (82.00 ± 123.74)	1.1
	Khartoum North	475 (19)	31.42 (29.68 ± -33.21)	67.09 (62.53-72.43)	1.0
	Omdurman	400 (16)	31.97 (30.50 ± 33.52)	68.28 (64.04 ± 73.26)	1.0
Lambdacyhalothrin 0.05%	Khartoum	350 (14)	27.95 (26.57 ± 29.39)	59.69 (55.97 ± 64.04)	1.0
	Omdurman	100 (4)	44.05 (41.40 ± 46.92)	78.26 (71.69 ± 86.92)	1.6

The knockdown resistance ratios (KRR) for *An. arabiensis* tested against the seven insecticides were relatively similar between the populations of the three administrative areas. The exceptions were for DDT 4%, propoxur 0.1% and lambdacyhalothrin 0.05%. DDT 4%, showed the highest KRR by 1.4 fold than in two administrative areas. Similarly, KRR due to propoxur 0.1% was highest in *An. arabiensis* of Khartoum by 1.4 fold than in the two other areas. Moreover, The KRR for *An. arabiensis* due to lambdacyhalothrin 0.05% in Omdurman area was higher as 1.6 fold of that of Khartoum area.

Knockdown times for populations of *An. arabiensis* from urban and periurban areas in Khartoum state exposed to seven insecticides during 2011 - 2013 are presented in table 4.13. No significant difference in knockdown times in *An. arabiensis* for all insecticide tested between urban and periurban areas. The exceptions were for the DDT 4% and lambdacyhalothrin 0.05% which were higher in populations of periurban than urban area (P = 0.001and P = 0.003 respectively).The knockdown resistance ratio (KRR) for DDT 4%, malathion 5%, permethrin 0.75%, deltamethrin 0.05% and lambdacyhalothrin 0.05% in the periurban areas were higher by 1.4, 1.1, 1.3, 1.1 and 1.4 folds respectively than in urban areas. In contrast, KRR for fenitrothion 1% in urban areas was 1.1 fold of that in the periurban areas. Whereas, no difference in KRR was observed for propoxur 0.1% between urban and periurban areas.

Table 4.13: Mean mortality rates and knockdown time of *Anopheles arabiensis* from the three administrative areas in Khartoum state exposed to insecticides during 2011 - 2013.

Insecticide used	Land use	No. fem. tested (replicates)	KDT_{50} (50% Cl)	KDT_{95} (95% Cl)	KDT_{50} Ratio
DDT 4%	Urban	1300(52)	36.16 (34.67±37.74)	84.54 (78.70±91.62)	1.0
	Periurban	400(16)	49.21 (47.25±51.36)	115.04 (106.36±125.73)	1.4
Fenitrothion 1%	Urban	1600(64)	70.57 (62.89±82.79)	161.00 (126.25±234.26)	1.1
	Periurban	200(8)	65.22 (58.18±76.05)	148.81 (117.44±213.99)	1.0
Malathion 5%	Urban	1700(68)	43.87 (41.29±46.77)	119.16 (106.57±135.76)	1.0
	Periurban	200(8)	47.84 (45.38±50.62)	129.99 (116.28-148.17)	1.1
Propoxur 0.1%	Urban	850(34)	49.48 (46.94±52.31)	90.93 (82.99±101.74)	1.0
	Periurban	200(8)	51.68 (49.58±54.08)	94.98 (87.02±105.71)	1.0
Permethrin 0.75%	Urban	400(16)	36.45 (36.45±33.15)	97.78 (82.04±123.60)	1.0
	Periurban	150(6)	46.29 (40.96±52.90)	124.18 (101.52±162.05)	1.3
Deltamethrin 0.05%	Urban	650(26)	28.08 (26.88±29.31)	59.70 (56.28±63.69)	1.0
	Periurban	375(15)	32.38 (31.16±33.65)	68.85 (65.04±73.32)	1.1
Lambdacyhalothrin 0.05%	Urban	200(8)	38.48 (36.73±40.29)	68.367 (63.55±74.72)	1.0
	Periurban	100(4)	44.05 (41.40±46.92)	78.26 (71.69±86.92)	1.4

4.3. Seasonal variation in *Anopheles arabiensis* susceptibility status

The overall results on temporal variation in insecticide susceptibility status of *An. arabiensis* from Khartoum state in different seasons are shown in table 4.14. Most of *An. arabiensis* collected from different sentinel sites in the cold dry, hot dry and wet seasons were tested against DDT 4%, fenitrothion 1%, malathion 5% and propoxur 0.1%. However, specimens collected in the wet season were only tested against permethrin 0.75% and lambdacyhalothrin 0.05%. Deltamethrin 0.05% was used to test *An. arabiensis* collected in dry cold and wet seasons. A significant difference in the mortality in *An. arabiensis* between the seasons were observed only for DDT 4% ($\chi^2 = 8.905$, df = 2, P = 0.012) and propoxur 0.1 ($\chi^2 = 7.96$, df = 2, P = 0.019). Variation in the mortality rates due to DDT 4% and propoxur 0.1% were observed in *An. arabiensis* from Khartoum state in different seasons (Fig. 4.6). *Anopheles arabiensis* was resistant to DDT during both cold and hot dry season (87 ±1.80 and 89 ±2.80 respectively), and suspected resistance in wet season. In contrast, this species was suspected resistance to malathion 5% in the cold dry season (90 ±2.80) and resistant in both other seasons (63 ± 12.0 and 86 ±2.00 respectively).

For the susceptibility status of *An. arabiensis* in the nine sentinel sites, three out of seven insecticides were used to test *An. arabiensis* in the three seasons. These were DDT 4%, fenitrothion 1% and malathion 5%. DDT 4% was used to test mosquitoes in the three seasons from only four out of the nine sentinel sites. These were from Soba West, Edekheinat, Shambat and Elmaygoma sites. Whereas, fenitrothion 1% and malathion 5% were used from two sentinel sites. These were Soba West and Elmaygoma for fenitrothion 1%, and Edekheinat and Elmaygoma for malathion 5%. The aforementioned insecticides beside the remaining ones were tested against *An. arabiensis* from different sentinel sites

either once or twice. The results of analysis showed that there are significant difference in the mean mortality due to DDT 4% in *An. arabiensis* from Edekheinat (χ^2= 9.439, df = 2, P = 0.009) and Shambat (χ^2 = 7.424, df = 2, P = 0.024) sites (Fig. 4.7). However, slight variation in susceptibility status in different season was observed in *An. arabiensis* from Soba west (Fig. 4.7). No variation in the mortality rate in *An. arabiensis* was observed between the seasons in the two sentinel sites investigated using fenitrothion 1% (P > 0.05). Although, *An. arabiensis* from Elmaygoma site was resistant to malathion 5% during the three seasons (Fig. 4.8), a significant difference was observed among specimens of tested mosquitoes in different seasons (χ^2= 6.783, df = 2, P = 0.034). No significant difference was observed in mortality rates in *An. arabiensis* from Edekheinat site (χ^2 = 6.783, df = 2, P = 0.058), however this species was suspected/resistance in both cold and hot dry seasons and susceptible in the wet season (Fig. 4.8).

When considering administrative areas, DDT 4% and fenitrothion 1% were the only insecticides tested against populations of *An. arabiensis* from the three areas. In contrast, malathion 5% was used to test *An. arabiensis* from Khartoum and Khartoum North during the three seasons, and those from Omdurman were tested in two seasons (dry cold and wet seasons). In contrast, propoxur 0.1% was used to test mosquitoes from each of the three areas during only two seasons. The three remaining insecticides (permethrin 0.75%, deltamethrin 0.05% and lambdacyhalothrin 0.05%) were used in each area to test mosquitoes in two different seasons. However, *An. arabiensis* from Khartoum North site was tested against lambdacyhalothrin 0.05% in two different seasons. Significant difference was observed in the mortality due to DDT 4% between the in population of *An. arabiensis* form Khartoum area (χ^2 = 6.536, df = 2, P = 0.038). *Anopheles*

arabiensis from Khartoum area was suspected/resistance in the cold dry (91 ± 2.66), in hot dry (99 ± 0.52) and resistant to DDT in the wet (85 ± 5.64) seasons (Fig. 4.9). Mosquitoes from Khartoum North were resistant to this insecticide during the three seasons whereas those from Omdurman were suspected/resistance during both cold and hot dray seasons and fully susceptible in the wet season (Fig. 4.9). Similarly, variation in mortality due to malathion 5% were observed between the three seasons in *An. arabiensis* from Khartoum (χ^2 = 11.264, df = 2, P = 0.004) and Khartoum North (χ^2 = 6.085, df = 2, P = 0.048) areas (Fig. 4.9). *Anopheles arabiensis* from Khartoum area was susceptible, suspected/resistance and resistant to malathion 5% in the cold dry, hot dry and wet seasons respectively (Fig. 4.10). No significant differences were observed between the seasons in mortality of *An. arabiensis* from the administrative areas due to fenitrothion 1% (P > 0.05).

The results on variation of susceptibility tests showed a significant difference between the three seasons in mortality rate of *An. arabiensis* from urban area due to DDT 4% (χ^2 = 9.419, df = 2, P = 0.009) and periurban area due to malathion 5% (χ^2 = 7.808, df = 2, P = 0.02). Although, the differences in mortality in *An. arabiensis* from urban area due to DDT 4% was not significant (P > 0.05), this species was resistant (84 ± 2.69) to this insecticide during the cold dry season and suspected/resistance in both dry hot and wet seasons (91 ± 2.48 and 91 ± 3.13 respectively) (Fig. 4.11). Similarly, *An. arabiensis* from urban area was resistant to malathion 5% in the cold dry season (78 ± 6.81) and suspected/resistance in hot dry and wet seasons (95 ± 1.50 and 95 ± 1.81 respectively) (Fig. 4.12). Similar mortality in *An. arabiensis* from both urban and periurban area were observed for fenitrothion 1% (P > 0.05).

Table 4.14: Mean ± SE mortality in *Anopheles arabiensis* from the three administrative areas in Khartoum state exposed to insecticides during 2011 - 2013.

Insecticide used	Cold dry season		Hot dry season		Wet season	
	No tested (repl)	Mean± SE	No tested (repl)	Mean± SE	No tested (repl)	Mean± SE
DDT 4%	625 (25)	87 ±1.80	425(17)	95 ±1.47	650(26)	89 ±2.80
Fenitrothion 1%	550 (20)	100 ±0.40	800(32)	100 ± 0.0	450(18)	100 ± 0.0
Malathion 5%	700 (28)	83 ±2.67	300(12)	86 ±4.04	900(36)	84 ±1.75
Propoxur 0.1%	400 (16)	90 ±2.80	200(8)	63 ±12.0	450(18)	86 ±2.00
Permethrin0.75%	**ND**	**ND**	**ND**	**ND**	400(16)	87.35±1.80
Deltamethrin0.05%	400 (16)	93 ±2.23	**ND**	**ND**	625(25)	95 ±1.82
Lambdacyhalothrin 0.05%	**ND**	**ND**	**ND**	**ND**	300(12)	99 ± 0.45

Note; Repl = Replicates; ND = Not done

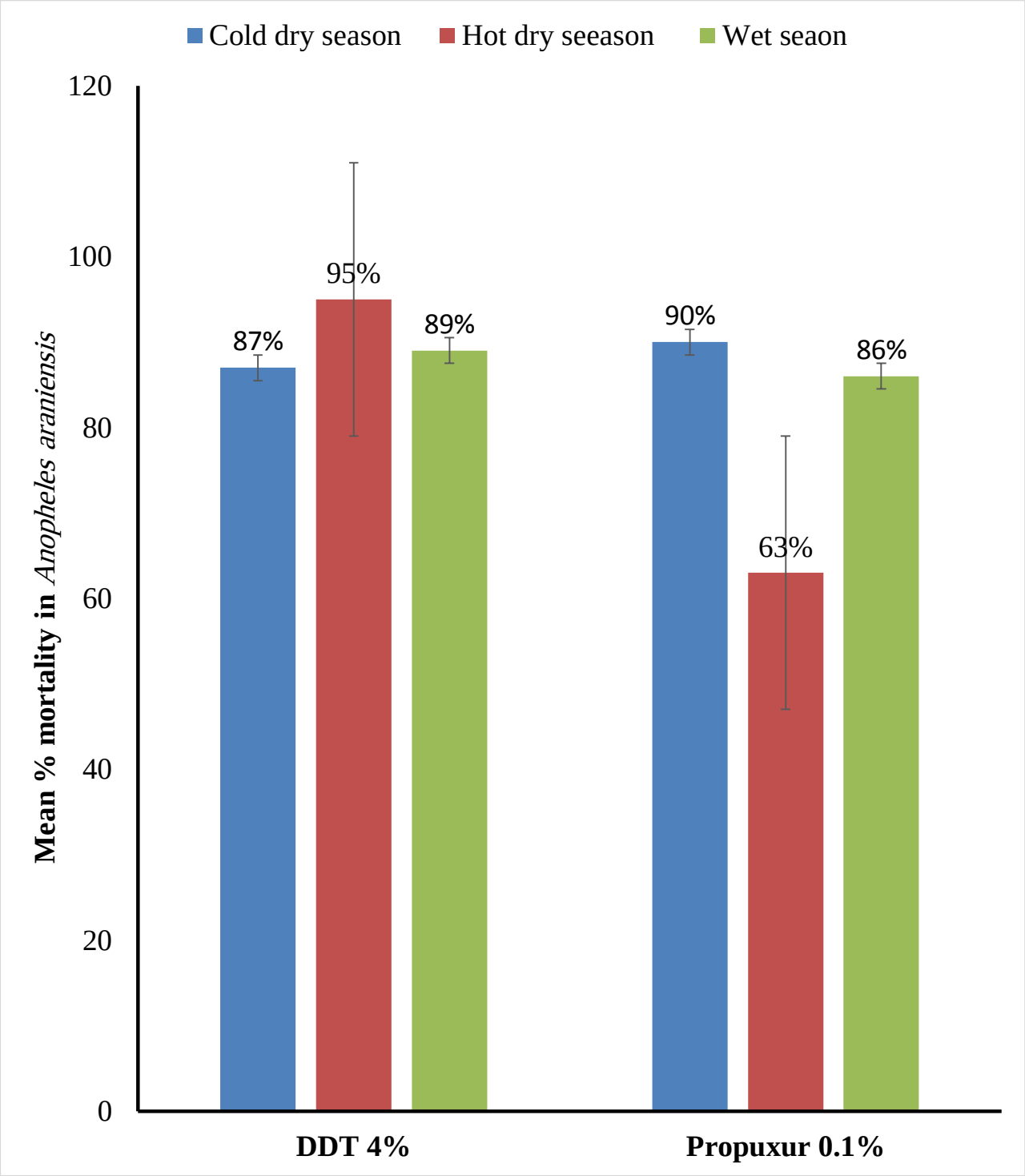

Figure 4.6: Mean percentage mortality in *Anopheles arabiensis* from Khartoum state exposed to DDT 4% and propoxur 0.1% in three different seasons during 2011 – 2013.

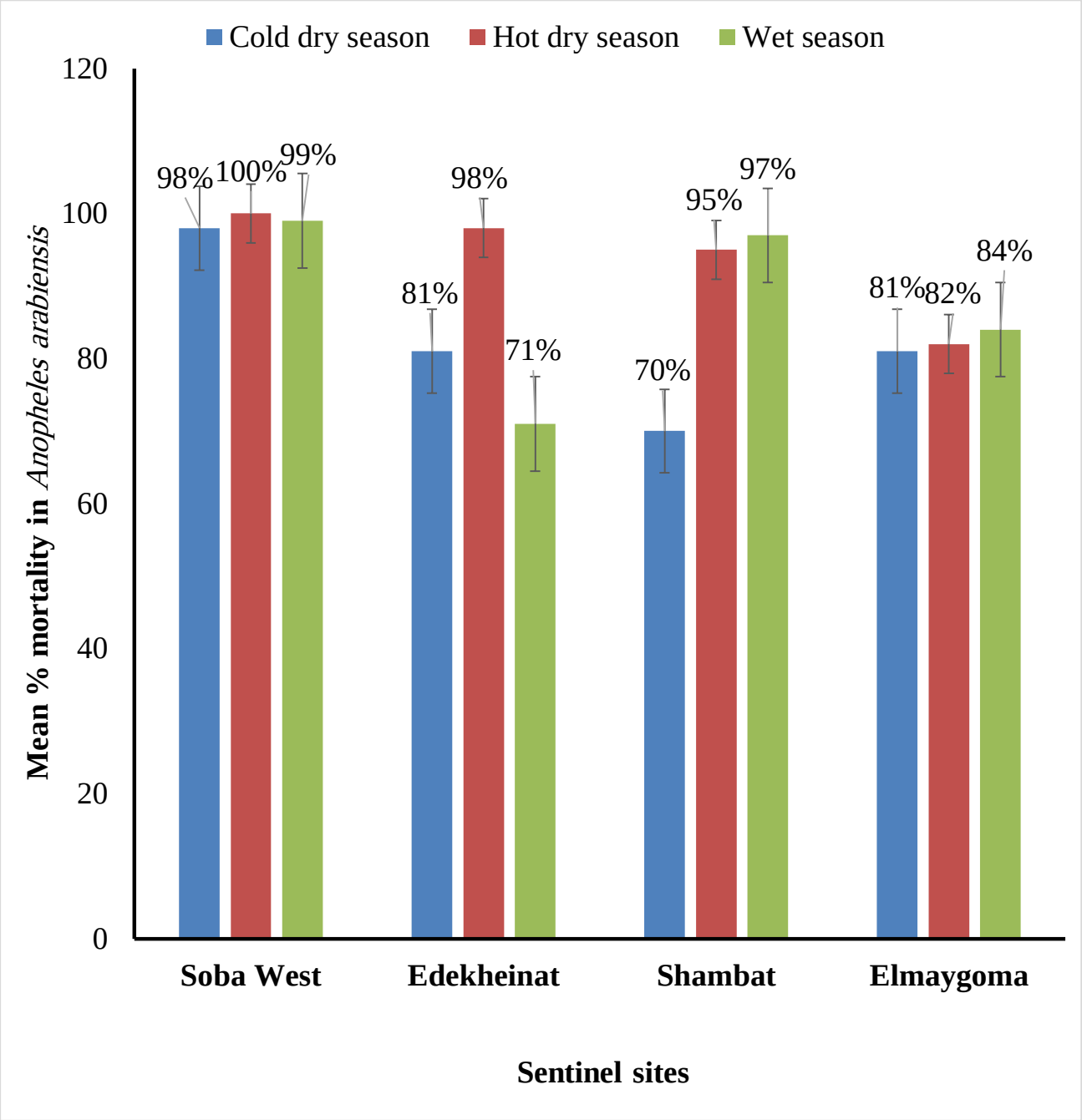

Figure 4.7: Mean percentage mortality in *Anopheles arabiensis* from different sentinel sites in Khartoum state exposed to DDT 4% during three different seasons during 2011 – 2013.

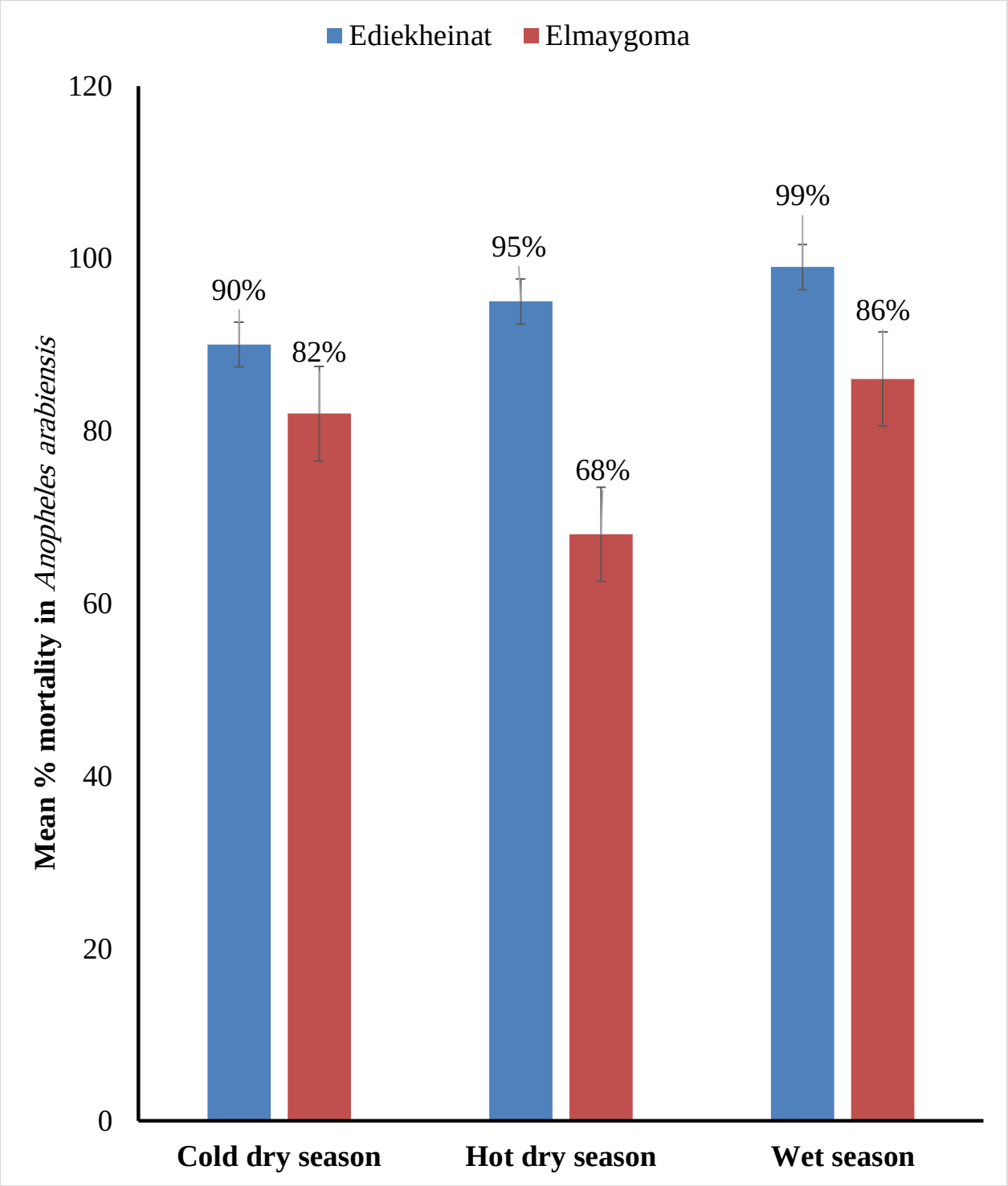

Figure 4.8: Mean percentage mortality in *Anopheles arabiensis* from different sentinel sites in Khartoum state exposed to malathion 5% during three different seasons during 2011 – 2013.

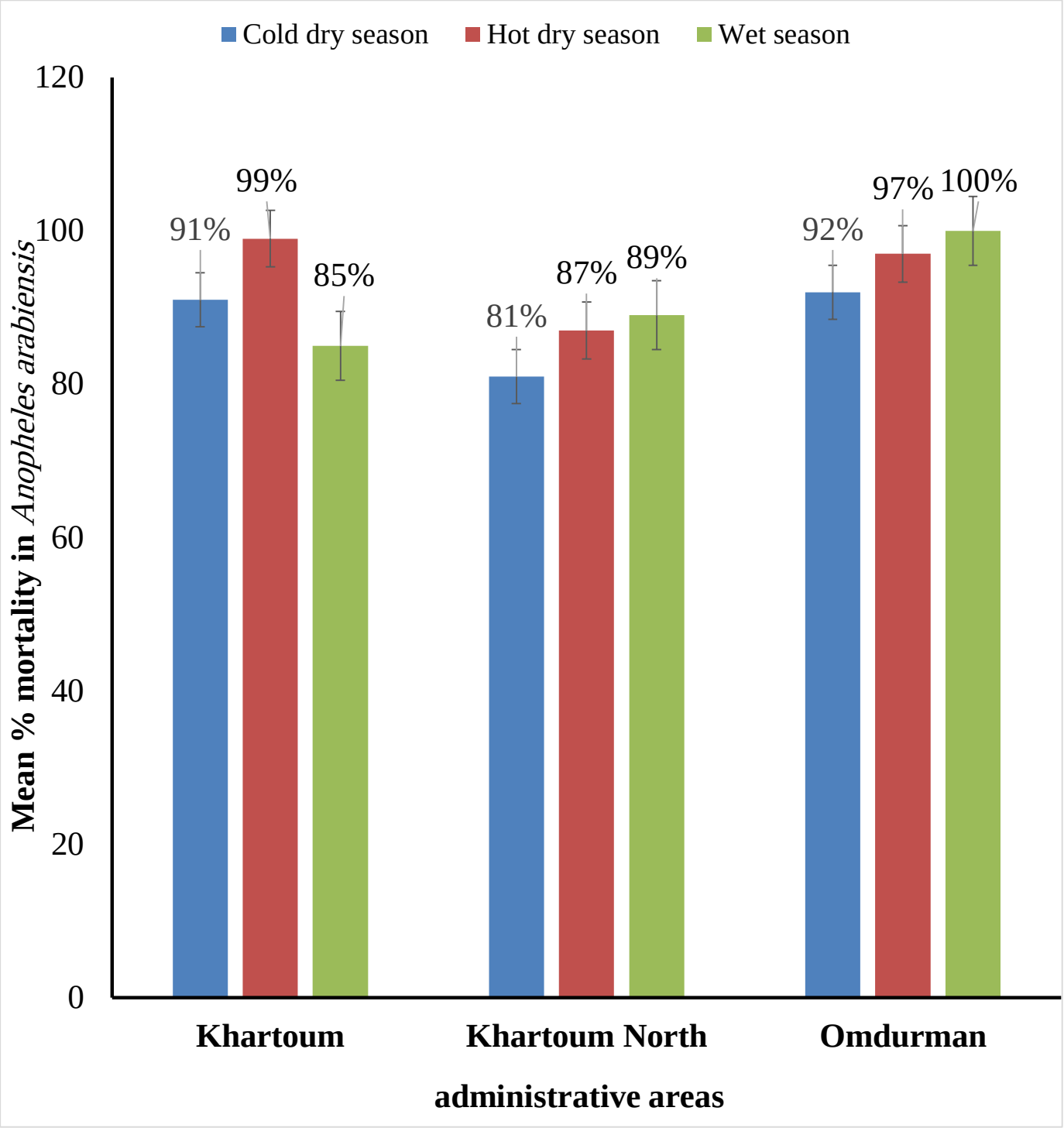

Figure 4.9: Mean percentage mortality in *Anopheles arabiensis* from three different administrative areas in Khartoum state exposed to DDT4% during three different seasons during 2011 – 2013.

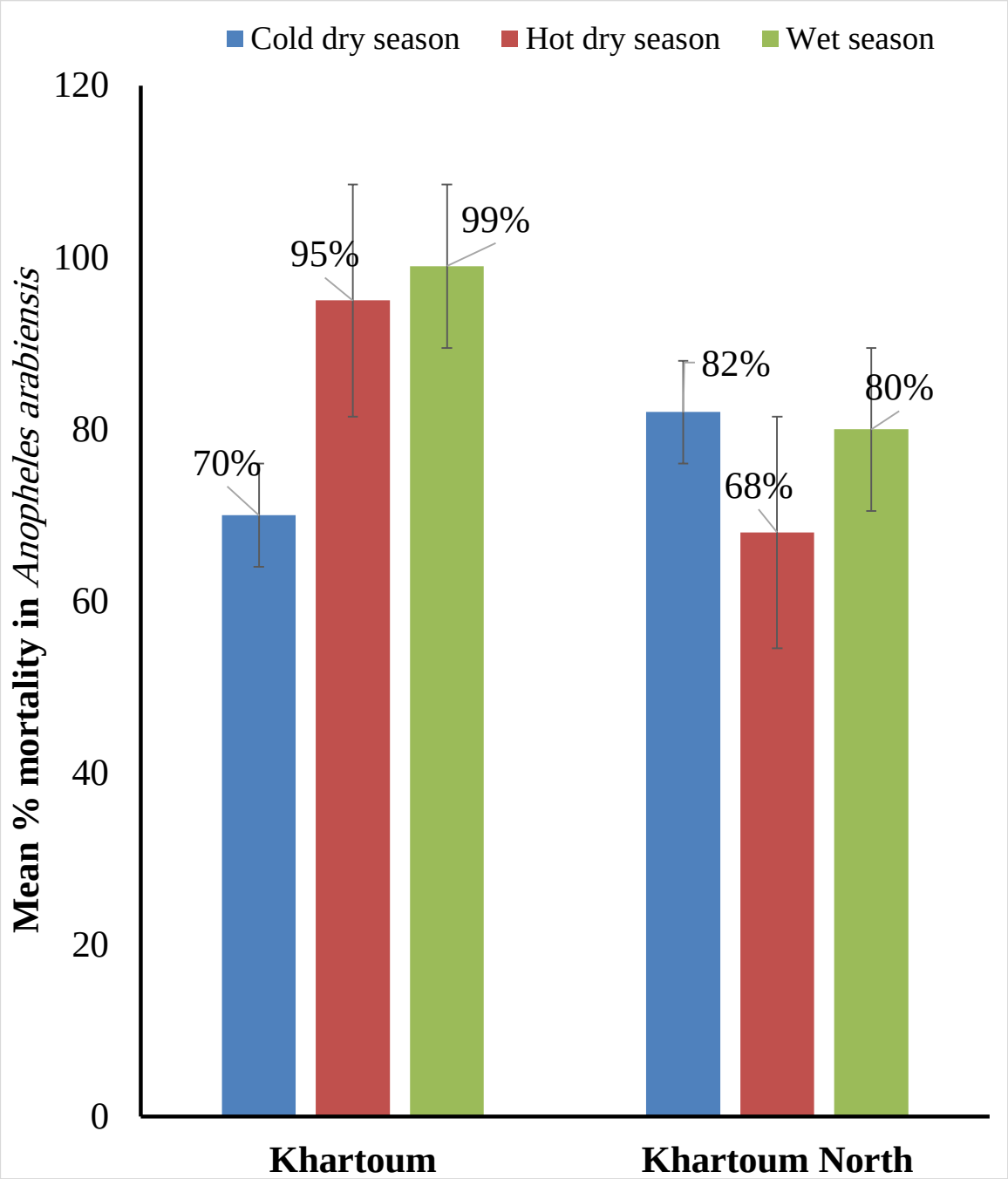

Figure 4.10: Mean percentage mortality in *Anopheles arabiensis* from three different administrative areas in Khartoum state exposed to malathion 5% during three different seasons during 2011 – 2013.

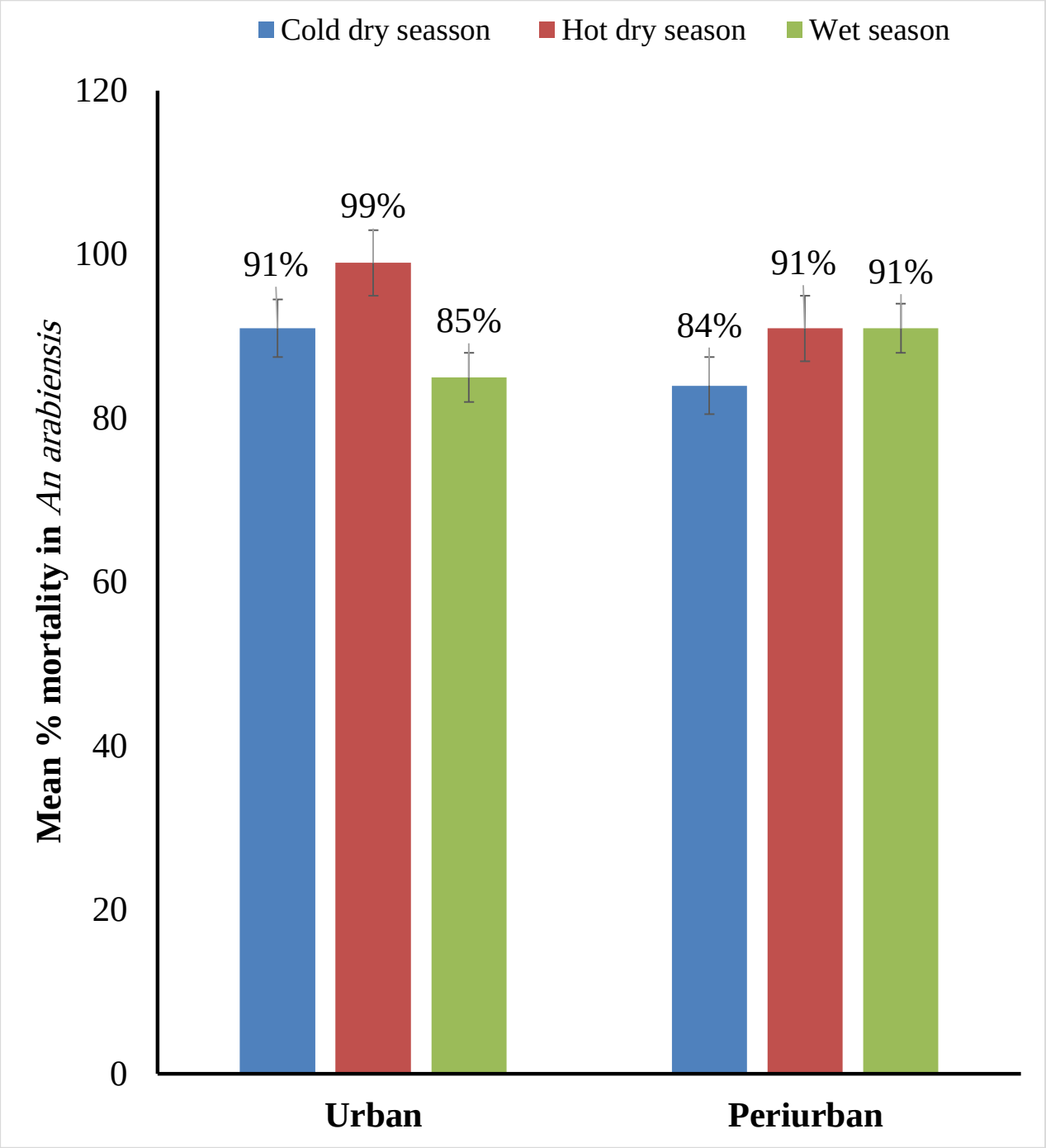

Figure 4.11: Mean percentage mortality in *Anopheles arabiensis* from two areas with different land use in Khartoum state exposed to DDT 4% during three different seasons during 2011 – 2013.

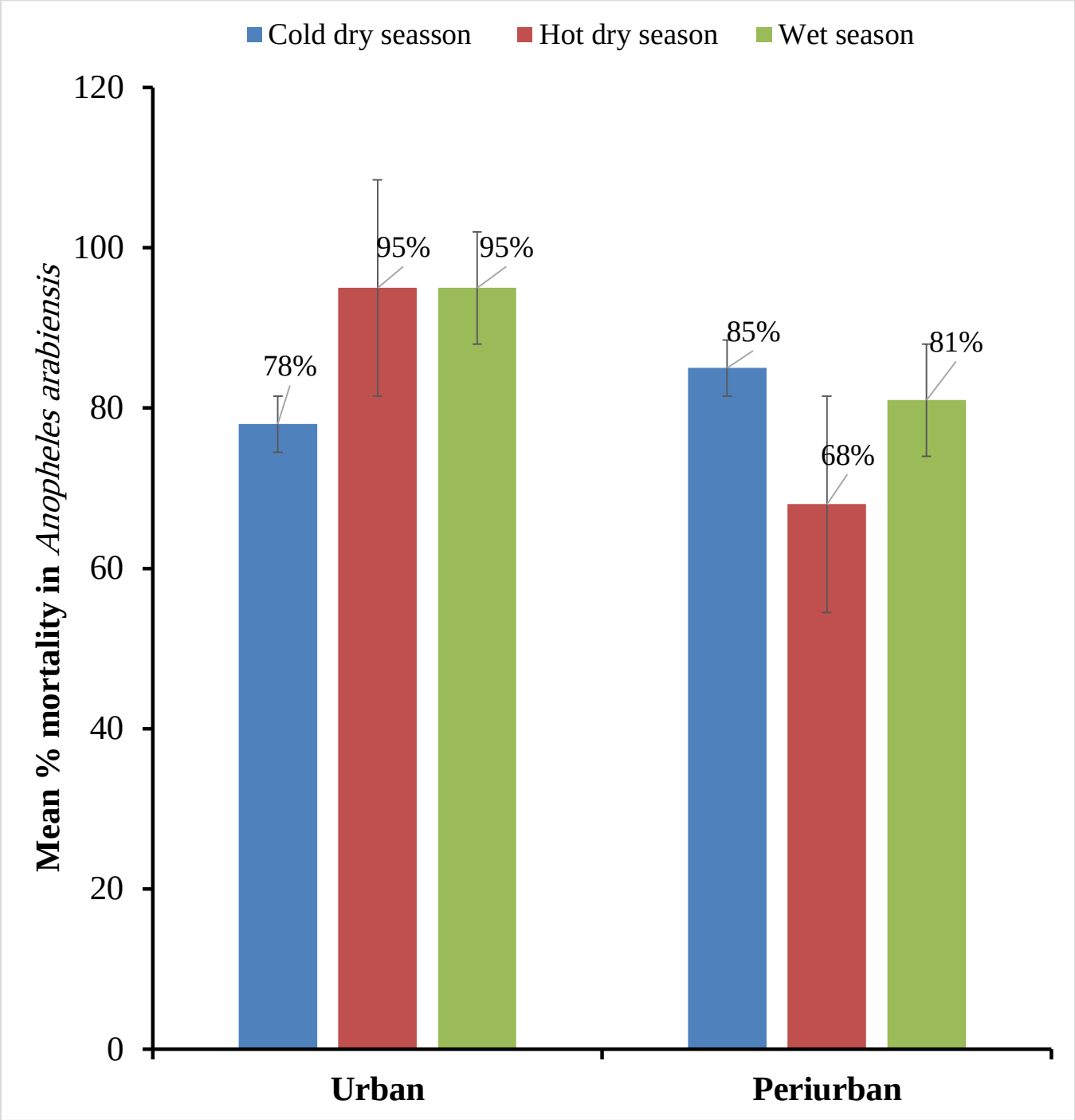

Figure 4.12: Mean percentage mortality in *Anopheles arabiensis* from two areas with different land use in Khartoum state exposed to malathion 5% during three different seasons during 2011 – 2013.

The results of analysis on the effect of sentinel sites, administrative areas and land use on the seasonal variation in susceptibility status in *An. arabiensis* are depicted in table 4.15. The generalized linear model (GLM) analysis indicated there was variations in mortality between the seasons in *An. arabiensis* due to all insecticides in the sentinel sites, administrative areas and land use (Table 4.15). The exceptions were fenitrothion 1% and lambdacyhalothrin 0.05% where the differences in mortality rates in mosquitoes were not significant (Table 4.15).

4.4. Spatial distribution of insecticide resistant strains of *Anopheles arabiensis* in Khartoum state during 2011 - 2013

The data obtained from WHO-susceptibility tests coupled with that of locations (GPS) were overlaid on maps of Khartoum state (Fig. 4.13 to 4.19). The distribution of resistant strains of *An. arabiensis* in Khartoum state during 2011-2013. Resistant specimens to DDT 4% were observed in populations from Edekheinat, Shambat, Elmaygoma and Abuseid sites (Fig. 4.13). Similarly, those resistant to malathion 5% were from Soba West, Shambat, Elmaygoma, Eltumanyat and Elsalamania West (Fig. 4.14). Resistance to propoxur 0.1% was in only two populations from Soba West and Edekheinat sites (Fig. 4.15) whereas the resistant strains of *An. arabiensis* to permethrin 0.75% were in Edekheinat, Elmaygoma and Elsalamania West Sites (Fig. 4.16). Deltamethrin resistant strains of *An. arabiensis* were from Soba West, Eltumanyat, Abuseid and Gizera Island sites (Fig. 4.17). For both fenitrothion 1% and lambdacyhalothrin 0.05%, no resistant strains of *An. arabiensis* were recorded in this study (Fig. 4.18 and 19).

Table 4.15: Generalized linear model testing the effects of sentinel sites, administrative areas, land use (urban and periurban) and seasons on mean mortality in *Anopheles arabiensis* due to seven insecticides in Khartoum state during 2011 - 2013.

Insecticide used/model tem	χ^2	df	P value
DDT 4%			
Sentinel sites	81.053	17	0.00
Administrative areas	32.533	5	0.00
Land use	19.757	5	0.001
Fenitrothion 1%			
Sentinel sites	0.0	9	1.0
Administrative areas	0.0	8	1.0
Land use	0.0	8	1.0
Malathion 5%			
Sentinel sites	118.56	13	0.00
Administrative areas	71.19	7	0.00
Land use	40.295	5	0.00
Propoxur 0.1%			
Sentinel sites	209.69	9	0.00
Administrative areas	153.36	5	0.00
Land use	150.64	4	0.00
Permethrin 0.75%			
Sentinel sites	51.59	4	0.00
Administrative areas	18.332	2	0.00
Land use	NA	NA	NA

Table 4.15 continued			
Deltamethrin 0.05%			
Sentinel sites	17.115	8	0.029
Administrative areas	4.597	3	0.204
Land use	1.93	3	0.587
Lambdacyhalothrin 0.05%			
Sentinel sites	0.027	2	0.987
Administrative areas	0.007	1	0.935
Land use	0.007	1	0.935

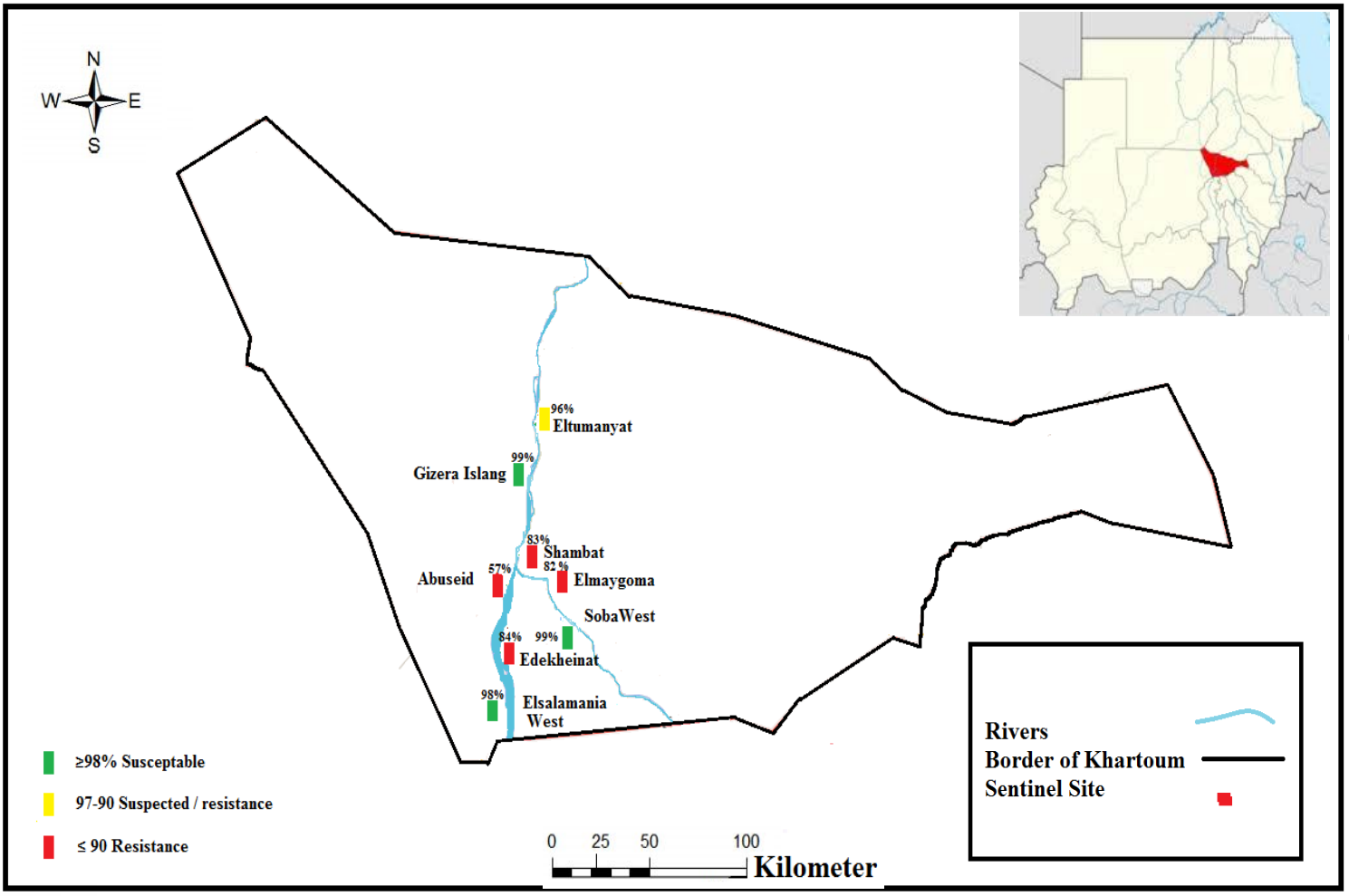

Figure 4.13: A map of Khartoum state showing distribution of susceptible, suspecetd/resistance and DDT-resistant strains of *Anopheles arabiensis* **in different sentinel sites during 2011 -2013.**

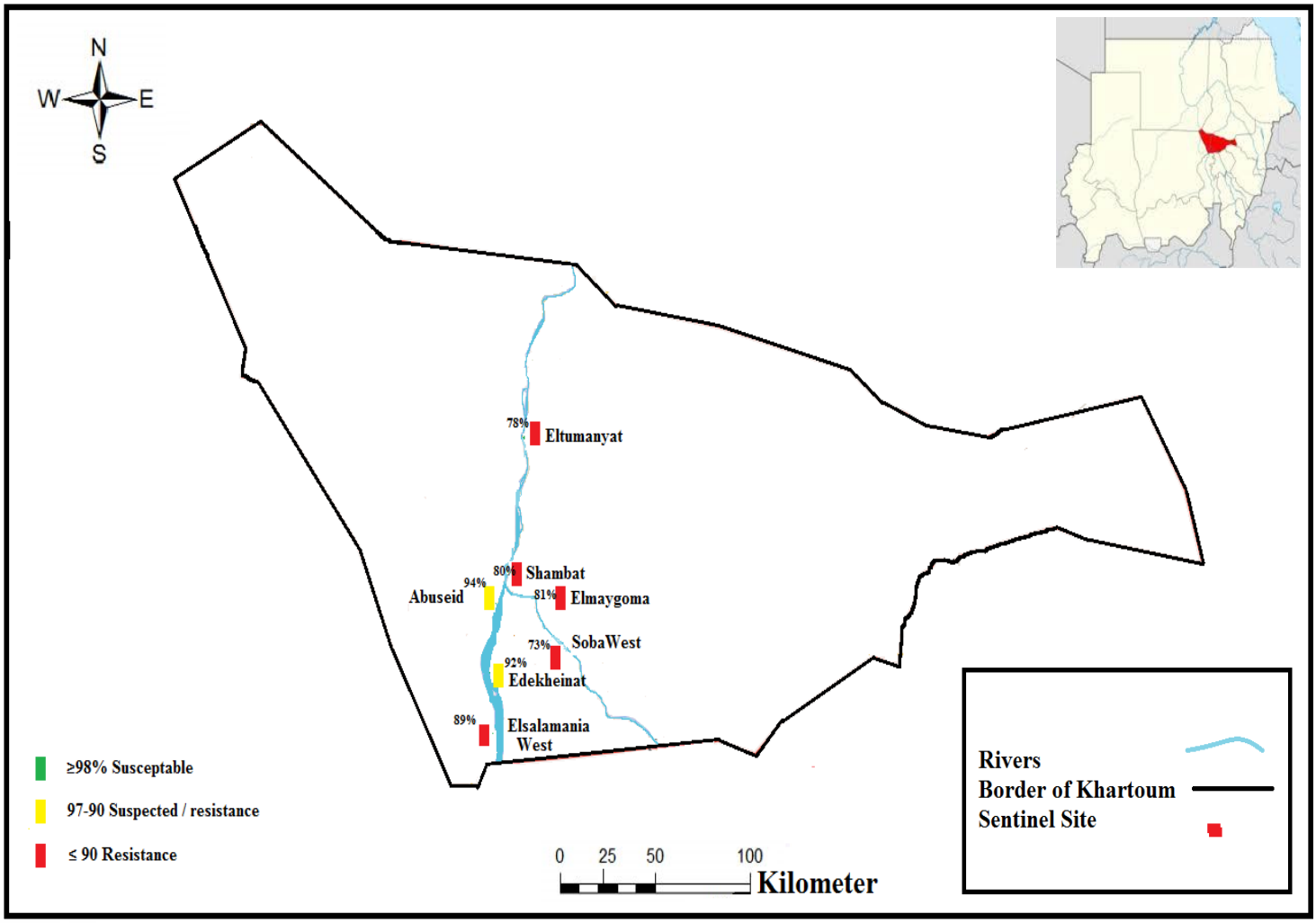

Figure 4.14: A map of Khartoum state showing distribution of susceptible suspecetd/resistance and malathion-resistant strains of *Anopheles arabiensis* in different sentinel sites during 2011 - 2013.

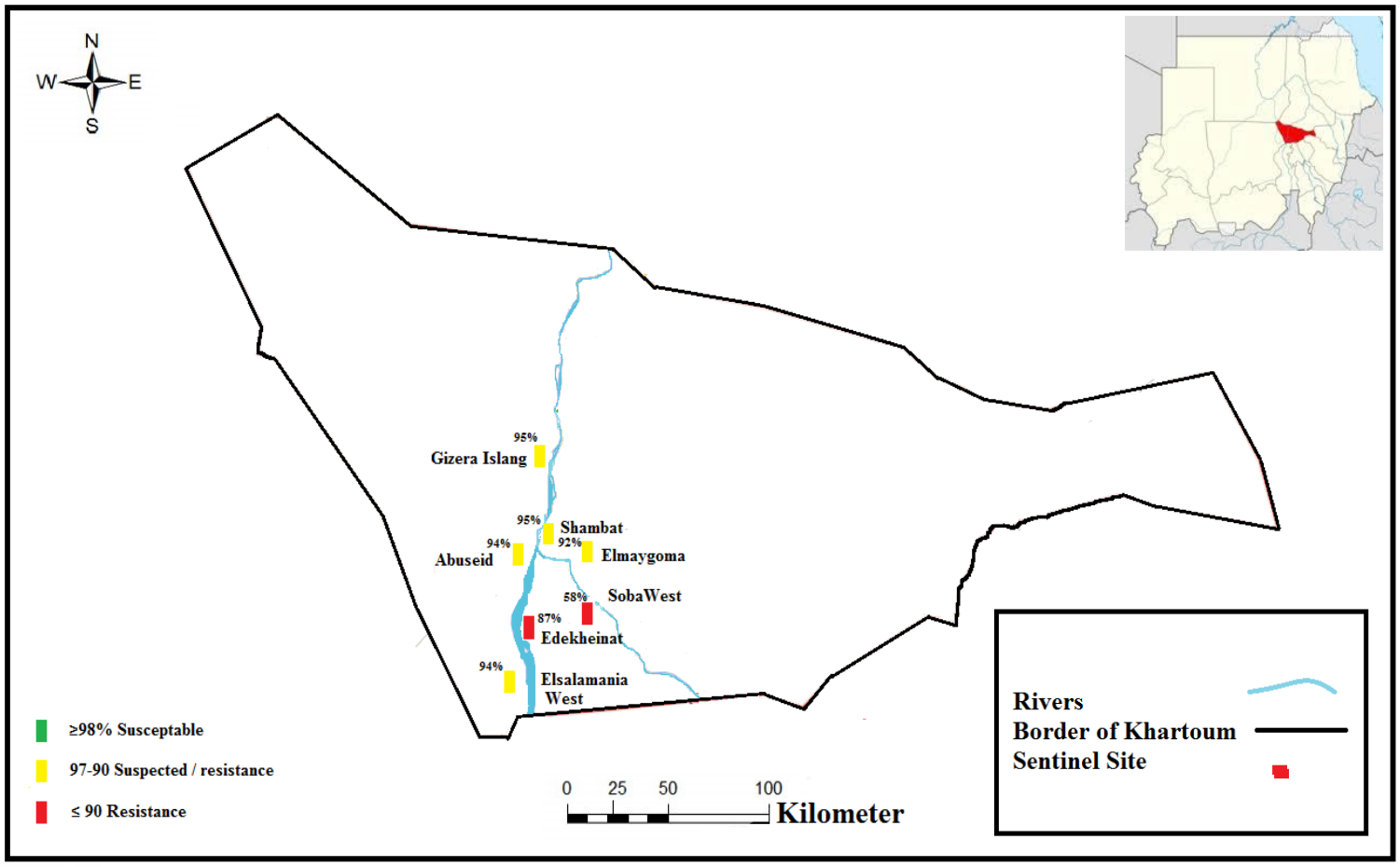

Figure 4.15: A map of Khartoum state showing distribution of susceptible, suspecetd/resistance and propoxur-resistant strains of *Anopheles arabiensis* in different sentinel sites during 2011 -2013.

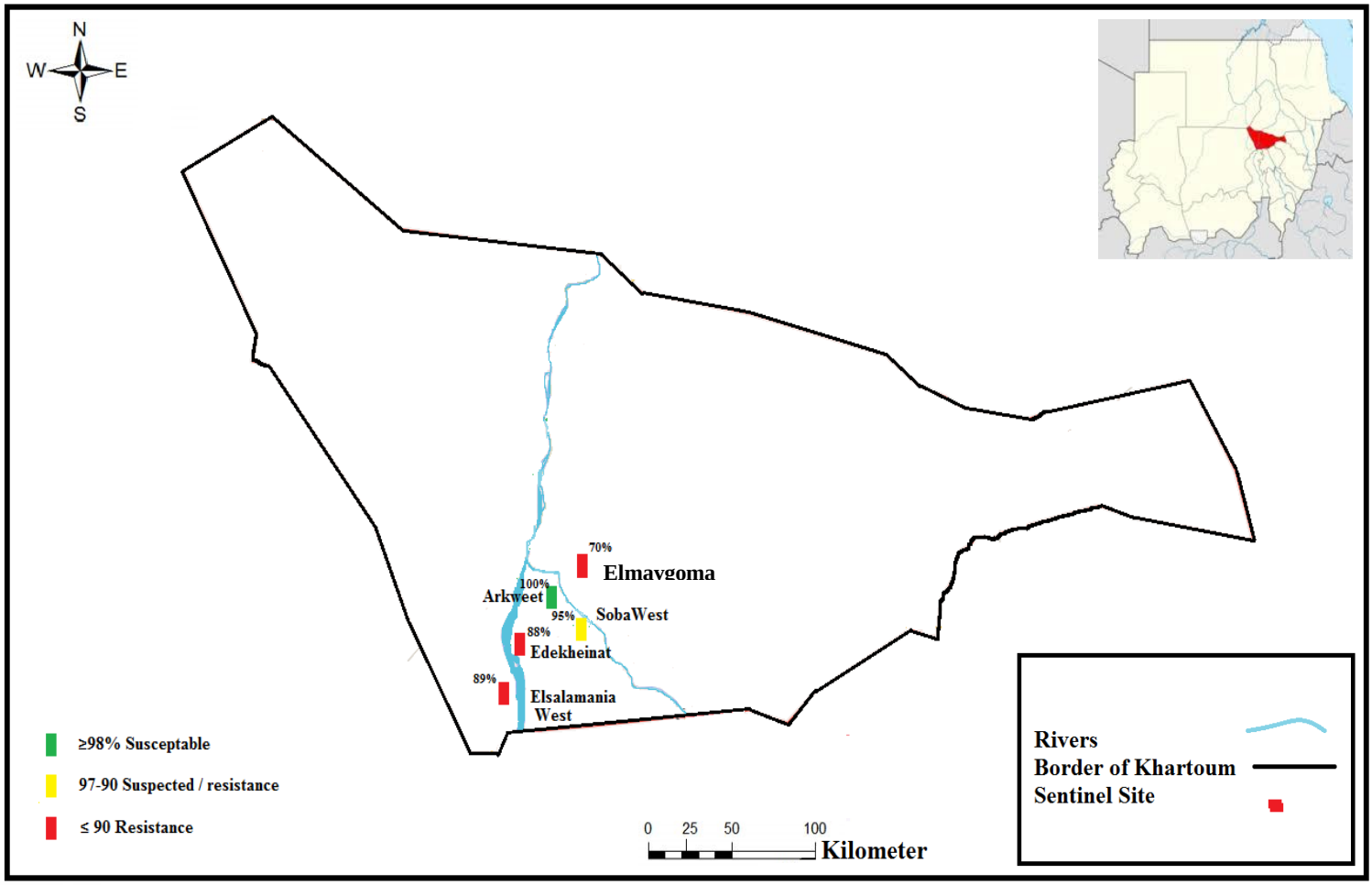

Figure 4.16: A map of Khartoum state showing distribution of susceptible, suspecetd/resistance and permrthrin-resistant strains of *Anopheles arabiensis* in different sentinel sites during 2011 -2013.

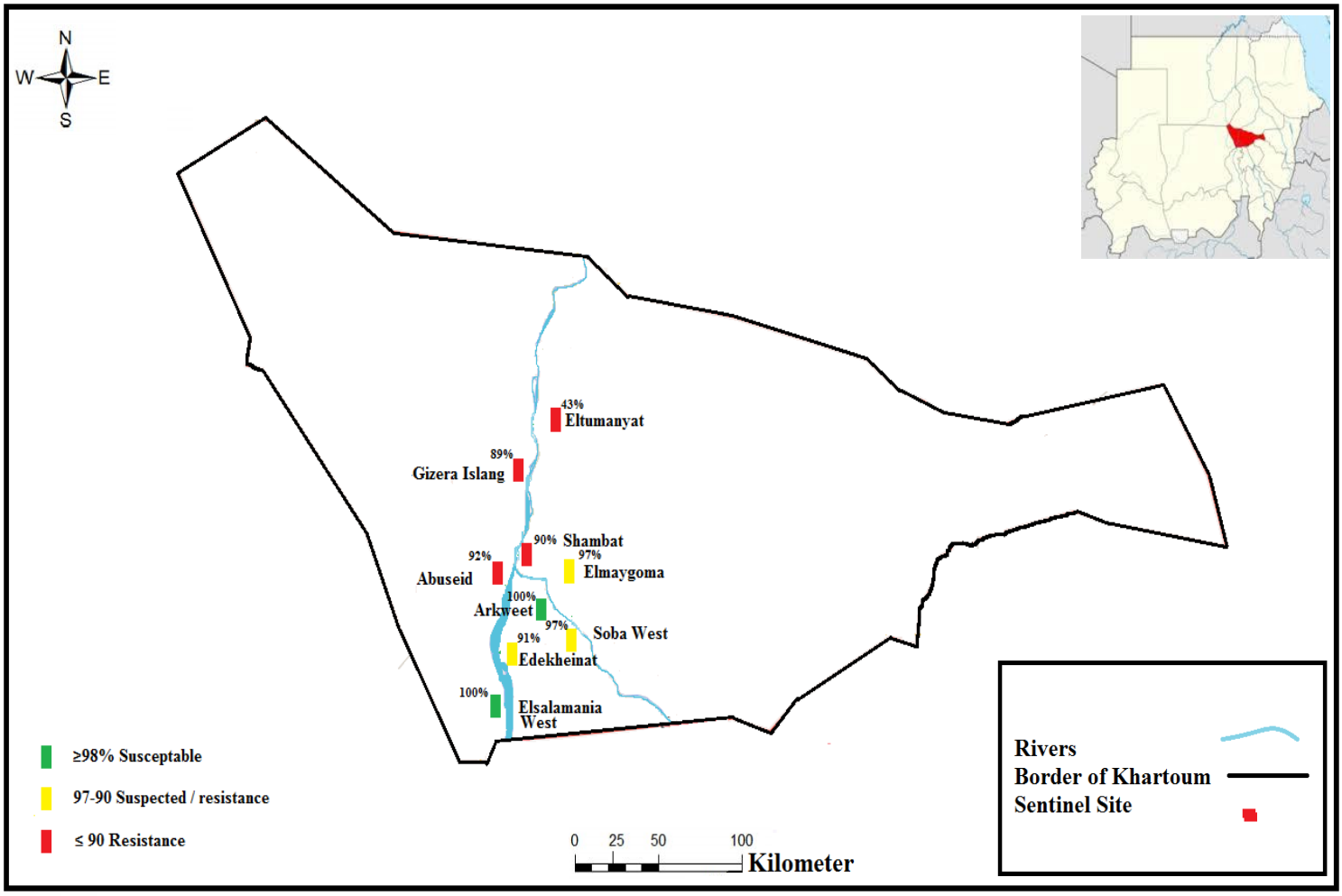

Figure 4.17: A map of Khartoum state showing distribution of susceptible, suspecetd/resistance and deltamethrin-resistant strains of *Anopheles arabiensis* in different sentinel sites during 2011 -2013.

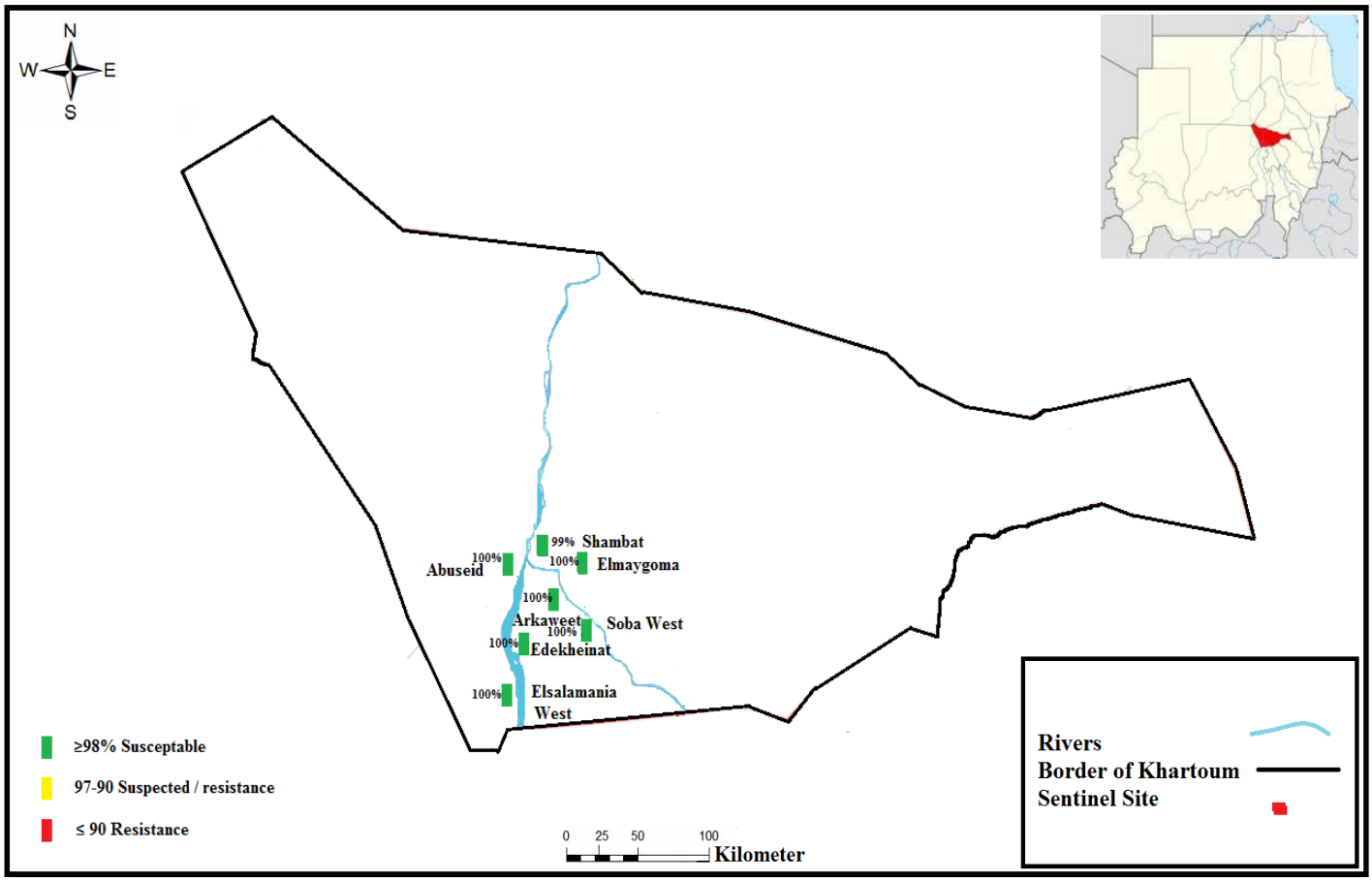

Figure 4.18: A distribution map of susceptibility status of strains *Anopheles arabiensis* **to frnitrothion 1% in sentinel sites in Khartoum state during 2011 -2013.**

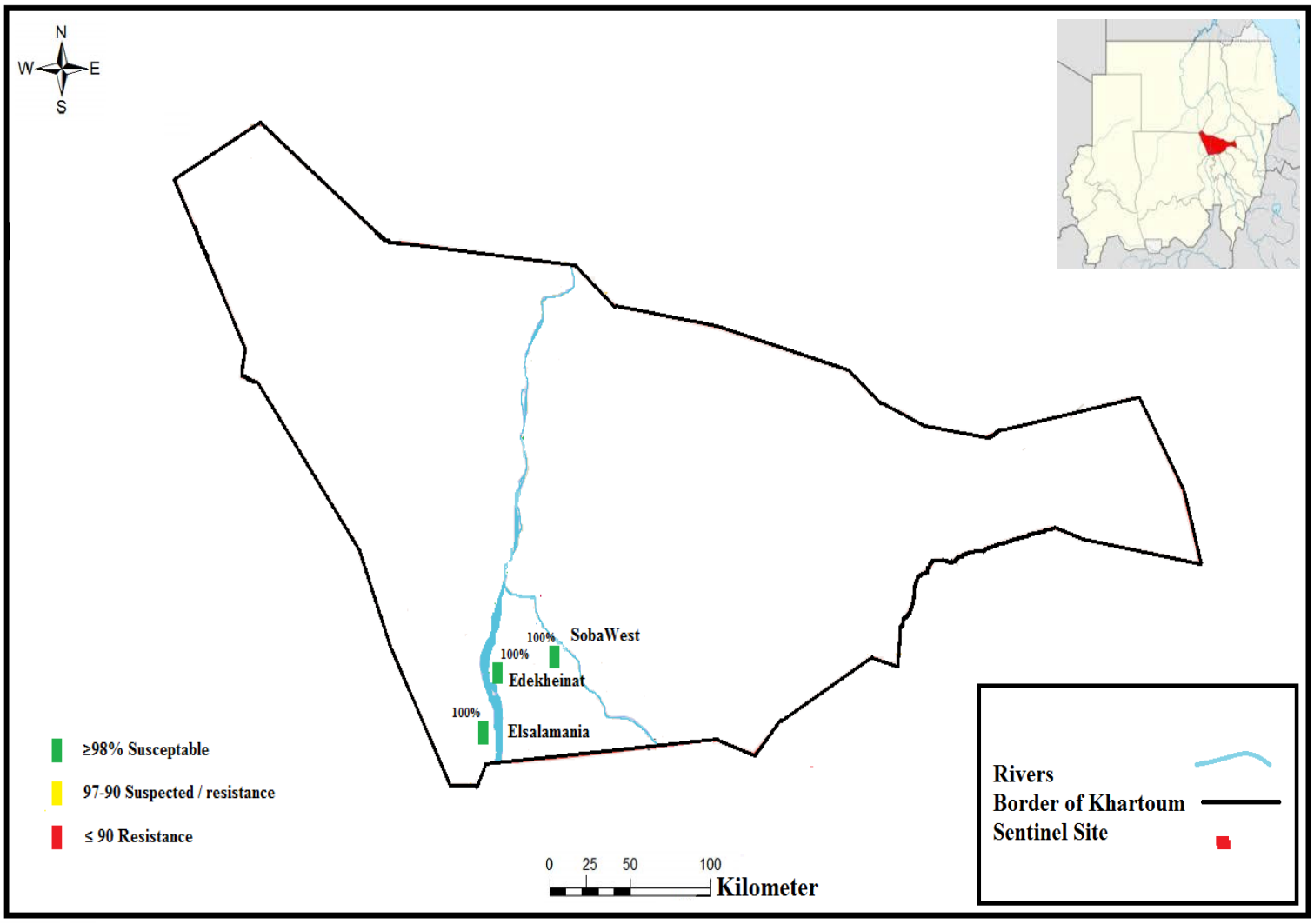

Figure 4.19: A distribution map of susceptibility status of strains *Anopheles arabiensis* **to lambdacyhalothrin 0.05% 1% in sentinel sites in Khartoum state during 2011 -2013.**

4.5. Occurrence, frequencies and distribution of *kdr* mutation in *Anopheles arabiensis* from different sentinel sites in Khartoum state

The allelic frequencies of *kdr* mutation in wild specimens of *An. arabiensis* are depicted in table 4.16. A total of 751 samples; 661 and 90 wild adult *An. arabiensis* collected in 2013 and 2014 respectively were screened for *kdr* mutation. Of the total 661 in 2013 examined, 640 (96.8%) samples gave PCR amplicons (Fig. 20) whereas 21 (3.2%) specimens were negative. The majority (88.4%) of the screened *An. arabiensis* individuals were susceptible (SS), 3.9% were homozygous resistant (RR) and 7.7% were heterozygous (RS) for L1014F - *kdr* allele (Fig. 4.21). Of PCR amplified samples, L1014F allele (West African *kdr*) was detected in 3 (0.4%) specimens, whereas the L1014S (East African *kdr*) in 22 (3.4%) of the tested *An. arabiensis* specimens. Out of the overall homozygous resistance alleles (RR), 12% were for L1014F and 88% for L1014S).

The allelic frequencies of L1014F and L1014S in *An. arabiensis* specimens collected from different sites ranged between 0-0.08 and 0-0.29 respectively (Table 4.16). The allelic frequency was significantly different between the sentinel sites (χ^2 = 163.2, df = 19, P = 0.00) where the highest values were observed in the Alshegelab and Edekheinat sites in Khartoum area and Edroshab site in Khartoum North area (0.29 and 0.25 respectively). However, no mutation was observed in population of Omdurman area.

The observed genotype frequencies of *kdr* in population of *An. arabiensis* was found to be deviated from the expected genotype frequencies predicted by the Hardy-Weinberg equilibrium (χ^2 =137.53, P = 0.00) with 0.08 variation in allelic frequency. However, when considering only L1014F, the frequency of the genotype in these population did not deviate from the expected genotype

frequencies predicted by the Hardy-Weinberg equilibrium (χ^2 = 2.82, P = 0.092) with 0.04 variation in allelic frequency. In contrast, when considering L1014S only, the allelic frequency of the genotype in these populations deviated from the expected genotype frequencies predicted by the Hardy-Weinberg equilibrium (χ^2 = 118.68, P = 0.00) with 0.07 variation in allelic frequency.

Figures 4.22 and 4.23 show the distribution of *kdr* mutations in wild *An. arabiensis* from different sites in Khartoum State during 2011 -2013. The L1014F and L1014S mutant alleles were detected in *An. arabiensis* specimens collected from only periurban areas. The L1014F allele mutation was detected in *An. arabiensis* from Alshegelab (1; 3.6%) and Edekheinat (2; 7.1%) sites in Khartoum administrative area (Fig. 4.24). The L1014S allele mutation was detected in samples collected from Edekheinat (5; 19.2%), and Alshegelab (6; 21.4%) sites in Khartoum area, and from Edroshab (7; 25.9%) and Eltumanyat (4; 14.3%) in Khartoum North area (Fig. 4.24). In contrast, none of the 90 specimens collected from four sites (Shambat, Alazeba, Elmaygoma and Buri) in 2014 had *kdr* mutations.

Likewise, of the 25 mosquito specimens randomly selected from the laboratory reared *An. arabiensis*, were all homozygous (SS) wild-type allele mutation.

Table 4.16: Allelic and genotypic frequencies at *kdr* L014F and L014F locus in wild populations of *Anopheles arabiensis* collected during March – June 2013 from different sites in Khartoum state, Sudan.

Sites	Number of mosquitoes	Genotypes			Allelic frequency		Samples with no result
		SS	RS	RR	L1014F	L1014S	
Soba West	30	28	2	0	0	0	0
Arkaweet	29	26	3	0	0	0	0
Edekheinat	30	14	3	7	0.08	0.21	6
Alshegelab	33	10	11	7	0.04	0.21	5
Alnasr	29	28	1	0	0	0	0
Alremaila	30	29	1	0	0	0	0
Jabra	33	32	1	0	0	0	0
Buri	36	35	1	0	0	0	0
Shambat	34	33	1	0	0	0	0
Elmaygoma	31	30	1	0	0	0	0
Edroshab	30	20	0	7	0	0.26	3
Eltumanyat	34	21	3	4	0	0.14	6
Alhalfaya	33	32	0	0	0	0	1
Alazeba	36	32	4	0	0	0	0
Algireef East	38	32	6	0	0	0	0
Soba East	38	32	6	0	0	0	0
Abu'siid	30	30	0	0	0	0	0
Alsarha	31	31	0	0	0	0	0
Algmayer	38	33	5	0	0	0	0
Angola	38	38	0	0	0	0	0

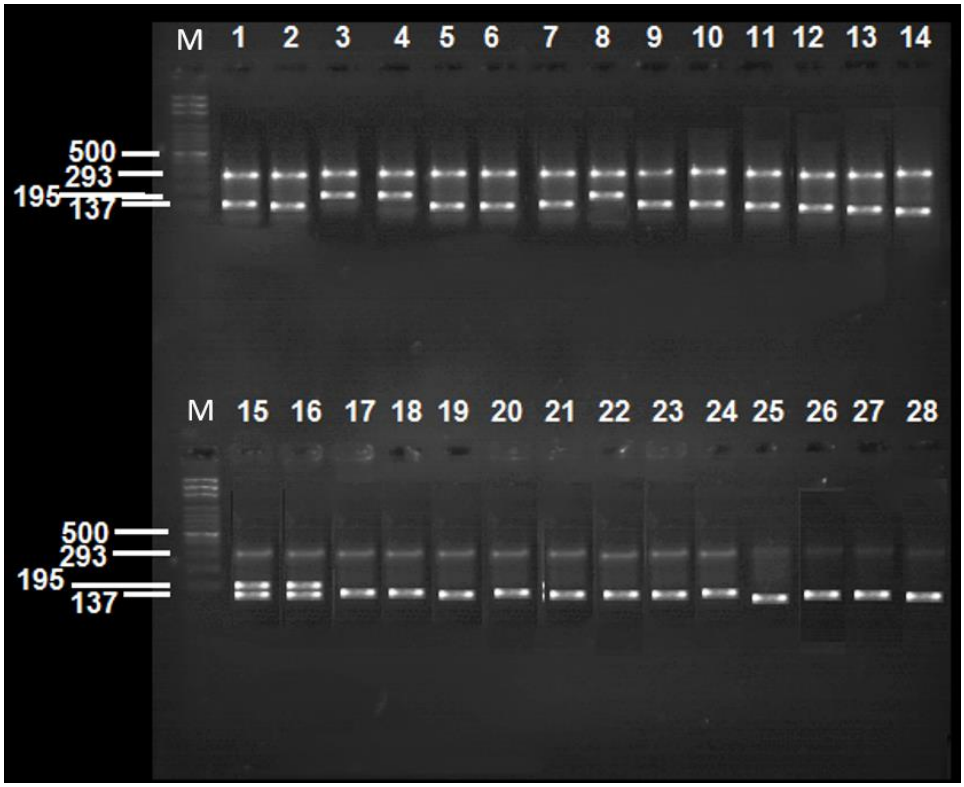

Figure 4.20: Amplicons produced by PCR-based diagnostic test for detection of knockdown resistance gene (*kdr*) in representative specimens of *Anopheles arabiensis* from different sites in Khartoum state. Primers create fragments of 293-bp internal control, 195-bp resistant, 137-bp susceptible). Lane M: 100 kb DNA molecular marker, lanes 1-2, 5-7 and 17-28: Homogenous susceptible (SS) samples, lanes 3 - 4 and 8: Homogenous resistance (RR) (for both L1014F and L1014S), lanes 15 -16: Heterogeneous resistance for L1014S alleles.

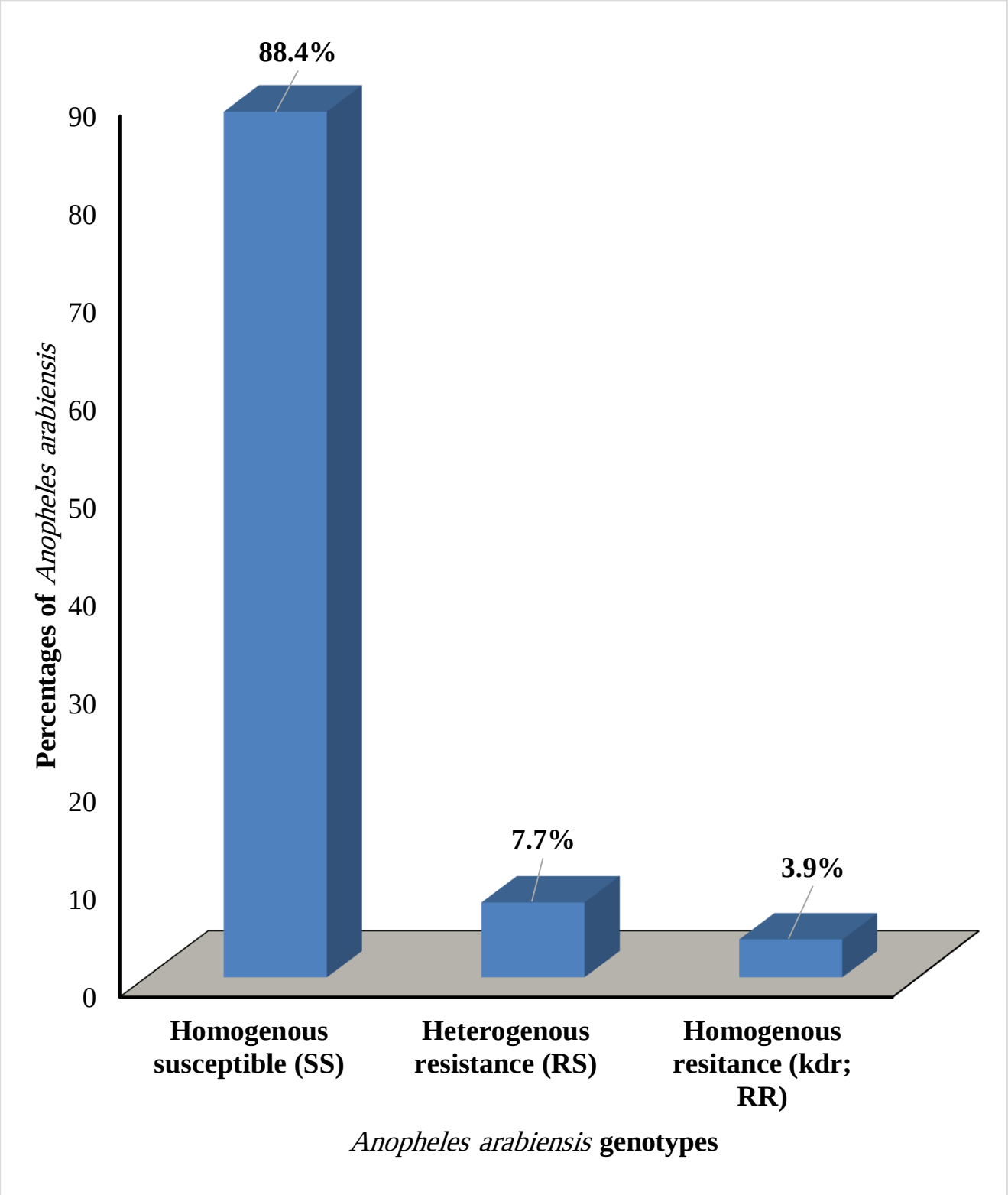

Figure 4.21: Percentage of different phenotypes of wild *Anopheles arabiensis* from Khartoum state during March –June 2013.

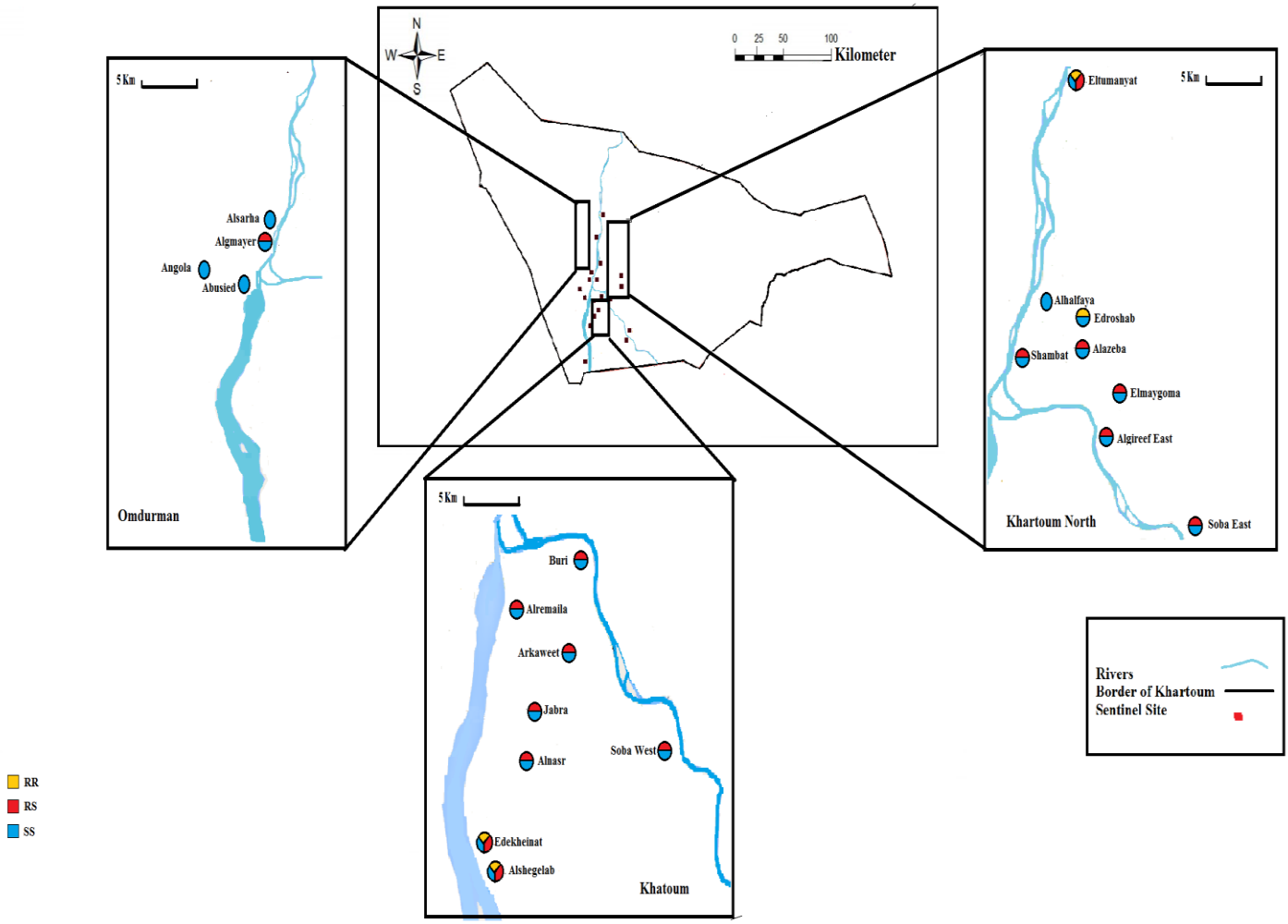

Figure 4.22: A distribution map of different genotypes of wild *Anopheles arabiensis* from sentinel sites in Khartoum state during March – June 2013.

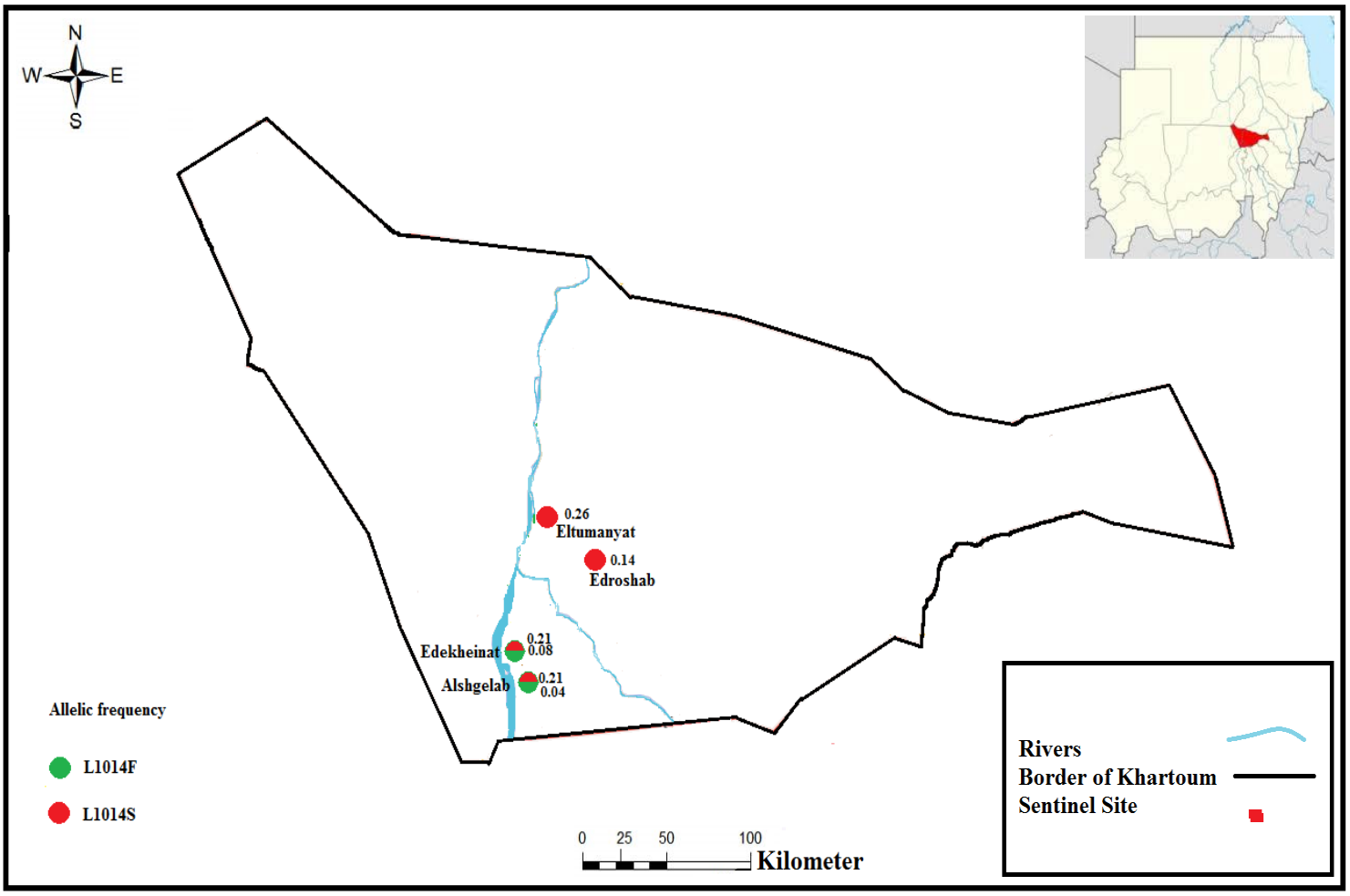

Figure 4.23: A map of Khartoum state showing frequencies of West (L1014F) and East (L1014S) *kdr* mutations in wild *Anopheles arabiensis* from sentinel sites in Khartoum during March –June 2013.

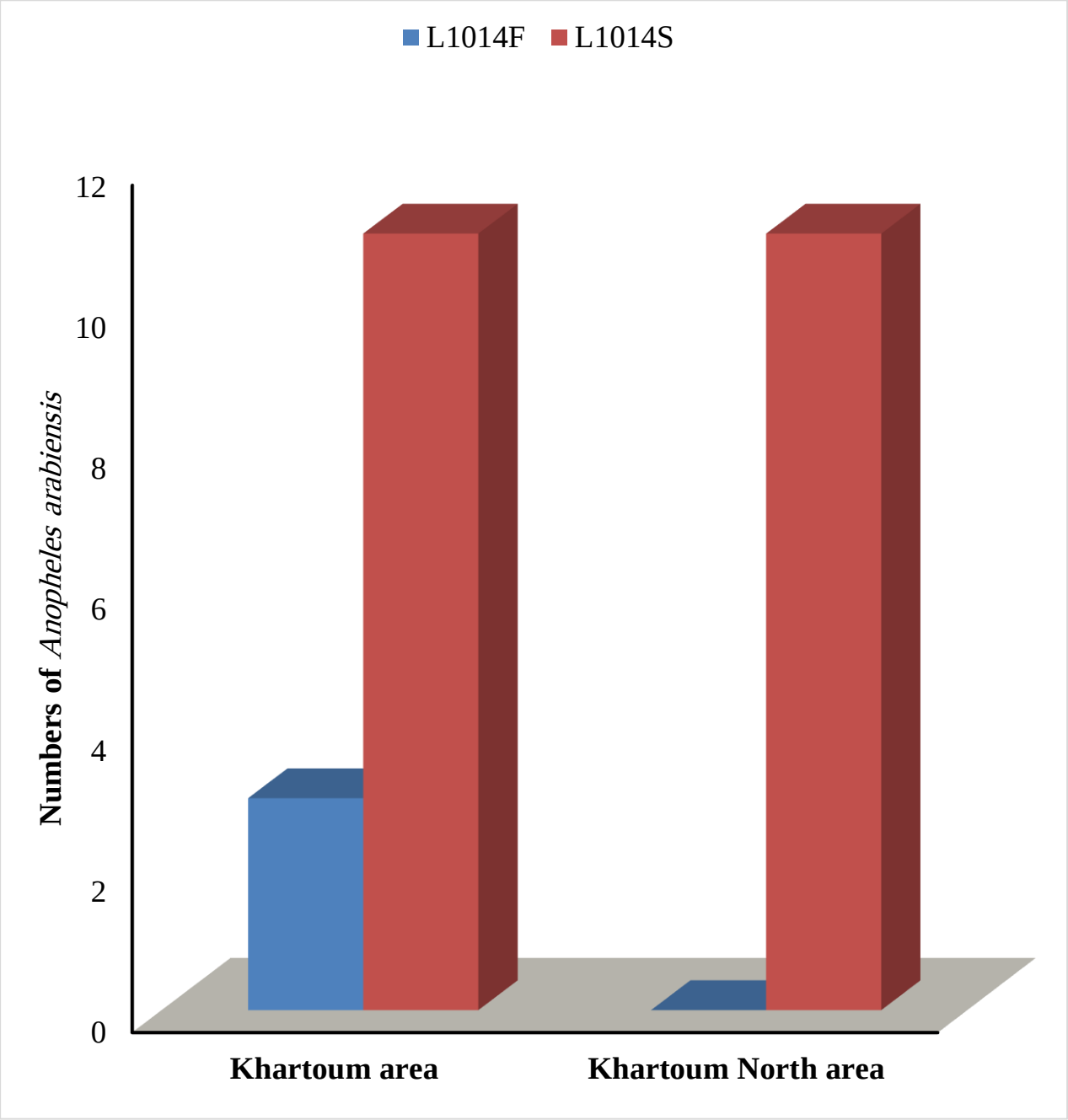

Figure 4.24: Numbers of wild *Anopheles arabiensis* with L1014F and L1014S-*kdr* mutation from administrative areas in Khartoum state during March –June 2013.

4.6. Occurrence and frequencies of acetylcholinesterase mutation in wild *Anopheles arabiensis* in Khartoum state

A total of 313 specimens of wild *An. arabiensis* from that assayed for *kdr* mutation were analyzed using IMP-PCR for occurrence of acetylcholinesterase mutation gene (ace-1^R mutation; ace.1 G119S) (Fig. 4.25). Almost, 209 (66.7%) samples successfully scored positive; 197 and 12 specimens were collected during March -June 2013 and October - December 2014 respectively. Out of the 197 PCR scored specimens, ace.1 G119S mutation were detected in 12 (5.1%) specimens of *An. arabiensis* from Khartoum state (Fig. 4.26). In contrast, none of the PCR scored from specimens of *An. arabiensis* collected in 2014 were with ace.1 G119S mutation. The overall specimens of wild *An. arabiensis* with ace.1 G119S mutation was 3.8%. *Anopheles arabiensis* with ace.1 G119S mutation were observed in specimens from only two sites; Al-Remeila in Khartoum and Shambat in Khartoum North area (Fig. 4.27). Both sites with ace.1 G119S mutant strain of *An. arabiensis* were urban areas.

The allelic frequencies of ace.1 G119S in *An. arabiensis* specimens in different sites varied between 0 - 0.8. The observed genotype frequencies in these populations of mosquitoes deviated from the expected genotype frequencies predicted by the Hardy-Weinberg equilibrium ($\chi^2 = 144.69$, $P = 0.00$) with 0.06 allelic variation.

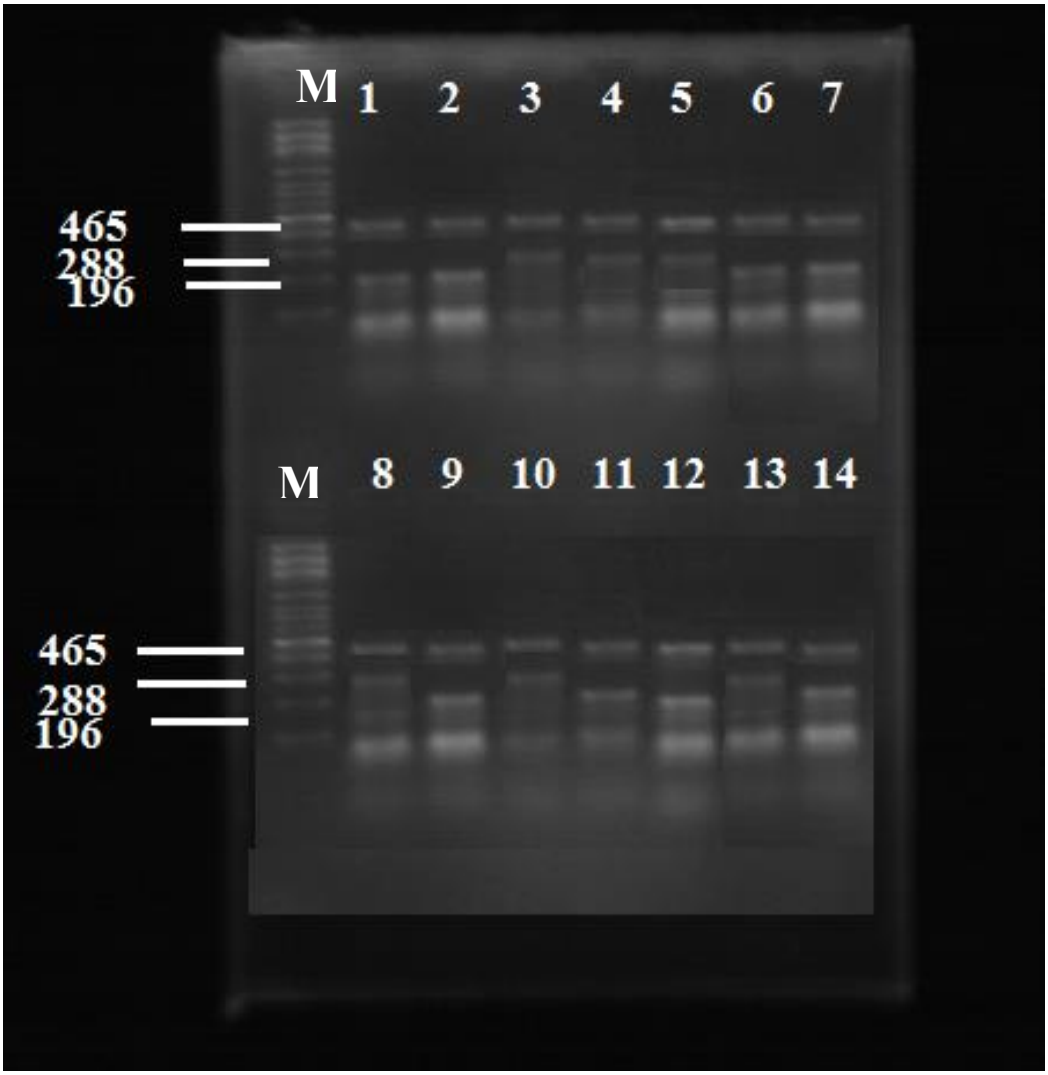

Figure 4.25: Amplicons produced by Polymerase Chain Reaction (PCR) G119S gene in wild *Anopheles arabiensis* **collected from different sites in Khartoum state. Primers create a 456-bp universal band, 288-bp for resistant individuals, and 196-bp for susceptible individuals. Lane M: 100 kb DNA molecular marker, lane 1: negative control (PCR water), lane 2 -4:** *Anopheles* **mosquitoes from different sites in Khartoum state, lanes 11: Reference strains from Dongola colony-reared** *An. arabiensis***.**

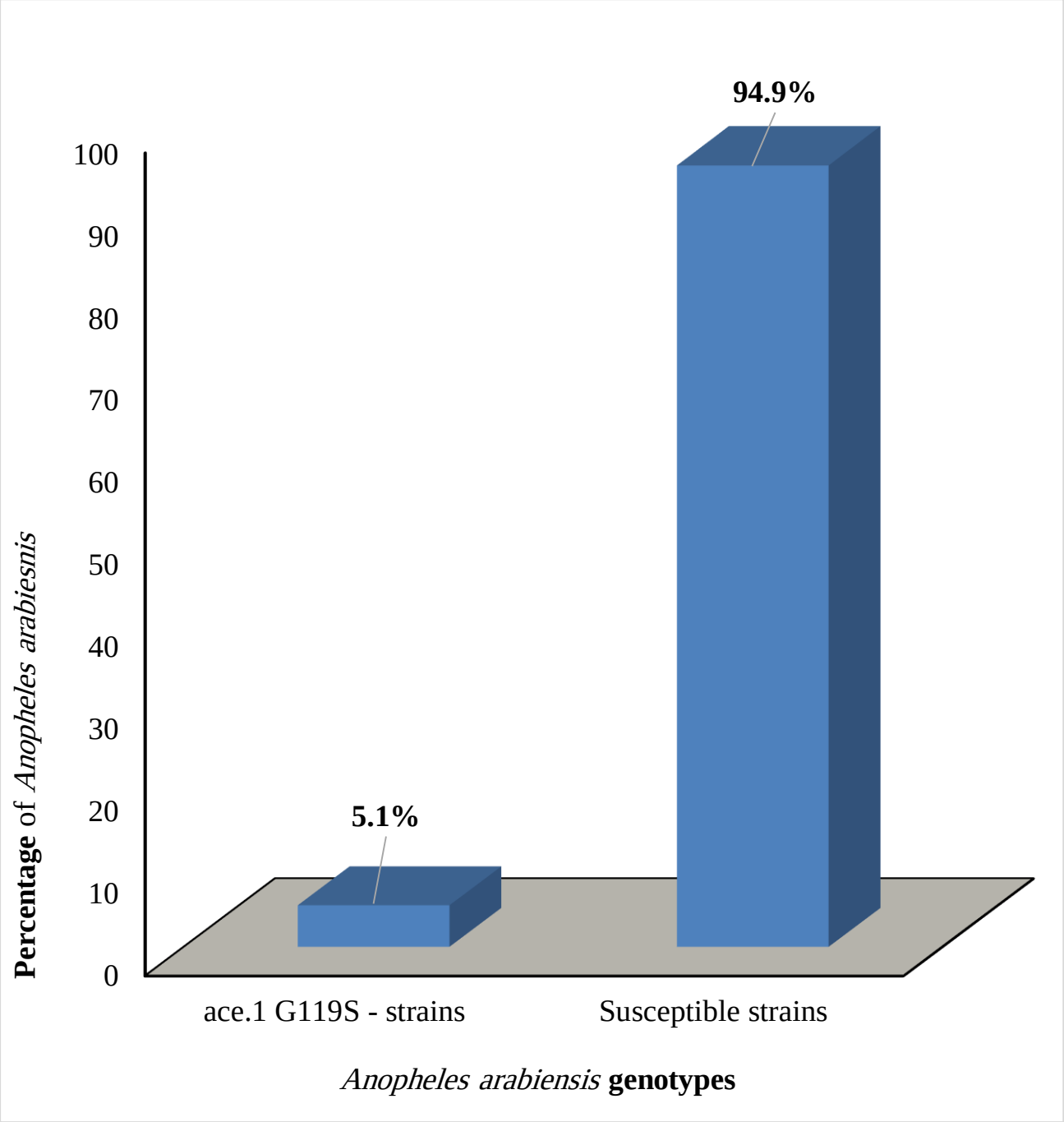

Figure 4.26: Percentages of wild *Anopheles arabiensis* with ace.1 G119S mutation in Khartoum state during March –June 2013.

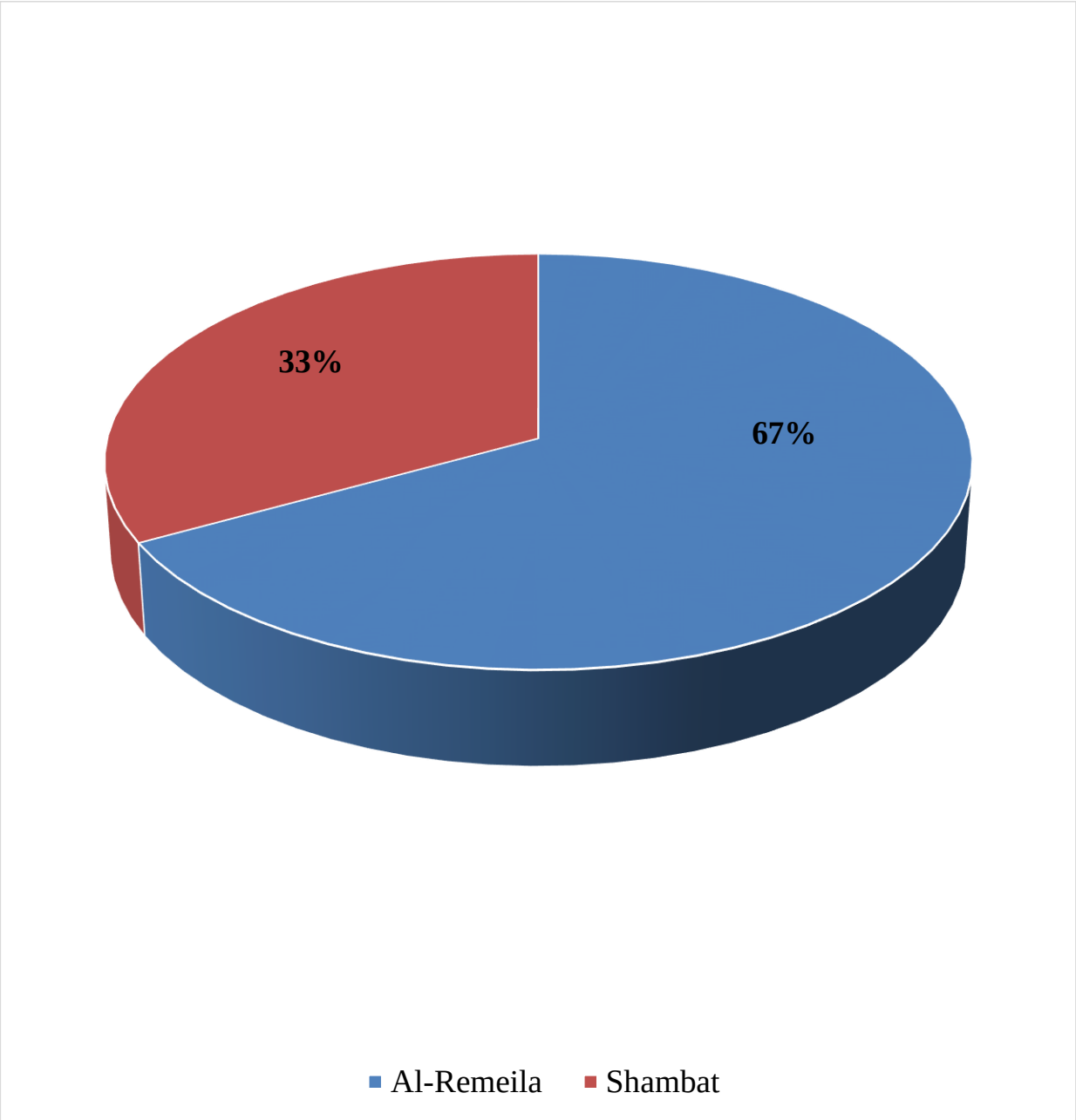

Figure 4.27: Percentage of wild *Anopheles arabiensis* with ace.1 G119S mutation from sentinel sites in Khartoum state during March – June 2013.

4.7. PCR detection of *Plasmodium* sporozoites in *Anopheles arabiensis*

Figure 4.28 shows PCR amplification for infection of wild adult *An. arabiensis* with *Plasmodium* sporozoites. A total of 751 *An. arabiensis* were assayed for infection with sporozoite of *Plasmodium* parasites. Of these, 661 and 90 specimens were collected during March - June 2013 and October -December 2014 respectively. No *Plasmodium* parasites were detected in all the samples of *An. arabiensis* (n = 661) collected during the year 2013. In contrast, 6 out of 90 (6.7%) of females collected during 2014 were infected with *P. falciparum*. Of the infected females *An. arabiensis*; 3 (6.8%; 3/44) were from Soba West, 2 (6.5%; 2/31) from Elmaygoma and 1 (14.3%; 1/7) from Alazeba sites (Table 4.17). The majority of infected females *An. arabiensis* were from periurban areas (5; 83.3%) (Fig. 4.29).

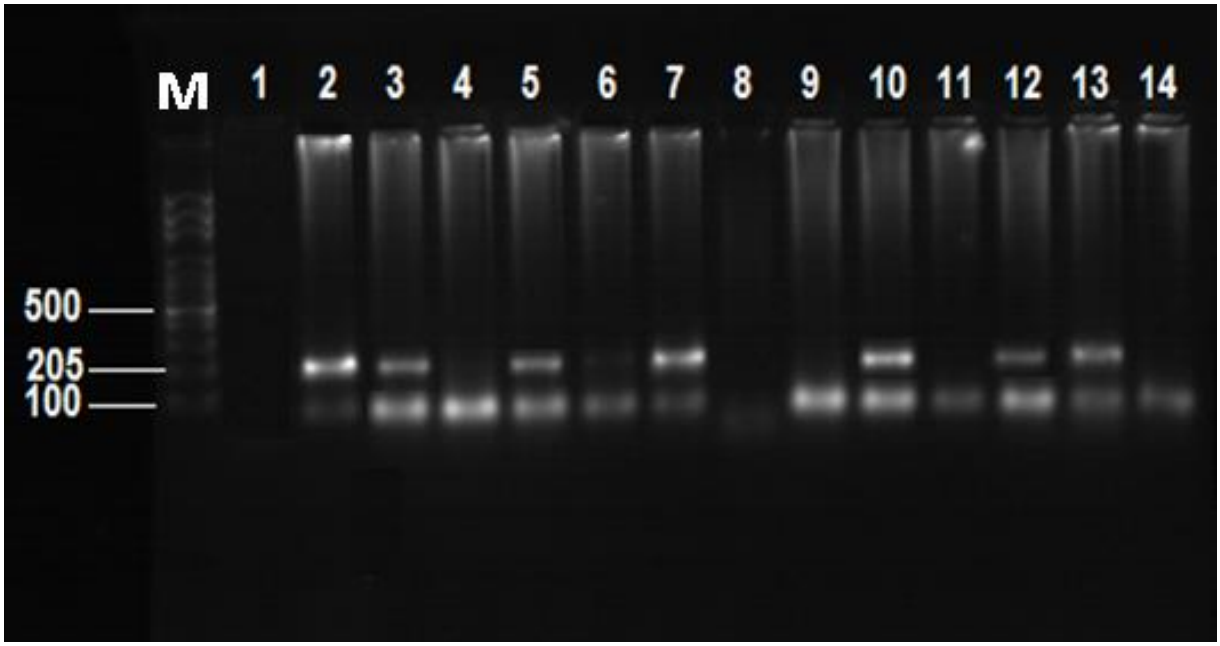

Figure 4.28: Amplicons produced by PCR using species-specific primers for identification of *Plasmodium falciparum* **and** *P. vivax* **collected from different sites in Khartoum state. Primers create a 205-bp for** *P. falciparum* **and 120-bp for** *P. vivax***. Lane M: 100 kb DNA molecular marker, lane 1: negative control (PCR water), lane 2: reference strain of** *P. falciparum***, lanes 3-7:** *An. arabiensis* **from Soba West site, Lanes 8-9: Mosquito specimens from Shambat, Lanes 10-11: Specimens of** *An. arabiensis* **from Alazeba, Lanes 12-14: Samples from Elmaygoma.**

Table 4.17: Numbers and percentages of infected wild *Anopheles arabiensis* with *Plasmodium falciparum* from four sites in Khartoum state during 2014 detected by PCR.

Sites	Total numbers assayed by PCR	Positive samples to *Plasmodium* sporozoites		Negative samples to *Plasmodium* sporozoites	
		Number	%	Number	%
Soba West	44	3	6.8	41	93.2
Shambat	8	0	0.0	8	100
Elmaygoma	31	2	6.5	29	93.5
Alazeba	7	1	14.3	6	85.7
Total	90	6	6.7	84	93.3

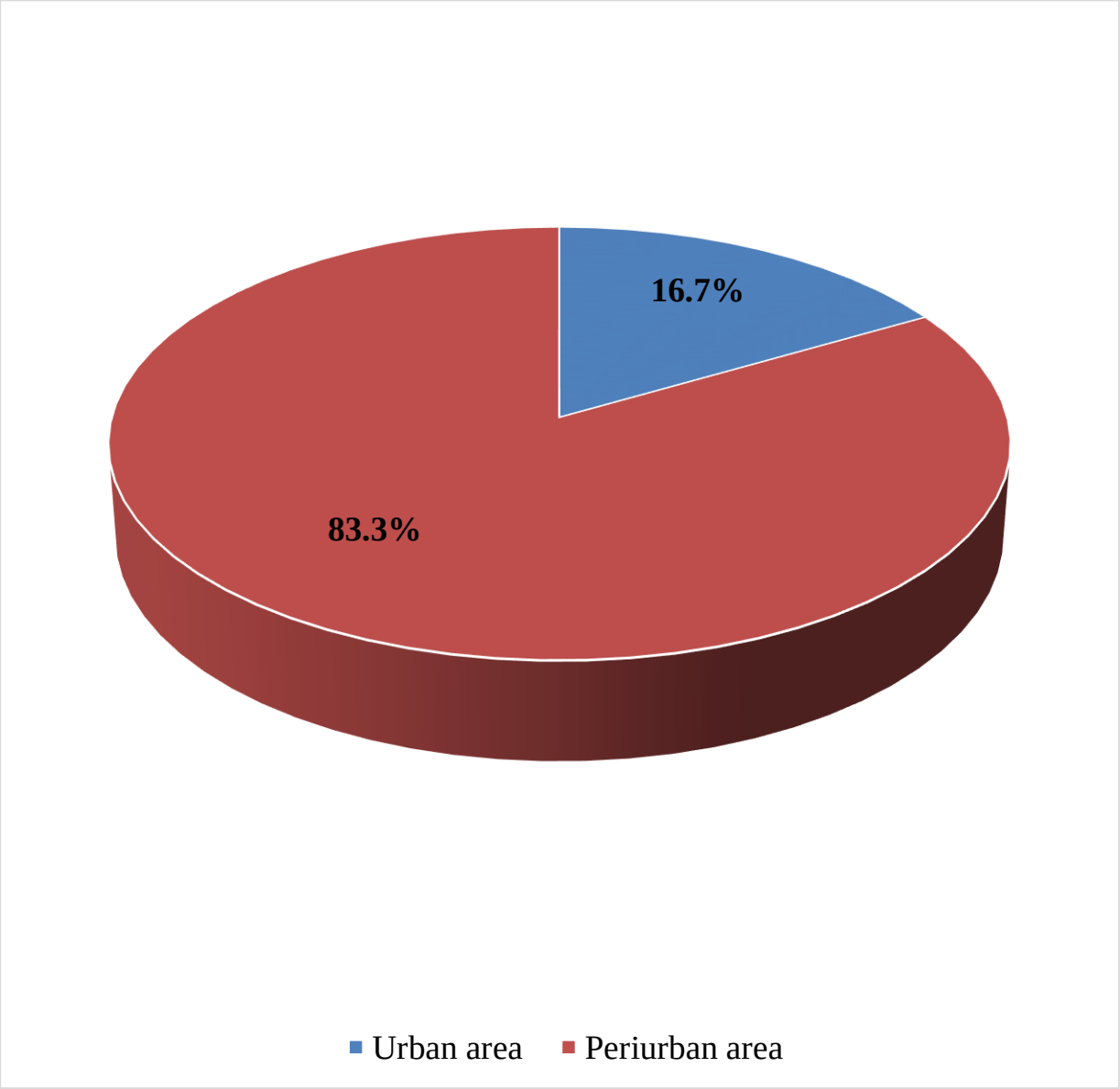

Figure 4.29: Percentage of wild *Anopheles arabiensis* infected with *Plasmodium falciparum* in urban and periurban in Khartoum state during October – December 2014.

4.8. The results of socio-economic questionnaire on the uses of insecticides in the public health and pesticides in the agricultural practices in Khartoum state

4.8.1. Qualitative data on pesticides uses

The public health insecticides and agricultural pesticides used in Khartoum state during the last 5 years are depicted in table 4.18. The KAP data showed that a total of 10 public health insecticides have been used as the main intervention for mosquito control in Arkaweet and Shambat sites. Out of the 17 pesticides registered for agricultural uses, 12 pesticides have been also used in Elmaygoma and Elsalamania sites (Table 4.18). The pesticides used in both practices mainly represented the four classes of insecticides; organochlorines, organophosphates, carbamates and pyrethroids. Of these, 5 pesticides representing three classes were used in both practices. These pesticides were DDT, malathion, permethrin, deltamethrin and lambdacyhalothrin. However, DDT, malathion and permethrin have been used extensively in agricultural practices during the last 5 years. Although, DDT and malathion were banned some decade ago, the farmers obtain this insecticide from illegal markets.

The KAP data also showed that several crops were cultivated in both Elmaygoma and Elsalamania sites with most commonly ones were for animal feed, vegetables and legumes in different seasons during the year. However, the season in both areas varied between 3 to 4 months in the year for each cultivated crop. During each season, the crop could be treated twice to four times with the above mentioned pesticides.

Table 4.18: The classes and names of pesticides used during the last five years in public and agriculture practices in Khartoum state

Classes	insecticide used	
	Public health	**Agriculture**
Organochlorines	DDT	DDT
	Gama Hexine	Endrin
Organophosphates	Abate (Temephos)	Folimat
	Diazinon	2,4 D (2,4-Dichlorophenoxyacetic acid)
	Malathion	Malathion
Carbamates	-	Bayleton
	-	Sevin USA
Pyrethroids	Permethrin	Permethrin
	Deltamethrin	Deltamethrin
	Lambdacyhalothrin	Lambdacyhalothrin
	-	Danitol

4.8.2. Knowledge, Attitude and Practice (KAP) on the uses of pesticides

4.8.2.1. Characteristics of the study population

Figure 4.30 shows the numbers of recruited health workers with different education levels. A significant difference was observed between the two surveyed sentinel sites in the educational level among the health workers ($\chi^2 = 23$, df = 21, P = 0.0). Unlike those in Arkaweet, all the health workers interviewed in Shambat site have been graduated from universities. In Arkaweet site, the health workers studied up to either primary (50%) or intermediate schools. Moreover, a significant difference was also observed between the two sites in numbers of health workers who attended training courses on the uses of insecticides ($\chi^2 = 6.462$, df = 20, P = 0.011). The results showed that 54% of the health workers in Shambat site had training courses with none in Arkaweet site.

The results on the education levels among the farmers in Elmaygoma and Elsalamania sites are shown in figure 4.31. No significant differences were observed among the study populations in the two areas neither in educational levels nor the training courses they previously had (P = 0.113 and 0.743 respectively). Relatively, similar numbers of farmers were illiterate or graduated from universities. Furthermore, 64% and 38% of the farmers in Elmaygoma and Elsalamania studied up to primary schools respectively. In addition, 31% of the farmers in Elsalamania site studied up to secondary schools compared to none in Elmaygoma site. Moreover, only 14% and 19% of the farmers in Elmaygoma and Elsalamania sites respectively attended training courses on uses of pesticides.

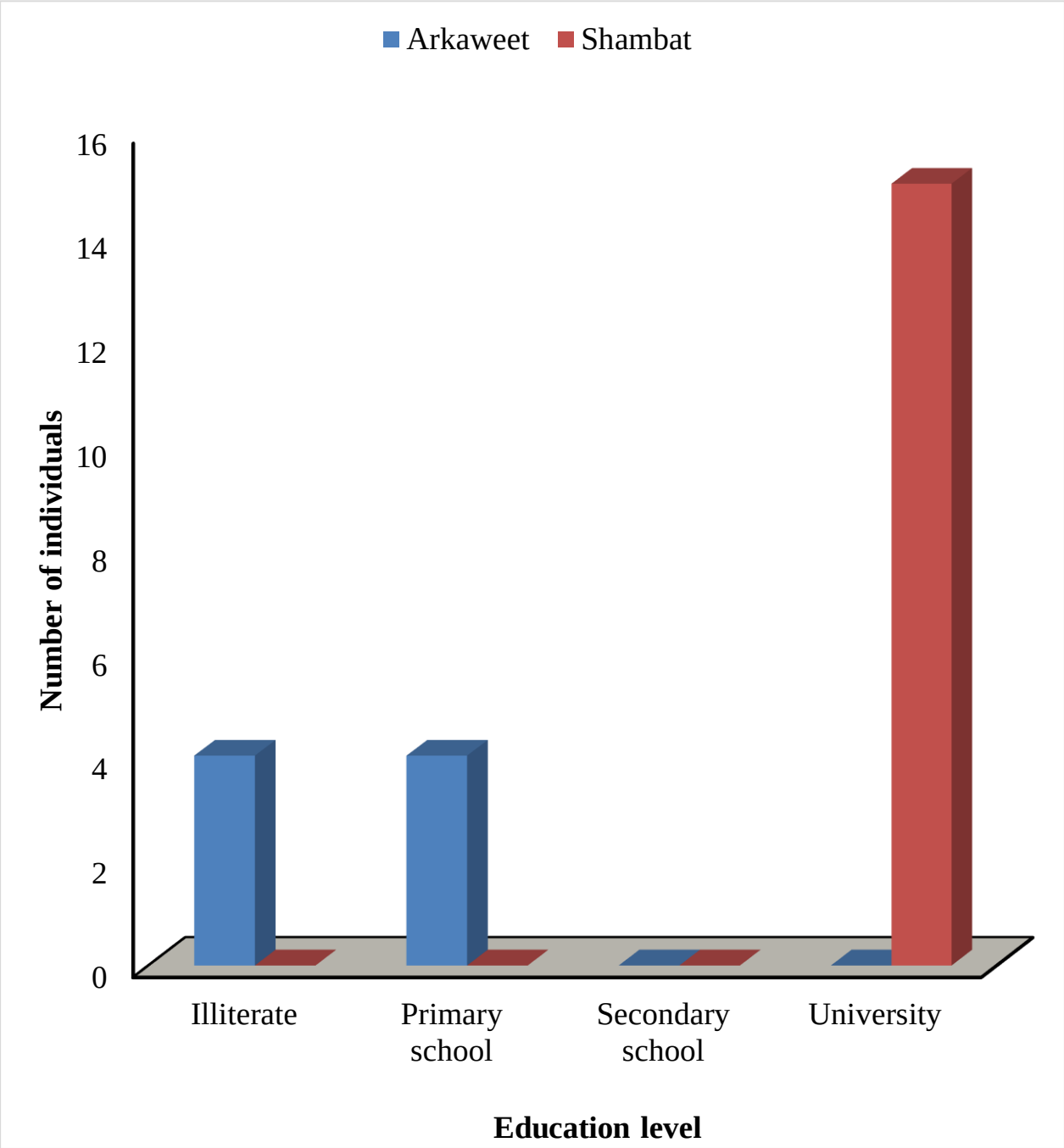

Table 4.30: Educational levels among health workers in two sentinel sites in Khartoum state, Sudan.

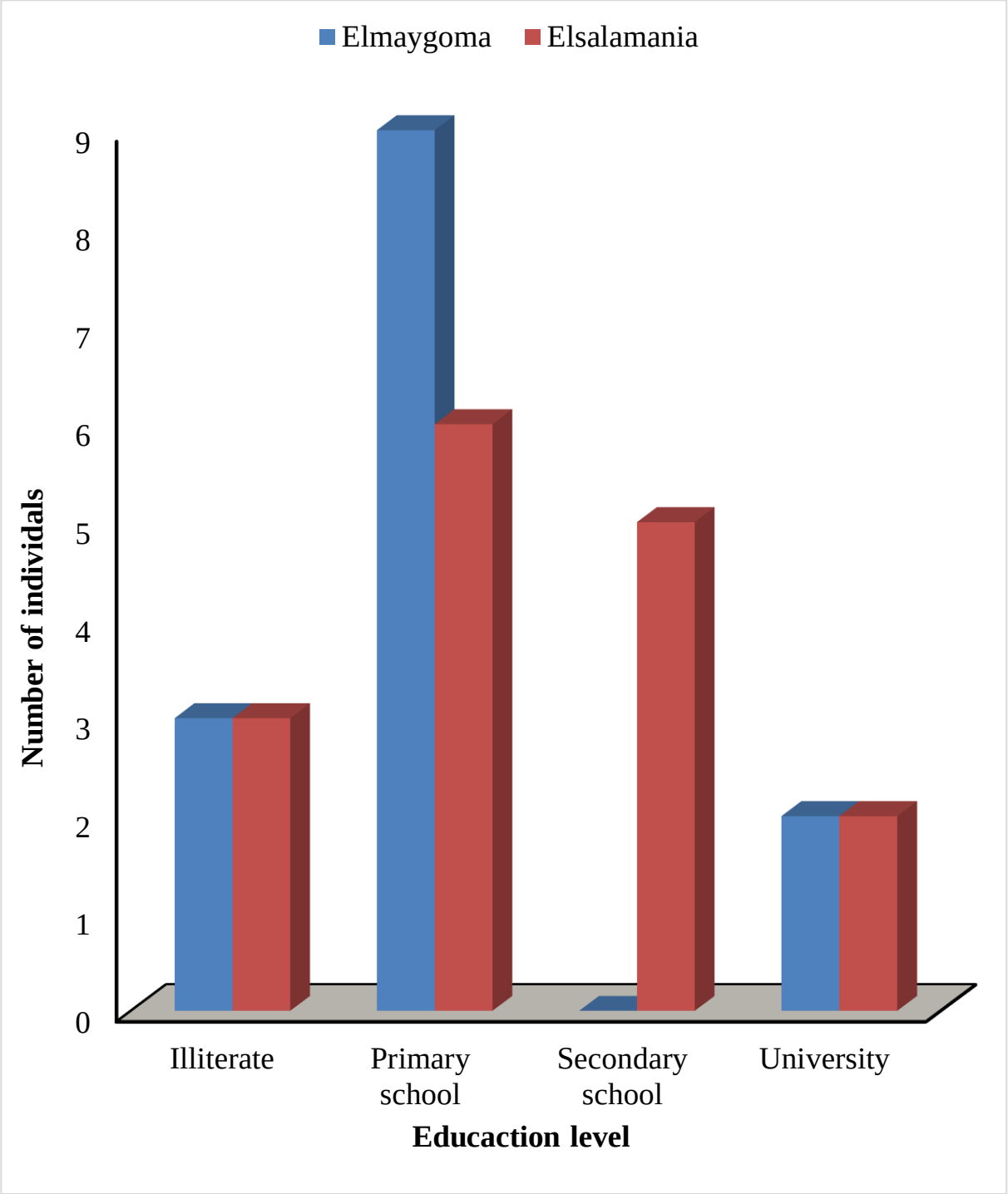

Table 4.31: Educational levels among farmers in two sentinel sites in Khartoum state, Sudan.

4.8.2.2. Knowledge of the health workers about the mosquito control

Table 4.19 shows the knowledge of health workers about the mosquito control in Arkaweet and Shambat sites in Khartoum state. Most of health workers (83.3%) conducted mosquito's routine surveys. Also, 83.3% of the health workers answered that they conducted surveys every month. The target was flying (adult) and aquatic (larvae) stages. All the respondents (100%) agree that there are interventions for mosquitoes control with 66.6% believed that the uses of insecticides is main control method. Moreover, Aerosol spraying (6.7%) and fogging (60%) or both methods (33.3%) were the main interventions used against adult mosquitoes as answered by the respondents. Most of the health workers (70%) apply insecticides against mosquito aquatic stages 3 times every month. Moreover, more than 80% of the respondents answered that there are private companies conducting insect control activities using insecticides in the two areas. The majority (86.7%) of the health workers answered that the malaria control programmes in their area follow-up these activities.

4.8.2.3. Knowledge and, related attitude and practices of the health workers about the uses of insecticides.

Table 4.20 shows data on the knowledge, attitude and practices of interviewed health workers about the uses of insecticides in Arkaweet and Shambat sites in Khartoum state. The results showed that 56.7% of the health workers in the two areas were aware of how to prepare the effective doses of used insecticides in mosquito control. Moreover, 76.7% of interviewed populations check the validity and the effective doses of insecticides in the field during conducting the interventions. In addition, 73.3% of the interviewed workers wash the excess prepared insecticide in the plumbs by water after they conducted the interventions. According to the interviewee, 70% of the respondents use only one

insecticide in intervention for mosquito control. Even for those who answered that they use more than one insecticide, they emphased on using them separately. Almost, more than 50% of the respondents, answered that the control programme in the area replace the insecticide with another every 4 years. Most of the interviewee (76.7%) answered that there are no insecticides rotation system in the area and 73.3% agree in that there is no coordination between the health and agriculture sectors in uses of the pesticides in their areas.

4.8.2.4. Knowledge, attitude and practices of the farmers about the uses of pesticides.

The data on the knowledge, attitude and practices of farmers on the uses of pesticides in Elmaygoma and Elsalamania sites in Khartoum state are depicted in table 4.21. The results showed that 96.7% of the farmers use pesticides for their crops. According to the interviewee, 70% use more than one pesticide in the agriculture season and most of farmers (86.7%) use these chemicals separately. The majority of the farmers (93.3%) check the validity and the effective doses of pesticides from the label attached before using them. Moreover, 76.7% of the respondents answered that they are used to prepare the working effective doses of pesticides from the stock by themselves. Furthermore, 73.3% of the farmers replace the pesticide by another when it becomes inefficient as by most of them (70%). Almost, 80% and 16.7% of the interviewed farmers answered that they obtain the pesticides from the market and agricultural sectors respectively. Approximately, about two third of the farmers do not use the pesticide rotation system in their farms. However, most of the farmers (66.7%) wash the excess pesticides by water after use and 20% burn them.

Table 4.19: Knowledge of the health workers about the mosquito control in Arkaweet and Shambat sites in Khartoum state.

Item	Categories	Study population (No and %)		Total
		Arkaweet	Shambat	
Do you have mosquito routine surveys in the area?	1. Yes	11 (78.5)	14 (87.5)	25 (83.3)
	2. No	3 (21.5)	2 (12.5)	3 (16.7)
How many surveys do you have a month?	1. Twice	12(85.7)	13(81.3)	25(83.3)
	2. More	2 (14.3)	3(18.7)	5(16.7)
What are the target stages of mosquitoes?	1. Aquatic stages	0 (0)	0 (0)	0 (0)
	2. Flying stages	0 (0)	2 (12.5)	2 (6.7)
	3. Flying and aquatic stages	14 (100)	14(87.5)	28 (93.3)
Do you have any interventions for mosquito control in the area?	1. Yes	14 (100)	16 (100)	30 (100)
	2. No	0 (0)	0 (0)	0 (0)
What are the main interventions used against mosquitoes?	1. Insecticides	11 (78.5)	9 (56.4)	20(66.6)
	2. Larval Source Management	3 (21.5)	5 (31.3)	8 (26.7)
	3. Others	0 (0)	2 (12.3)	2 (6.7)
What are the interventions used for flying stages?	1. Aerosol spray	0 (0)	2 (12.3)	2 (6.7)
	2. Fogging	14 (100)	4(25)	18(60)
	3. All	0 (0)	10(62.5)	10(33.3)
How frequent do you use insecticides against aquatic stages a month?	1. Twice	2 (14.3)	0 (0)	2 (6.7)
	2. Three times	12 (85.7)	9 (56.3)	21 (70)
	3. Four times	0 (0)	7 (43.7)	7 (23.3)

Table 4.19: Continued

Are there activities by private companies working in insect control in the area?	1. Yes	14 (100)	12 (75)	26 (86.7)
	2. No	0 (0)	4 (25)	4 (13.3)
If yes, is there a follow up from malaria control program to such activities?	1. Yes	14 (100)	12 (75)	26 (86.7)
	2. No	0 (0)	4 (25)	4 (13.3)

Table 4.20: Knowledge and related attitude and practices of the health workers about the uses of insecticides in Arkaweet and Shambat sites in Khartoum state.

Item	Categories	Study population (No and %)		Total
		Arkaweet	Shambat	
Do you prepare the effective doses of insecticides yourself?	1. Yes	12 (85.7)	5 (31.3)	17 (56.7)
	2. No	2 (14.3)	11 (68.7)	13 (43.3)
Do you check the validity and doses of the insecticides before use them?	1. Yes	10 (71.4)	14 (87.5)	24 (80)
	2. No	4 (28.6)	2 (12.5)	6 (20)
How do you check the validity and the effective doses of insecticides?	1. From the label tagged	2 (14.3)	1 (6.3)	3(10)
	2. In the laboratory	0 (0)	4 (25)	4 (13.3)
	3. In the field	12(85.7)	11(68.7)	23(76.7)
How do you get rid of the excess prepared insecticide after use them?	1. By burning	0 (0)	0 (0)	0 (0)
	2. Washing with water	8(57.1)	14 (87.5)	22(73.3)
	3. Bore it in wells	0 (0)	0 (0)	0 (0)
	4. Bore it in fishbowls	0 (0)	0 (0)	0 (0)
	5. Others	6(42.9)	2(12.5)	7 (26.7)
How many insecticides do you use?	1. One insecticide	9 (64.3)	12 (75)	21 (70)
	2. More	5(35.7)	4 (25)	9(30)
If more, how do you use them?	1. Separately	14(100)	16(100)	7 (100)
	2. Mixture	0 (0)	0 (0)	0 (0)

Table 4.20: Continued

Do you replace the insecticides?	1. Yes	3 (21.5)	14 (87.5)	17 (56.7)
	2. No	11 (78.5)	2 (12.5)	13 (43.3)
How frequent do you replace the insecticide?	1. One year	0 (0)	0 (0)	0 (0)
	2. Two years	0 (0)	3 (18.7)	3 (10)
	3. Three years	0 (0)	1 (6.3)	1 (3.3)
	4. Four years	14 (100)	12 (75)	26 (86.7)
Do you use a rotation system for the uses of insecticides?	1. Yes	0 (0)	7 (46.7)	7 (23.3)
	2. No	14 (100)	9 (52.3)	23 (76.7)
Is there any coordination between health and the agricultural sectors?	1. Yes	0 (0)	8 (50)	8 (26.7)
	2. No	14 (100)	8 (50)	22 (73.3)

Table 4.21: Knowledge and related attitude and practices of the farmer about the uses of agricultural pesticides in Elmaygoma and Elsalamania sites in Khartoum state.

Item	Categories	Study population (No and %)		Total
		Elmaygoma	Elsalamania West	
Do you use pesticides?	1. Yes	15 (100)	14 (93.3)	29 (96.7)
	2. No	0 (0)	1 (6.7)	1 (3.3)
How many pesticides	1. One	7 (46.7)	2 (13.3)	9 (30)
do you use in the	2. More than one	8 (53.3)	13 (86.7)	21 (70)
agriculture season?				
If more, how do you use	1. Separately	15 (100)	11 (73.3)	26 (86.7)
them?	2. Mixture	0 (0)	4 (26.7)	4 (13.3)
Do you check the	1. Yes	14 (93.3)	14 (93.3)	28 (93.3)
validity and the doses	2. No	1 (6.7)	1 (6.7)	2 (6.7)
of pesticides?				
Do you prepare the	1. Yes	9 (60)	14 (93.3)	23 (76.7)
used pesticides	2. No	6 (40)	1 (6.7)	7 (23.3)
yourself?				
Do you replace the	1. Yes	12 (80)	10 (66.7)	22 (73.3)
pesticide?	2. No	3 (20)	5 (33.3)	8 (26.7)
If yes, why do you	1. inefficient	10 (66.7)	11 (73.3)	21 (70)
replace them?	2. Difference crops	5 (33.3)	4 (26.7)	9 (30)
	3. Difference seasons	0 (0)	0 ()	0 (0)
From where do you get	1. Markets	10 (66.7)	14 (93.3)	24 (80)
the pesticides?	2. Companies	0 (0)	1 (6.7)	1 (3.3)
	3. Agriculture section	5 (33.3)	0 (0)	5 (16.7)

Table 21: Continued

Do you use rotation system of pesticides?	1. Yes	9 (60)	11 (73.3)	20 (66.7)
	2. No	6 (40)	4 (26.7)	10 (33.3)
How do you get rid of the excess prepared pesticides?	1. By burning	5 (33.3)	1 (6.7)	6 (20)
	2. Washing with water	8 (53.4)	12 (80)	20 (66.7)
	4. Discard it in wells	2 (13.3)	2 (13.3)	4 (13.3)
	5. Discard it in fishbowls	0 (0)	0 (0)	0 (0)

4.8.2.5. Knowledge of the farmers about the mosquito vectors

Table 4.22 illustrates the results on the knowledge of the farmers about the mosquito vectors in Elmaygoma and Elsalamania sites in Khartoum state. Ninety percent of the farmers spend the night in their farms. Where all of them answered that there are biting insects during the night. Moreover, all the interviewed farmers' emphasis that the only biting insects are mosquitoes and it occur mainly during the rainy season (autumn). According to the interviewee, 56.7% and 40% of the respondents protect themselves from the mosquito bites by spraying insecticides in their huts (the places where they sleep) and uses of ITNs respectively

Table 4.22: Knowledge of farmers about mosquito vectors in Elmaygoma and Elsalamania sites in Khartoum state.

Item	Categories	Study population (No and %)		Total
		Elmaygoma	Elsalamania West	
Do you spend the in the night in the farm?	1. Yes	12 (80)	15 (100)	27 (90)
	2. No	3 (20)	0 (0)	3 (10)
If yes, is there any biting insect?	1. Yes	15 (100)	15 (100)	30 (100)
	2. No	0 (0)	0 (0)	0 (0)
If yes, what are these insects?	1. Mosquitoes	15 (100)	15 (100)	30 (100)
	2. Sand flies	0 (0)	0 (0)	0 (0)
	3. Others	0 (0)	0 (0)	0 (0)
At which season mosquitoes present more?	1. Winter	0 (0)	0 (0)	0 (0)
	2. Summer	5 (33.3)	4 (26.7)	9 (30)
	3. Autumn	10 (66.7)	11 (73.3)	21 (70)
How do you protect yourself from mosquito bites?	1. Uses of insecticides	8 (53,3)	9 (60)	17 (56.7)
	2. Use of ITNs	6 (40)	6 (40)	12 (40)
	3. Others	1 (6.7)	0 (0)	1 (3.3)

CHAPTER FIVE

5. DISCUSSION

To date, several studies to assess the susceptibility/resistance status in *An. arabiensis* have been published from different parts of Sudan (Abdalla *et al.*, 2008; Himeidan *et al.*, 2007, 2011a; Mohammed *et al.*, 2015). Therefore, the present study was carried out in Khartoum state to determine the susceptibility/resistance status of *An. arabiensis* to the major classes of insecticides used and to determine the frequency of *kdr* and ace-1 G119S mutant genes. Moreover, attempt was made to detect the *Plasmodium* sporozoites infection in concomitance with *kdr* and ace-1 G119S mutation in wild adults *An. arabiensis*.

To perform WHO-susceptibility tests, anopheline larvae were collected from the nine sentinel sites. The study revealed two types of larval habitats; these were drinking water pipe leakage and irrigation canals. The larval habitats formed by pipe leakage were observed in urban areas whereas those from irrigation canals were in periurban areas. This finding is consistent with the results reported by Abuelmaali *et al.* (2013) who found that larval habitats observed in the urban sites were road puddles, pools and broken pipelines pools while in peri-urban sites were mainly irrigation canals and hoof prints. Moreover, in Northern Sudan, Ageep *et al.* (2009) found a significant number of larval habitats of *An. arabiensis* in Northern Sudan associated largely with artificial habitats which were mainly formed by leaking pipes and brickworks. However, in Northern Sudan, Dukeen and Omer (1986) reported that most of larval habitats occurred by the riverside. Similarly, other studies indicated that of vector breeding habitats were in the vicinity of major streams or rivers (Sogoba *et al*., 2007; Majambere *et al.*, 2008; Ageep *et al.*, 2009). The results obtained from our surveys on larval habitats will

help to design more effective and efficient vector control programme in the future in Khartoum state.

In Sudan, there are 29 anopheline mosquito species that have been recorded (Lewis, 1958; Nugud *et al.*, 1997; El-Rayah, 2007). The results of the morphological identification showed that anopheline mosquitoes collected belonged to the *An. gambiae* complex. Furthermore, the molecular analysis revealed that *An. arabiensis* was the only anopheline mosquito detected in Khartoum state. This finding is consistent with previous studies which showed that *An. arabiensis* is the only malaria vector in Khartoum state (Petrarca *et al.*, 2000; El Sayed *et al.*, 2000). Currently, *An. arabiensis* the member of the *An. gambiae* s.l. is the only malaria vector in the country (Dukeen *et al.*, 1986; Hamad *et al.*, 2002; Ageep *et al.*, 2009). This species is the most widespread and dominant anopheline species throughout the arid regions in Sudan (Dukeen and Omer, 1986). It is distributed over dry savannah and semi-arid parts, extending northwards along the River Nile to 20^{o} N in Sudan (Dukeen and Omer, 1986; Ageep *et al.*, 2009). Although in northern Sudan, *An. arabiensis* is found highly zoophilic and exophilic (Dukeen and Omer, 1986), it showed some degree of anthropophilic and endophilic behaviour in Khartoum state (El Sayed *et al*., 2000). Furthermore, this species was found to have a high vectorial capacity, which makes it an efficient malaria vector (Nugud and El Sayed, 2001).

Susceptibility tests were carried out for mosquito specimens collected from nine sentinels following the WHO standard protocol using seven insecticides (DDT4%, fenitrothion 1%, malathion5%, propoxur 0.1%), permethrin 0.75%, deltamethrin 0.05% and lambdacyhalothrin 0.05%. Assessment of susceptibility/resistance status of *An. arabiensis* to the above mentioned insecticides was done for populations from 9 sentinel sites in Khartoum state. During this study, field populations of *An. arabiensis* from three out of nine

sentinel sites were tested against the seven insecticides used. However, *An. arabiensis* specimens from the other six sentinel sites were tested with varied numbers of insecticides (3- 7 insecticides). This variation was due to the availability of larval habitats and/or the density of larvae in the habitats investigated in sentinel sites during certain months of collection. The reasons of that were the differences in the types of larval habitats between the investigated areas. The larval habitats in urban (residential) areas are mainly formed of leakage of water pipes whereas in the peri-urban (agricultural) areas are formed of water canal seepage. Often, larval habitats formed of canal seepage are more likely suitable for breeding of *An. arabiensis* than those formed by broken pipes (Ageep *et al.,* 2009).

In Khartoum state, the uses of agricultural pesticides in periurban areas and insecticides for control of mosquito vectors in both urban and periurban areas apparently have a major impact on the development of resistance in the wild populations of malaria vectors. This might be the reason that the populations of *An. arabiensis* showed differences in their susceptibility status. However, the results of overall mean mortality revealed an evidence for resistance to DDT, malathion, propoxur, permethrin and deltamethrin in *An. arabiensis* in Khartoum state. On the other hand, this species was susceptible to only fenitrothion and lambdacyhalothrin. The results obtained in this study are in line with a previous study in Khartoum state which also showed that *An. arabiensis* is fully susceptible to fenitrothion and lambdacyhalothrin (Seidahmed *et al.*, 2012; Abuelmaali *et al.*, 2013). These authors found that *An. arabiensis* was resistance to only malathion and suspected/resistant to DDT. Nevertheless, Abuelmaali *et al.* (2013) found confirmed resistance of *An. arabiensis* to pyrethroids in this state.

According to WHO (2013) criteria for determination of resistance, the results revealed that populations of *An. arabiensis* from three sentinel sites were

resistant to DDT, malathion and permethrin and propoxur in two sites (see table 3.4). Likewise, specimens of *An. arabiensis* collected from the nine sites were resistant to deltamethrin with exception of those from Arkaweet and Elsalamania West sites which were fully susceptible (100% for each). Resistance in *An. arabiensis* from different sentinel sites to most of insecticides probably might be due to extensive use of massive numbers of insecticides of different classes in agriculture against domestic pests coupled with that used in the public health practice.

The detection of confirmed resistance of *An. arabiensis* to DDT observed in this study is not surprising in view of the long history of its use in both agriculture and health practices. Selection for resistance to this insecticide might be started as far back as 1970s when DDT was used to control agricultural pests and mosquito vectors in Sudan. Although, DDT was banned decades ago, the WHO announced in 2006 its re- application on a limited and controlled scale to maximize impact on malaria vectors (Sadasivaiah *et al.*, 2007). Furthermore, DDT has been illegally used in agriculture where it could be purchased from illegal markets in Sudan. Although, previous studies showed that *An. arabiensis* in Khartoum state is susceptible to this insecticide (Seidahmed *et al.*, 2012), currently resistance to DDT in *An. arabiensis* has been reported from Gizera and Sennar states (Abdalla *et al.*, 2008), White Nile state (Ismail *et al.*, 2012), Rahad area in Gedarif state (Yagoop *et al.*, 2013) and New Half in Kassala state in eastern Sudan (Himeidan *et al.*, 2007, 2011a). Moreover, previous studies also showed that the resistance to DDT in populations of malaria vectors was due to the longstanding and extensive use of DDT in the IRS programmes (Lines, 1988; Protopopoff *et al.*, 2008). Likewise, resistance in *An. arabiensis* to DDT were reported from other African countries such as in South Africa (Nardini *et al.*, 2013), Burkina Faso (Jones *et al.*, 2012) and Ethiopia (Balkew *et al.*, 2010).

The results obtained in this study on resistance of *An. arabiensis* to malathion agree with historical and recent studies from neighboring Gezira and White Nile states (Lines, 1988; Abdalla *et al.,* 2008; Ismail *et al.,* 2012). Moreover, resistance of *An. arabiensis* to malathion was previously confirmed in Khartoum state (Seidahmed *et al.*, 2012; Abuelmaali *et al.,* 2013; Mohammed *et al.,* 2015). Malathion was banned seven years ago by the Khartoum state Malaria Control Program (KSMCP) due to development of resistance in *An. arabiensis* (KMFP, personal communication). Currently, resistance of *An. arabiensis* to malathion has become widely spread in different region of Sudan including Khartoum, Gizera, White Nile and Gedarif states (Himeidan *et al.,* 2007; Abdalla *et al.,* 2008, Ismail *et al.,* 2012; Yagoop *et al.,* 2013). Moreover, a survey conducted in Khartoum state showed that organophosphates and carbamates were the most commonly used pesticides in agriculture practice (Seidahmed *et al.,* 2012). This might indicate that malathion is used in agriculture practice although it has previously been suggested that resistance to malathion in *An. arabiensis* in Gizera state was due to house spraying rather than aerial crop spraying (Lines, 1988).

The confirmed resistance to permthrin and reduced susceptibility to deltamethrin is of concern for the Integrated Vector Management which depends on ITNs/LLINs as a protection measure against malaria vector. Malaria vector control has been very dependent on the pyrethroids. These insecticides are the only classes approved for use on insecticide treated nettings (WHO, 2014) and are being increasingly deployed in IRS programmes in Africa. Besides, pyrethroids are also widely used in the control of agricultural pests worldwide (UN, 2006). A previous study conducted in Khartoum state Showed that *An. arabiensis* is fully susceptible to permethrin (Seidahmed *et al.*, 2012). In contrast, Abuelmaali *et al.* (2013) found that this species is suspected resistance to pyrethroids in this state.

Permethrin resistance is now well established where *An. arabiensis* has been detected with high resistance to this insecticide in different regions in Sudan including Gizera, White Nile, Sennar Blue Nile, Kassala and Gedarif states (Abdalla *et al.*, 2008; Himeidan *et al.*, 2011a; Ismail *et al.*, 2012; Yagoop *et al.*, 213). Resistance to pyrethroids especially permethrin and deltamethrin insecticides in *An. arabiensis* and other malaria vectors have been reported from different African countries (Hargreaves *et al.*, 2000; Etang *et al.*, 2003; Casimiro *et al.*, 2006). Pyrethroid, permethrin has along back history of uses in IRS for public health purposes in Sudan (Himeidan *et al.*, 2007; Abdalla *et al.*, 2008). This insecticide was then replaced by bendiocarb in 2007 when *An. arabiensis* showed strong resistance (Abdalla *et al.*, 2008). Moreover, pyrethroid insecticides have been extensively used in agriculture (Seidahmed *et al.*, 2012; Abuelmaali *et al.*, 2013). Therefore, the development of resistance might be due to high selective pressure imposed on mosquito vectors through the indiscriminate usage of pyrethroids by farmers to control agricultural crop pests at the time of spraying for mosquito vector control by the vector control programmes. The development of resistance in malaria vector to pyrethroid insecticides has a serious implication on malaria control strategies in the country. Pyrethroids, deltamethrin and lambdacyhalothrin are the main insecticides being used for ITNs/LLINs to control malaria vectors elsewhere (WHO, 2014).

Unlike populations of Khartoum and Omdurman areas, *An. arabiensis* from Khartoum North area was resistant to most of the insecticides tested. This finding might be due to that most of the agricultural schemes and riverine farms in Khartoum state are located in Khartoum North area (Elkhalifa *et al.*, 2008). Likewise, in a study in Gezira state *An. arabiensis* from urban areas with agriculture practices has been found more resistant to insecticides than those from settlement areas (Abdalla *et al.*, 2014). The presence of multiple insecticide

resistance in populations of *An. arabiensis* in this administrative area is consistent with previous studies (Matambo *et al.*, 2007; Abdalla *et al.*, 2008; Himeidan *et al.*, 2011a). However, a steadily and fast development of urban agriculture has recently taken place in many areas in the Khartoum state. Besides, the ongoing malaria control programme, the development of farming system will increase and accelerate the development of insecticide resistance in malaria vector in the state. Similarly, association between the farming and development of insecticide resistance in malaria vectors has been reported from different African countries (Yadouleton *et al.*, 2009; Antonio-Nkondjio *et al.*, 2011).

In Khartoum state, both urban and peri-urban agricultural areas have been expanded due to high demand for accommodation and food. This expansion was due to steadily immigration of people from other states in Sudan. However, the expansion in the periurban agriculture was greater than those in the urban areas, therefore, this situation has led to extensive use of pesticides in these areas. In addition to that, the uses of insecticides especially organophosphate temephos for public heath purposes in both urban and periurban areas has increased and accelerated insecticide resistance in the malaria vector. In this study, it was apparent that there was an impact of agricultural pesticides on susceptibility of *An. arabiensis* as shown by differences in the insecticide susceptibility of these populations in agricultural periurban areas and non-agricultural urban ones. This finding is in agreement with the results obtained previously by Seidahmed *et al.* (2012) and Abuelmaali *et al.* (2013). The authors found that *An. arabiensis* from periurban areas are more likely to develop insecticide resistance than those in urban areas. This probably might be due to extensive use of massive numbers of insecticides of different classes in agriculture against domestic pests coupled with that used in the public health practice. This suggestion can be supported by the

results obtained from the surveys on insecticides used by farmers in agricultural and public health workers in the public health practices.

In this study, relatively, little variations were observed in the knockdown times for each insecticides among the population of *An. arabiensis* from different sentinel sites, the three administrative areas and from the urban and periurban areas. The KDT_{50} and KDT_{95} for all insecticides in the current study was markedly lower than those reported for populations of *An. arabiensis* in different regions in Sudan (Himeidan *et al.,* 2007, 2011a; Abdalla *et al.,* 2008; Ismail *et al.,* 2012; Yagoop *et al.,* 2013). For example, in this study, the knockdown time 50% for DDT was higher in Shambat and Elmaygoma sites by 1.7 folds than in Abuseid site. Moreover, KDT_{50} for permethrin, was higher in Edekheinat, Elmaygoma and Elsalamania West sites by 2.7, 2.1 and 2.1 folds respectively than in Arkaweet site. This finding suggested that a knockdown resistance mechanism could be operating in this mosquito population. The KDT_{50} and KDT_{95} for DDT in this study was much lower than those reported for a population from New eastern and central Sudan (Himeidan *et al.*, 2007, 2011a; Abdalla *et al.*, 2008; Ismail *et al.*, 2012; Yagoop *et al.*, 2013).

In this study, the variation in mortality rates in *An. arabiensis* due to insecticides between the three different seasons (dry cold, dry hot and wet) showed some differences especially for DDT 4%, malathion 5% and propoxur 0.1%. For example, in Khartoum state, DDT and propoxur resistance was high with mortality rates of 87% and 83% respectively during the cold dry season. During the hot dry season, the mortality rates increased to 95% and 86% for DDT and propoxur respectively. The same trend was observed for DDT in populations of Edekheinat and Shambat sites. However, for malathion variation of mortality rates in *An. arabiensis* from Elmaygoma sites was higher in hot dry season. Although, there was variations in mortality rates in population of *An. arabiensis*

from administrative areas or different land use (urban and periurban areas) due to DDT and malathion between the season, these differences did not follow fixed trends. However, seasonal variations in susceptibility of *An. arabiensis* to insecticides have been previously reported from Khartoum and Gizera states in Sudan (Abuelmaali *et al.,* 2013; Abdalla *et al.,* 2014). Likewise such variations have been observed in susceptibility of *Anopheles* mosquitoes to insecticides in different African countries (Diabate *et al.,* 2002; Ranson *et al.,* 2009; Djegbe *et al.,* 2011).

In Africa, there have been efforts to map the insecticide resistance of the main malaria vectors at nation or continental scale (WHO/ANVR, 2005; Coleman *et al*., 2006; Santolamazza *et al*., 2008; Coetzee and Koekemoer, 2013). Such information is necessary to monitor, detect and manage insecticide resistance in mosquito vectors. In this study, resistance to DDT, malathion, propoxur, permethrin and deltamethrin were observed in populations of *An. arabiensis* from certain sentinel sites representing both urban and periurban areas in the three administrative areas in Khartoum state. Nevertheless, the resistances to these insecticides are not uniformly distributed among the populations of *An. arabiensis* from different nine sentinel sites. For example, DDT-resistant strains of *An. arabiensis* were observed in only four of the mentioned sentinel sites (see fig. 4.31). These sites were Edekheinat (periurban area) in Khartoum, Shambat (urban area) and Elmaygoma (periurban area) in Khartoum North and Abuseid site in Omdurman administrative area. Insecticide resistance has been reported in the main malaria vectors worldwide. Resistance is however not uniformly distributed among vector species and can greatly differ from one village, province, country, region and continent to another. Unfortunately, the highest levels of insecticide resistance were reported in Africa where malaria burden is still the highest in the world (WHO, 2011). The number of studies examining insecticide susceptibility

and resistance mechanisms in *Anopheles* malaria vectors in African countries is growing rapidly. In Sudan, several previous and current studies to determine the susceptibility status of *An. arabiensis* to different agricultural pesticides and public health insecticides has been published (Himeidan *et al.,* 2007, 2011a; Abdalla *et al.,* 2008, 2014; Ismail *et al.,* 2012; Seidahmed *et al.,* 2012; Yagoop *et al.,* 2013; Abuelmaali *et al.,* 2013). These studies elucidated the distribution of resistance and susceptible strains of *An. arabiensis* to insecticides in different parts of studies areas, these include Kassala (Himeidan *et al.,* 2007, 2011a) and Gedarif states (Yagoop *et al.,* 2013) in eastern Sudan, Gezira (Abdalla *et al.,* 2008, 2014) and White Nile states (Himeidan *et al.,* 2011a; Ismail *et al.,* 2012) in Central Sudan and Khartoum state (Himeidan *et al.,* 2011a; Seidahmed *et al.,* 2012; Abuelmaali *et al.,* 2013; Mohammed *et al.,* 2015). Likewise, mapping and distribution of insecticide resistance in malaria vectors in different African countries have been interviewed and published (WHO/ANVR, 2005; Santolamazza *et al.,* 2008; Ranson *et al.,* 2011; Gnanguenon *et al.,* 2015).

The ability to determine the resistance status of mosquito vectors is essential to guide the use of insecticides in the malaria control programme in the country. It allows for a rational choice of insecticide to be made, based on the type and extent of resistance present. Knockdown resistance has been detected in *An. gambiae* s.s. and *An. arabiensis* (Martinez-Torres *et al.,* 1998; Ranson *et al.,* 2000; Diabaté *et al.,* 2004; Stump *et al.,* 2004; Balkew *et al.,* 2010; Yewhalaw *et al.,* 2011; Dabire´ *et al.,* 2014; Toé *et al.,* 2015). The screening for the L1014S and L1014F *kdr* mutation, causing the knockdown resistance, is commonly performed using two different AS-PCR assays (Martinez-Torres *et al.,* 1998; Ranson *et al.,* 2000). These assays provide a cheap mean for determining the *kdr* allele frequencies in *An. gambiae* s.s. and *An. arabiensis* populations.

In this study, both L1014F and L1014S mutations were detected in populations of *An. arabiensis* collected from four sites representing periurban areas. The L1014F was detected in populations of *An. arabiensis* from two sites Edekheinat (two samples) and Alshegelab (one sample) in Khartoum administrative area. Whereas, L1014S mutations were detected in specimens from four sites, these were Edekheinat (5; 16.6%) and Alshegelab (6; 18.2%) and, Edroshab (7; 23.3%) and Eltumanyat (4; 11.8) in Khartoum North area. Likewise, the occurrence of L1014F mutation was reported in very low frequency in populations of *An. arabiensis* from different areas in Khartoum state (Himeidan *et al.*, 2011a; Abuelmaali *et al.*, 2013) and in other geographic regions in Sudan including Gizera, Sennar, Kassala and White Nile states (Abdalla *et al.*, 2008, 2014; Yagoop *et al.*, 2013). In contrast, The L1014S *kdr* mutation has previously been reported in a very low frequency in a population of *An. arabiensis* from Kassala state in eastern Sudan (Himeidan *et al.*, 2007). The results on the L1014S mutation from this study and from the previous reports suggested that this type of mutation is so limited.

The *kdr* (L1014F and L1014S) mutation is mainly associated with resistance to pyrethroids and organochlorines insecticides (i.e. DDT). Although, DDT was stopped for uses in agricultural and public health practices some decades ago, this insecticide has illegally been used in agricultural practice (Seidahmed *et al.*, 2012). In contrast, pyrethroid insecticides are currently in use in Sudan including Khartoum state where permethrin and deltamethrin are used in ITNs/LLINs and in a very low scale in IRS respectively (personal comm. Department of IVM unit, FMOH, Sudan). Therefore, the *kdr* mutation detected in populations of *An. arabiensis* in Khartoum state in this study might be due to the selected cross-resistance due to pyrethroids rather than organochlorines. However, the role of the organochlorines DDT cannot be neglected since it has

been used in agriculture practice. The L1014F mutation observed in this study was lower than that previously reported in *An. arabiensis* from Khartoum state (Himeidan *et al.,* 2011a; Abuelmaali *et al.,* 2013) and different regions in Sudan (Himeidan *et al.,* 2011a; Abdalla *et al.*, 2014). The L1014S allele was detected for the first time in Khartoum state. This type of mutation is scare in *An. arabiensis* population in most of East African countries including Sudan although it is originally reported from Kenya but in *An. gambiae* (Stump *et al.*, 2004). In this study, the L1014S *kdr* mutation was observed in a relatively high frequency compared to that previously reported by Himeidan *et al*. (2007) who recorded mutation in *An. arabiensis* from eastern Sudan.

Resistance mechanisms testing for malaria vectors has largely focused on target site mutations (*kdr* and insensitive acetylcholinesterase G119S (Ace-1^R) with relatively few resistant populations assessed for metabolic mechanisms. In this study, we report, for the first time, the presence of the ace.1 G119S mutation in *An. arabiensis* populations from two sites: Alremaila and Shambat in Khartoum and Khartoum North area respectively. To confirm this presence of ace.1 G119S mutation, several PCR amplification were done for this allele in the *An. arabiensis* specimens, and as a control, 30 specimens reference susceptible strain of Dongola colony-reared *An. Arabiensis* were used. The ace.1R allele observed in this study in *An. arabiensis* was in a very low frequency; however, no comparative studies have been conducted in this species in Sudan. However, a single study was previously conducted on *An. arabiensis* to detect acetylcholinesterase enzyme level based on isoenzyme analysis (Abdalla *et al.,* 2008). The authors found an elevated enzyme which indicates a metabolic-based resistance in this species which commonly leads to resistance to organophosphates and carbamates insecticides. The ace.1 G119S mutation has been observed in malaria vectors in West African countries especially *An. gambiae* and *An. arabiensis* (Djogbenou *et*

al., 2008; Dabire´ *et al.,* 2014; Namountougou *et al.,* 2013).The emergence of the ace-1R mutation in *An. arabiensis* populations in Khartoum state may be linked to the use of organophosphates and carbamates insecticides in agricultural and public health practices. Bioassays performed in this study and previous ones indicated a high resistance to the carbamates, malathion in Khartoum state (Seidahmed *et al.,* 2012; Mohammed *et al.,* 2015).These authors also reported that this insecticide has been used for control of agricultural crop pests and insect vectors in Khartoum state. However, further bioassays on a wider scale are now required in order to understand the implications of the current status of the ace-1R mutation for the efficacy of organophosphates and carbamates insecticides in vector control in Sudan. The information provided by bioassays coupled with the genetic data obtained in this study is a prerequisite for the informed use of both insecticide classes' based-combinations for bed net impregnation and/or indoor residual spraying.

Anopheles arabiensis was the only member of *An. gambiae* complex recorded in the study area. This species is the sole malaria vector in Sudan (Petrarca *et al.*, 2000; Hamad *et al.*, 2002). Further confirmation was obtained by detection of *P. falciparum* in *An. arabiensis*. Previous studies on *Plasmodium* sporozoites infection in *An. arabiensis* were conducted using ELISA technique (Hamad *et al.,* 2002; Himeidan *et al.,* 2011b; Elmahdi *et al.,* 2012). All infected females detected here were with *P. falciparum,* the most common and widely spread malaria parasites in Sudan (Elhassan *et al.,* 1995). Nevertheless, no *Plasmodium* parasites infection was detected in concomitance with *kdr* allele mutation in *An. arabiensis* in this study. Despite epidemiological importance, the impact of insecticide resistance on vector-parasite interactions and malaria transmission is poorly understood. Few studies were conducted to assess the effect of insecticide resistance in *Plasmodium* sporozoites infection and competence of

malaria vectors (Alout *et al.,* 2012, 2014; Saddler *et al.,* 2015). Saddler *et al.* (2015) suggested that, continued use of insecticides in a population of insecticide-resistant mosquitoes could select them to be more susceptible to *Plasmodium* infection. In another study, the sensitivity to DDT was found higher in mosquitoes infected by *Plasmodium* (Alout *et al.,* 2014).

Information from knowledge, attitudes, and practices surveys are always important to design or improve malaria control programs, and to identify indicators for a program's effectiveness (Vijayakumar *et al.,* 2009). In addition, data from KAP studies can be incorporated into the decision-making processes, the design of sustainable interventions with active community participation, and the implementation of educational systems (Nieto *et al.,* 1999). Therefore, in this study surveys were carried out to assess the knowledge, attitude and practices of public health workers and farmers in selected sites in Khartoum state towards the uses of insecticides.

The resistance of the malaria vectors to most insecticides used in different sentinel sites categorized as urban and peri-urban areas in the three administrative areas of Khartoum state observed in this study and has previously been reported (Himeidan *et al.,* 2011a; Mohammed *et al.,* 2015) is not surprising. The results of KAP surveys revealed that 11 and 9 pesticides have been used during the last 5 years. Of which 5 pesticides representing three major classes have been used for both practices in the same area. The uses of a massive numbers of insecticides of different classes in agriculture against domestic pests coupled with that used in the public health practice might probably cause a development of resistance in malaria vector to most common insecticides in this state. The uses of the same pesticides for control of agricultural crops and the public health insect vectors have been previously reported in Khartoum state (Seidahmed *et al.,* 2012; Abuelmaali *et al.,* 2013). In this study, the KAP results showed that pyrethroids

are used in both practices. Likewise, in Ethiopia, Balkew *et al.* (2010) found that pyrethroids have been in use for the control of both livestock and crop pests. It is known that the pyrethroids are the insecticides used for ITNs/LLINs for personal and community-based protection against malaria vectors elsewhere (WHO, 2014). *Anopheles arabiensis,* the malaria vector in many African countries including Sudan showed resistance conferring alleles against DDT, permethrin and deltamethrin (Balkew *et al.,* 2010; Himeidan *et al.,* 2011a; Yewhalaw *et al.,* 2011; Abdalla *et al.,* 2014; Dabire´ *et al.,* 2014; Toé *et al.,* 2015). Moreover, several studies pointed out that the past and current agricultural use of DDT then pyrethroids for crop protection to have led to the selection of resistant mosquitoes through insecticide residues accumulated in breeding sites around agricultural areas (N'Guessan *et al.,* 2007; Diabaté *et al.,* 2002; Dongus *et al.,* 2009; Ranson *et al.,* 2009; Yadouleton *et al.,* 2011).

In this study, the result of the KAP study showed that the health workers have a good experience about malaria vector control. Despite good knowledge of malaria vector interventions, time of application used were poorly associated with the proper control and prevention of the disease in the state. Although, the uses of larval source management (LSM) and aerosol insecticides spray are useful for the control of malaria vectors, uses of IRS and LLINs remain the main malaria vector control recommended by the WHO (WHO, 2014). However, Elkhalifa *et al.* (2008) reported that the main control measures for malaria vector in Khartoum rely on the LSM through larvicides treatment and LSM in both settlement and agricultural area respectively. Unlike other states in Sudan, IRS and LLINs are not used for mosquito vectors control in Khartoum state (NMCP, 2014).

The results on the knowledge and related attitude and practices among both health workers and farmers on the uses of pesticides showed a relatively poor or superficial knowledge about how to prepare the working doses, check the validity

and effective dosage, and to get rid of the excess insecticides after usage. This finding might be due to moderate level of education among these respondents as shown by the results of the surveys. In addition, few numbers of these respondents had training course on the uses of insecticides. Nevertheless, there is no available information on the KAP among both health workers and farmers about the uses of pesticides in Khartoum state. A single study was previously conducted to assess the KAP among farmers in urban and periurban area in Khartoum state (Abuelmaali *et al.,* 2013). The authors found that farmers in urban areas more likely to dispose off pesticides by an approved method than in periurban areas in Khartoum state, although farmers from both areas showed a poor pesticide application practice. Almost, a better knowledge on the uses of agricultural pesticides and public health insecticides is crucial for insecticide management in malaria vectors.

In both study areas, farmers most commonly complained of mosquito bites at night at their farms where they spend the night. They answered that they received the highest mosquito bites during the rainy season where the highest mosquito density. Most of the farmers protect themselves from mosquito bites either by ITNs or by spraying agricultural pesticides for mosquito control inside the places where they sleep. Likewise, Abuelmaali *et al.,* (2013) found that majority of the farms used agricultural products for mosquito control in the home. However, the author found that the urban farmer perceived high mosquito bites during the summer season. They also, suggested that the high density of mosquito was due to the different types of larval habitats in the area such as; road puddles, pools and broken pipelines pools while in peri-urban sites were mainly irrigation canals and hoof prints.

CHAPTER SIX

6.1. CONCLUSIONS

The present study was carried out during March 2011 to February 2014, to investigate the occurrence and distribution of phenotypic and genotypic resistance strain of *An. arabiensis* in concomitance with malaria parasites infection in this species in Khartoum state, Sudan.

Anopheles arabiensis was the only member of *An. gambiae* complex in Khartoum state and it bred in two main types of larval habitat formed by drinking water pipes leakage or irrigation canals. The collection of this species from only two types of larval habitats could be due to extensive LSM activities conducted by KMFP during the last 7 years in this state.

The findings reported here also reduce the possibility of the use of most of the insecticides tested except for fenitrothion and lambdacyhalothrin. Both insecticides could be used in malaria vectors managements in Khartoum state and other regions in Sudan.

This study reports the first evidence of occurrence of L014S and ace-1^R (G119S) mutations in *An. arabiensis* in Khartoum State. The presence of combined molecular (*kdr* and G119S) and bioassay data show a wide spectrum of multiple and cross-resistance in *An. arabiensis*. The occurrence of multiple as well cross-resistance could significantly affect the malaria vector control in this state.

As exemplified in this study, infection of *An. arabiensis* with *P. falciparum* indicates that this species is the only malaria vector in Khartoum state. This finding also confirm that *P. falciparum* is the most common and widely spread malaria parasite in Sudan.

The KAP of farmers and public health workers was relatively low towards proper uses of insecticides which might threaten the activities of malaria control programme in the state.

6.2. RECOMMEDATIONS

The current study added more information on the susceptibility status, occurrence of both *kdr* and G119S as well *Plasmodium* infection in *An. arabiensis* in Khartoum state, more future studies are need to verify the following aspects:

1. Detailed species composition of anopheline mosquitoes and their larval habitats in Khartoum state.
2. Spatio-temporal variation in susceptibility status of *An. arabiensis* to all commonly used insecticides in agricultural and public health practices in Khartoum state. For example, susceptibility status of this vector to bendiocarb is crucial, as this is main insecticide used for IRS in Sudan.
3. Wide scale surveys to determine the occurrence and distribution of both type of mutation (*kdr* and G119S) in *An. arabiensis*.
4. The magnitude of malaria transmission through collection of large numbers of *An. arabiensis* specimens from different sites in this state.
5. More training courses and educations for both public health workers and farmers in Khartoum state on insecticide resistance management programmes in insect vectors.
6. More intersectoral collaboration between the Federal Ministry of Health and Ministry of agricultural to manage and monitor the growing insecticide resistance in malaria vector due to extensive use of the same pesticides in agricultural and public health practices.

REFERENCES

Abdalla, H., Matambo, T. S., Koekemoer, L. L., Hunt, R. H. and Coetzee, M. (2008). Insecticide susceptibility and vector status of natural populations of *Anopheles arabiensis* from Sudan. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **102:** 263-271.

Abdalla, H., Wilding, C. S., Nardini, L., Pignatelli, P., Koekemoer, L. L. Ranson, H. and Coetzee, M. (2014). Insecticide resistance in *Anopheles arabiensis* in Sudan: temporal trends and underlying Mechanisms. *Parasites and Vectors,* **7:** 213.

Abdalla, S. I., Malik, E. M. and Ali, M. (2007). The burden of malaria in Sudan: incidence, mortality and disability - adjusted life -years. *Malaria Journal,* **6:** 97.

Abdalla, T. M., Abdeen, M. T., Ahmed, I. S., Hamdan, H. Z., Magzoub, M. and Adam, I. (2013). Severe *Plasmodium falciparum* and *Plasmodium vivax* malaria among adults at Kassala Hospital, eastern Sudan. *Malaria Journal*, **12:** 148.

Abuelmaali, S. A., Elaagip, A. H., Basheer, M. A., Frah, E. A., Ahmed, F. T. A., Elhaj, H. F. A., Seidahmed, O. M. E., Weetman, D. and Abdel Hamid, M. M. (2013). Impacts of Agricultural Practices on Insecticide Resistance in the Malaria Vector *Anopheles arabiensis* in Khartoum State, Sudan. *PLOS ONE*, **8(11):** e80549.

Ageep, T. B., Cox, J., Hassan, M. M., Knols, B. G. J., Benedict, M. Q., Malcolm, C. A., Babiker, A. and El Sayed, B. B. (2009). Spatial and temporal distribution of the malaria mosquito *Anopheles arabiensis* in northern Sudan: influence of environmental factors and implications for vector control. *Malaria Journal,* **8:** 123.

Akogbéto, M. (2000). Lagoonal and coastal malaria at Cotonou: entomological findings. *Sante*, **10(4):** 267-275.

Aldridge, W. N. (1953). Serum esterases. I. Two types of esterase (A and B) hydrolysing p-nitrophenyl acetate, propionate and butyrate and a method for their determination. *Biochemical Journal*, **53:** 110-117.

Alout, H., Ndam, N. T., Sandeu, M. M., Dje´gbe, I., Chandre, F., Dabire´, R. K., Djogbe´nou, L. S., Corbel, V. and Cohuet, A. (2013). Insecticide Resistance Alleles Affect Vector Competence of *Anopheles gambiae*s.s. for *Plasmodium falciparum* Field Isolates. *PLOS ONE*, **8(5):** e63849.

Alout, H., Yameogo, B., Djogbénou, L. S., Chandre, F., Dabiré, R. K., Corbel, V. and Cohuet, A. (2014). Interplay Between *Plasmodium* Infection and Resistance to Insecticides in Vector Mosquitoes. *Journal of Infectious Diseases*, **210(9):** 1464-1470.

Aly, A. S. I., Vaughan, A. M. and Kappe. S. H. I. (2009). Malaria Parasite Development in the Mosquito and Infection of the Mammalian Host. *Annual Review of Microbiology*, **63:** 195-221.

Antonio-Nkondjio C, Simard F, Cohuet A, Fontenille D. (2002). Morphological variability in the malaria vector, *Anopheles moucheti*, is not indicative of speciation: evidences from sympatric south Cameroon populations. *Infection, Genetics and Evolution*, **2(1):** 69-72.

Antonio-Nkondjio, C., Fossog, B. T., Ndo, C., Djantio, B. M., Togouet, S. Z., Awono-Ambene, P., Costantini, C., Wondji, C. S. and Ranson, H. (2011). *Anopheles gambiae* Distribution and Insecticide Resistance in the Cities of Douala and Yaounde (Cameroon): Influence of Urban Agriculture and Pollution. *Malaria Journal*, **10**, 154.

Antonio-Nkondjio, C., Kerah, C. H., Simard, F., Awono-Ambene, P., Chouaibou, M., Tchuinkam, T. and Fontenille, D. (2006). Complexity of the malaria

vectorial system in Cameroon: contribution of secondary vectors to malaria transmission. *Journal of Medical Entomology*, **43:** 1215-1221.

Anyanwu, G. I., Molyneux, D. H. and Phillips, A. (2000). Variation in Cuticular Hydrocarbons among Strains of the *Anopheles gambiae sensu stricto* by Analysis of Cuticular Hydrocarbons Using Gas Liquid Chromatography of Larvae. *Memorias Institute Oswaldo Cruz*, **95(3):** 295-300.

Balkew, M., Ibrahim, M., Koekemoer, L., Brooke, B.D., Engers, H., Aseffa, A., Gebre-Michael, T. and Elhassen, I. (2010). Insecticide Resistance in *Anopheles arabiensis* (Diptera: Culicidae) from Villages in Central, Northern and South West Ethiopia and Detection of kdr Mutation. *Parasites and Vectors*, **3:** 40.

Barnes, K. I. (2005). Effect of artemether-lumefantrine policy and improved vector control on malaria burden in KwaZulu-Natal, South Africa. *PLOS Medicine*, **2:** e330.

Bass, C., Nikou, D., Donnelly, M. J., Williamson, M. S., Ranson, H., Ball, A., Vontas, J. and Field, L. M. (2007). Detection of knockdown resistance (*kdr*) mutations in *Anopheles gambiae*: a comparison of two new high-throughput assays with existing methods. *Malaria Journal*, **6:** 111.

Bayoh, M. N. and Lindsay, S. W. (2003). Effect of temperature on the development of the aquatic stages of *Anopheles gambiae* sensu stricto (Diptera: Culicidae). *Bulletin of Entomological Research,* **93:** 375-381.

Becker, N. and Ascher, K. R. S. (1998). The use of Bacillus thuringiensis subsp. israelensis (Bti) against mosquitoes, with special emphasis on the ecological impact. *Israel Journal of Entomology*, **32:** 63-69.

Bhattarai A, Ali, A. S., Kachur, S. P., Mårtensson, A., Abbas, A. K., Khatib, R., Al-Mafazy, A. W., Ramsan, M., Rotllant, G., Gerstenmaier, J. F., Molteni,

F., Abdulla, S., Montgomery, S. M., Kaneko, A. and Björkman, A. (2007). Impact of artemisinin-based combination therapy and insecticide-treated nets on malaria burden in Zanzibar. *PLOS Medicine*, **4(11):** e309.

Bøgh, C., Clarke, S. E., Pinder, M., Sanyang, F. and Lindsay, S. W. (2001). Effect of passive zooprophylaxis on malaria transmission in The Gambia. *Journal of Medical Entomology*, **38:** 822-828.

Breman, J. G., Alilio, M. S. and White. N. J. (2007). Defining and defeating the intolerable burden of malaria III. Progress and perspectives. *American Journal of Tropical Medicine and Hygiene*, **77(suppl 6):** vi-xi.

Brogdon, W. and Chan, A. (2010). Guidelines for Evaluating Insecticide Resistance in Vectors using the CDC Bottle Bioassay/ Methods in *Anopheles* research. Second edition. CDC Atlanta USA: CDC technical report, 343.

Brogdon, W. G. and McAllister J. C. (1998b). Insecticide resistance and vector control. *Emerging Infectious Diseases*, **4:** 605-613.

Brogdon, W. G. and McAllister, J. C. (1998a). Simplification of adult mosquito bioassays through use of time-mortality determinations in glass bottles. *Journal of American Mosquito Control Association*, **14(2):** 159-164.

Brown, T. M. and Brogdon, W. G. (1987). Improved detection of insecticide resistance through conventional and molecular techniques. *Annual Review of Entomology*, **32:** 145-162.

Busvine, J. (1951). Mechanism of resistance to insecticide in houseflies. *Nature*, **168:** 193-195.

Cano, J., Berzosa, P. J., Roche, J., Rubio, J. M., Moyano, E., Guerra-Neira, A., Brochero, H., Mico, M., Edu, M. and Benito, A. (2004). Malaria vectors in the Bioko Island (Equatorial Guinea): estimation of vector dynamics and transmission intensities. *Journal of Medical Entomology*, **41:** 158-161.

Carlson, D. A. and Service, M. W. (1980). Identification of mosquitoes of *Anopheles gambiae* species complex A and B by analysis of cuticular components. *Science*, **207(4435):** 1089-1091.

Casimiro, S., Coleman, M., Hemingway J. and Sharp, B. (2006). Insecticide resistance in *Anopheles arabiensis* and *Anopheles gambiae* from Mozambique. *Journal of Medical Entomology*, **43:** 276-282.

Catteruccia, F., Crisanti, A. and Wimmer, E. A. (2009). Transgenic technologies to induce sterility. *Malaria Journal*, **8(Suppl 2):** S7.

Chanda, E., Masaninga, F., Coleman, M., Sikaala, C., Katebe, C., MacDonald, M., Baboo, K. S., Govere, J. and Manga, L. (2008). Integrated vector management: The Zambian experience. *Malaria Journal*, **7:** 164.

Chandre, F., Darriet, F., Manga, L., Akogbeto, M., Faye, O., Mouchet, J. and Guillet, P. (1999). Status of Pyrethroid Resistance in *Anopheles gambiae sensu lato*. *Bulletin of World Health Organization*, **77:** 230-234.

Chareonviriyaphap, T., Roberts, D. R., Andre, R. G., Harlan, H. J., Manguin, S. and Bangs, M. J. (1997). Pesticide avoidance behavior in *Anopheles albimanus*, a malaria vector in the Americas. *Journal of American Mosquito Control Association*, **13(2):** 171-183.

Charlwood, J. D., Smith, T., Kihonda, J., Heiz. B., Billingsley, P. F. and Takken, W. (1995). Density independent feeding success of malaria vectors (Diptera: Culicidae) in Tanzania. *Bulletin of Entomological Research*, **85:** 29-35.

Chauhan, V., Negi, R. C., Verma, B. and Thakur, S (2009). Transfusion Transmitted Malaria in a Non-Endemic Area. *Journal of the Association of Physicians of India*, **57:** 653-654.

Cianchi, R., Urbanelli, S., Villani, F., Sabatini, A. and Bullini, L. (1983). Electrophoretic studies in mosquitoes: recent advances. *Parasitologia*, **27**: 157-167.

Coetzee, M. (2004). Distribution of the African malaria vectors of the *Anopheles gambiae* complex. *American Journal of Tropical Medicine and Hygiene*, **70:** 103-104.

Coetzee, M. and Fontenille, D. (2004). Advances in the study of *Anopheles funestus*, a major vector of malaria in Africa. *Insect Biochemistry and Molecular Biology*, **34:** 599-605.

Coetzee, M., Craig, M. and le Sueur, D. (2000). Distribution of African malaria mosquitoes belonging to the *Anopheles gambiae* complex. *Parasitology Today*, **16:** 74-77.

Coetzee, M., Hunt, R. H., Brack, L. E. O., Davidson, G. (1993). Distribution of mosquitoes belonging to the Anopheles gambiae complex including malaria vector, south of latitude, 15ºS. *South African Journal of Science*, **89:** 227-231.

Coetzee, M., Hut, R. H., Wilkerson, R., della Torre, A., Coulibaly, M. B. and. Besansky, N. J. (2013). *Anopheles coluzzii* and *Anopheles amharicus*, new members of the *Anopheles gambiae* complex. *Zootaxa*, **3619(3):** 246-274.

Cohuet, A., Simard, F., Wondji, C. S., Antonio-Nkondjio, C., Awono-Ambene, P. and Fontenille, D. (2004). High malaria transmission intensity due to *Anopheles funestus* (Diptera: Culicidae) in a village of savannah-forest transition area in Cameroon. *Journal of Medical Entomology*, **41:** 901-905.

Collins, F. H., and Besansky, N. J. (1994). Vector biology and the control of malaria in Africa. *Science*, **264:** 1874-1875.

Coluzzi M. (1964). Morphological divergence in the *Anopheles gambiae* complex. *Rivista di Malariologia*, **43:** 197-232.

Coluzzi, M. (1984). Heterogeneities of the malaria vectorial system in tropical Africa and their significance in malaria epidemiology and control. *Bulletin of the World Health Organization*, **62(Suppl):** 107-113.

Coluzzi, M., Sabatini, A., Petrarca, V. and Di Deco, M. A. (1979). Chromosomal differentiation and adaptation to human environments in the *Anopheles gambiae* complex. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **73:** 483-497.

Coluzzi, M. (1993).Advances in the study of Afrotropical malaria vectors. *Parassitologia*, **35(Suppl):** 23-29.

Corbett, J. R. (1974). The Biochemical Mode of Action of Pesticides, pp. 102–130. Academic Press, London, UK.

Cox-Singh, J., Davis, T. M. E., Sung, L. K., Shamsul, S. S. G., Matusop, A., Ratnam, S., Rahman, H. A., Conway, D. J. and Singh, B. (2008). *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. *Clinical Infectious Diseases*, **46:** 165-171.

Curtis, C. F. (2002). Should the use of DDT be revived for malaria vector control? *Biomédica revista del Instituto Nacional de Salud*, **22(4):** 455-461.

Dabire´, R. K., Namountougou, M., Diabate´, A., Soma, D. D., Bado, J., Toe, H. K., Bass, C. and Combary, P. (2014). Distribution and Frequency of kdr Mutations within *Anopheles gambiae* s.l. Populations and First Report of the Ace.1G119S Mutation in *Anopheles arabiensis* from Burkina Faso (West Africa). *PLOS ONE*, **9(7):** e101484.

Darriet, F., Duchon, S., Hougard, J. M. (2005). Spinosad: a new larvicide against insecticide-resistant mosquito larvae. *Journal of American Mosquito Control Association*, **21:** 495-496.

Davidson, G., Paterson, H. E., Coluzzi, M., Mason, G.F. and Micks, D.W. (1967). The *Anopheles gambiae* complex. In: Wright, J. W. & Pal, R. (Eds), Genetics of Insect Vectors of Disease. Elsevier Publishers, Amsterdam, pp. 211-250.

Davies, T. G., Field, L. M., Usherwood, P. N. and Williamson, M. S. (2007). DDT, pyrethrins, pyrethroids and insect sodium channels. *IUBMB Life*, **59(3):** 151-162.

De Meillon, B. (1936). The control of malaria in South Africa by measures directed against the adult mosquitoes in habitations, Quart. *Bulletin of World Health Organization,* **5:** 134-137.

della Torre, A., Costantini, C., Besansky, N. J., Caccone, A., Petrarca, V., Powell, J. R. and Coluzzi, M. (2002) Speciation within *Anopheles gambiae*-the glass is half full. *Science*, **298:** 115-117.

della Torre, A., Fanello, C., Akogbeto, M., Dossou-yovo, J., Favia, G., Petrarca, V. and Coluzzi, M. (2001). Molecular evidence of incipient speciation within *Anopheles gambiae* s.s. in West Africa. *Insect Molecular Biology*, **10:** 9-18.

Diabaté, A., Baldet, T., Chandre, F., Akogbeto, M., Guiguemde, T., Darriet, F., Brengues, C., Guillet, P., Hemingway, J., Small, G. J. and Hougard, J. M. (2002). The Role of Agricultural Use of Insecticides in Resistance to Pyrethroids in *Anopheles gambiae* s.l. in Burkina Faso. *American Journal of Tropical Medicine and Hygiene*, **67**, 617-622.

Diabaté, A., Brengues, C., Baldet, T., Dabiré, K. R., Hougard, J. M., Akogbeto, M., Kengne, P., Simard, F., Guillet, P., Hemingway, J. and Chandre, F. (2004). The spread of the Leu-Phe *kdr* mutation through *Anopheles gambiae* complex in Burkina Faso: genetic introgression and *de novo* phenomena. *Tropical Medicine and International Health*, **9:** 1267-1273.

Diatta, M., Spiegel, A., Lochouarn, L. and Fontenille, D. (1998). Similar feeding preferences of *Anopheles gambiae* and *A. arabiensis* in Senegal. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **92:** 270-272.

Ding, Y., Ortelli, F., Rossiter, L.C., Hemingway, J., Ranson, H. (2003). The *Anopheles gambiae* glutathione transferase supergene family: annotation, phylogeny and expression profiles. *BMC Genomics* 4: 35-50.

Djegbe, I., Boussari, O., Sidick, A., Martin, T., Ranson, H., Chandre, F., Akogbeto, M. and Corbel, V. (2011). Dynamics of insecticide resistance in malaria vectors in Benin: first evidence of the presence of L1014S *kdr* mutation in *Anopheles gambiae* from West Africa. *Malaria Journal*, **10(1):** 261.

Djogbenou, L., Dabire, R., Diabate, A., Kengne, P., Akogbeto, M., Hougard, J. M. and Chandre, F. (2008). Identification and geographic distribution of the ACE-1R mutation in the malaria vector *Anopheles gambiae* in south-western Burkina Faso, West Africa. *American Journal of Tropical Medicine and Hygiene*, **78(2):** 298-302.

Dongus, S., Nyika, D., Kannady, K., Mtasiwa, D., Mshinda, H., Gosoniu, L., Drescher, A. W., Fillinger, U. Tanner, M., Killeen, G. F. and Castro, M. C. (2009). Urban Agriculture and *Anopheles* Habitats in Dar es Salaam, Tanzania. *Geospatial Health*, **3**: 189-210.

Du, W., Awolola, T. S., Howell, P., Koekemoer, L. L., Brooke, B. D., Benedict, M. Q., Coetzee, M. and Zheng, L. (2005). Independent mutations in the Rdl locus confer dieldrin resistance to *Anopheles gambiae* and *An. arabiensis*. *Insect Molecular Biology*, **14(2):** 179-183.

Dukeen, M. Y. and Omer, S. M. (1986). Ecology of malaria vector Anopheles arabiensis Patton (Diptera: Culicidae) by the Nile of northern Sudan. *Bulletin of Entomological Research*, **76:** 451-467.

Dyck, V. A., Hendrichs, J. and Robinson, A. S. (2005). The Sterile Insect Technique: Principles and practice in area-wide integrated pest management. Springer Publishers, Dordrecht.

El Gadal, A. A., Haridi, A. M., Hassan, F. T. and Hussein, H. (1985). Malaria Control in the Gezira- Managil Irrigated Scheme of the Sudan. *The Journal of Tropical Medicine and Hygiene*, **88:** 153-159.

El Sayed, B. B., Arnot, D., Mukhtar, M., Baraka, O., Dafalla, A., Elnaiem, D. and Nugud, A. (2000). Astudy of urban malaria transmission problem in Khartoum. *Acta Tropica*, **75:** 163-171.

El Sayed, B. B., Malcolm, C. A., Babiker, A., Malik, E. M., El Tayeb, M. A. H., Nageeb, S., Nugud, A. D. and Knols, B. G. J. (2009). Ethical, legal and social aspects of the approach in Sudan. *Malaria Journal*, **8(Suppl 2):** S3.

Elhassan, I. M., Hviid, L., Jakobsen, P. H., Giha, H., Satti, G. M. H., Arnot, D. E., Jensen, J. B. and Theander, T. G. (1995). High Proportion of Subclinical *Plasmodium falciparum* Infections in an Area of Seasonal and Unstable Malaria in Sudan. *American Journal of Tropical Medicine and Hygiene*, **53:** 78-83.

Elkhalifa, S. M., Mustafa, I. O., Wais, M., Malik, E. M. (2008). Malaria control in an urban area: a success story from Khartoum, 1995-2004. *East Mediterranean Health Journal*, **14:** 206-215.

Elmahdi, Z. A., Nugud, A. A. and Elhassan, I. M. (2012). Estimation of malaria transmission intensity in Sennar state, central Sudan. *East Mediterranean Health Journal*, **18(9):** 951-956.

Elmardi, K. A., Noor, A. M., Githinji, S., Abdelgadir, T. M., Malik, E. M. and Snow R. W. (2011). Self-reported fever, treatment actions and malaria infection prevalence in the northern states of Sudan. *Malaria Journal*, **10:** 128.

El-Rayah, E. (2007). Mosquitoes of Sudan. *Sudan Notes and Records*, **6:** 153-187.

Enayati, A, A, Ranson, H. and Hemingway, J. (2005). Insect glutathione-S-transferases and insecticide resistance. *Insect Molecular Biology*, **14(1):** 3-8.

Enayati, A. and Hemingway, J. (2010). Malaria management: past, present, and future. *Annual Review of Entomology*, **55:** 569-591.

Etang, J., Manga, L., Chandre, F., Guillet, P., Fondjo, E., Mimpfoundi, R., Toto, J. C. and Fontenille, D. (2003). Insecticide susceptibility status of *Anopheles gambiae* s.l. (Diptera: Culicidae) in the Republic of Cameroon. *Journal of Medical Entomology*, **40:** 491-497.

Fanello, C., Santolamazza, F. and della Torre, A. (2002) Simultaneous identification of species and molecular forms of the *Anopheles gambiae* complex by PCR-RFLP. *Medical and Veterinary Entomology*, **16:** 461-464.

Farenhorst, M., Farina, D., Scholte, E-J, Takken, W., Hunt, R. H., Coetzee, M. and Knols, B. G. J. (2008) African water storage pots for the delivery of the entomopathogenic fungus *Metarhizium anisopliae* to the malaria vectors *Anopheles gambiae* s.s. and *Anopheles funestus*. *American Journal of Tropical Medicine and Hygiene*, **78:** 910-916.

Favia, G., Della Torre, A., Bagyaoko, M., Lanfrancotti, A., Sagnon, N. 'F., Toure, Y. T. and Coluzzi, M. (1997). Molecular identifications of sympatric chromosomal forms of *Anopheles gambiae* and further evidence of their reproductive isolation. *Insect Molecular Biology*, **6:** 377-383.

Feachem, R. and Sabot, O. (2008). A new global malaria eradication strategy. *The Lancet*, **371:** 1633-1635.

Ferreira, P. E., Culleton, R., Gil, J. P. and Meshnick, S. R. (2013). Artemisinin resistance in *Plasmodium falciparum:* what is it really? *Trends in Parasitology*, **29(7):** 318-320.

Fillinger, U, Sonye, G., Killeen, G., Knols, B. G. J. and Becker, N. (2004). The practical importance of permanent and semipermanent habitats for controlling aquatic stages of *Anopheles gambiae* sensu lato mosquitoes: operational observations from a rural town in western Kenya. *Tropical Medicine and International Health*, **9:** 1274-1289.

Fillinger, U. and Lindsay, S.W. (2006) Suppression of exposure to malaria vectors by an order of magnitude using microbial larvicides in rural Kenya. *Tropical Medicine and International Health*, **11:** 1629-1642.

Fillinger, U., Knols, B. G. J. and Becker, N. (2003). Efficacy and efficiency of new *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* formulations against Afrotropical anophelines in Western Kenya. *Tropical Medicine and International Health*, **8:** 37-47.

Fillinger, U., Sombroek, H., Majambere, S., van Loon, E., Willem Takken, W. and Lindsay, S. W. (2009). Identifying the most productive breeding sites for malaria mosquitoes in The Gambia. *Malaria Journal*, **8:** 62.

Fontenille, D. and Carnevale P. (2006). Malaria and dengue vector biology and control in West and Central Africa, pp. 91-99. In B. G. J. Knols and C. Louis (eds.), Bridging Laboratory and Field Research for Genetic Control of Disease Vectors. Frontis, Wageningen.

Fournet, F., Cussac, M., Ouari, A., Meyer, P-E., Toé, H. K., Gouagna, L. C., Dabiré, R. K. (2010). Diversity in anopheline larval habitats and adult composition during the dry and wet seasons in Ouagadougou (Burkina Faso). *Malaria Journal*, **9:** 78.

Franz, A. W. E., Sanchez-Vargas, I., Adelman, Z. N., Blair, C. D., Beaty, B. J., James, A. A. and Olson, K. E. (2006). Engineering RNA interference-based resistance to dengue virus type 2 in genetically modified *Aedes aegypti*. *Proceedings of the National Academy of Sciences of the United States of America*, **103:** 4198-4203.

Gadalla, N. B., Abdallah, T. M., Atwal, S., Sutherland, C. J, Adam. I. (2013). Selection of pfdhfr/pfdhps alleles and declining artesunate/sulphadoxine-pyrimethamine efficacy against *Plasmodium falciparum* eight years after deployment in eastern Sudan. *Malaria Journal*, **12:** 255.

Gadalla, N. B., Elzaki, S. E., Mukhtar, E., Warhurst, D. C., El-Sayed, B. and Sutherland, C. J. (2010). Dynamics of pfcrt alleles CVMNK and CVIET in chloroquine-treated Sudanese patients infected with *Plasmodium falciparum*. *Malaria Journal*, **9:** 74.

Gale, K. R. and Crampton, J. M. (1987). DNA probes for species identification of mosquitoes in the *Anopheles gambiae* complex. *Medical and Veterinary Entomology*, **l:** r27-136.

Garros, C., Harbach, R. E. and Manguin, S. (2005). Morphological assessment and molecular phylogenetics of the Funestus and Minimus groups of *Anopheles* (Cellia). *Journal of Medical Entomology*, **42:** 522-536.

Gentile, G., Slotman, M., Ketmaier, V., Powell, J. R. and Caccone, A. (2001) Attempts to molecularly distinguish cryptic taxa in *Anopheles gambiae* s.s. *Insect Molecular Biology*, **10:** 25-32.

Gillies, M. T. (1955). Studies in house leaving and outside resting of Anopheles gambiae Giles and Anopheles funestus Giles in East Africa. Part II. The exodus from houses and the house resting population. *Bulletin of Entomological Research*, **45:** 375-387.

Gillies, M. T. (1988). Anopheline mosquitoes: vector behaviour and bionomics. *In* W. H. Wernsdorfer, and I. Sir McGregor (eds.), Malaria. Principles and Practice of Malariology. Churchill Livingstone, Edinburgh.

Gillies, M. T. and Coetzee, M. (1987). A Supplement to the Anophelinae of Africa South of the Sahara (Afrotropical Region). *Publications of the South African Institute for medical Research*, no. 55.

Gillies, M. T. and De Meillon, B. (1968). The Anophelinae of Africa South of the Sahara. *Publications of the South African Institute for medical Research*, no. 54.

Gnanguenon, V., Agossa, F. R., Badirou, K., Renaud Govoetchan, R., Anagonou, R., Oke-Agbo, F., Azondekon, R., Youssouf, R. A., Attolou, R., Tokponnon, F. T., Aïkpon, R., Ossè, R., and Akogbeto, M. C. (2015). Malaria vectors resistance to insecticides in Benin: current trends and mechanisms involved. *Parasites and Vectors*, **8:** 223.

Goma, L. K. H. (1966): The mosquito Hutchinson & Co (Publisher) Ltd, London, 144pp.

Gone, T. Balkew, M. and Teshome G Michael, T. G. (2014). Comparative entomological study on ecology and behaviour of *Anopheles* mosquitoes in highland and lowland localities of Derashe District, southern Ethiopia. *Parasites and Vectors*, **7**: 483.

Govella, N. J., Okumu, F. O. and Killeen, G. F. (2010). Insecticide-treated nets can reduce malaria transmission by mosquitoes which feed outdoors. *American Journal of Tropical Medicine and Hygiene*, **82:** 415-419.

Greenwood B. M., Fidock D. A., Kyle D. E., Kappe S. H. I., Alonso P. L., Collins F. H. and Duffy P. E. (2008). Malaria: progress, perils, and prospects for eradication. *Journal of Clinical Investigation*, **118(4):** 1266-1276.

Guelbeogo, W. M., Sagnon, N., Liu, F., Besansky, N. J. and Carlo Costantini, C. (2014). Behavioural divergence of sympatric *Anopheles funestus* populations in Burkina Faso. *Malaria Journal*, **13:** 65.

Gupta, I. and Guin, P. (2010). Communicable diseases in the South-East Asia Region of the World Health Organization: towards a more effective response. *Bulletin of the World Health Organization*, **88:** 199-205.

Hamad, A. A., Nugud, A. H., Arnot, D. E., Giha, H. A., Abdel-Muhsin, A. M., Satti, G. M., Theander, T. G., Creasey, A. M., Babiker, H. A. and Elnaiem, D. E. (2002). A marked seasonality of malaria transmission in two rural sites in eastern Sudan. *Acta Tropica*, **831:** 71-82.

Harbach, R. E. (1988). The Mosquitoes of the Subgenus *Culex* in Southwestern Asia and Egypt (Diptera: Culicidae). *Contributions of the American Entomological Institute*, **24(1):** 1-101.

Harbach, R. E., Townson, H., Mukwaya, L. G. and Adeniran, T. (1997). Use of rDNA-PCR to investigate the ecological distribution of *Anophelesbwambae* in relation to other members of the *An. gambiae* complex of mosquitoes in Bwamba County. *Uganda Medical and Veterinary Entomology*, **11:** 329-334.

Hargreaves, K., Hunt, R. H., Brooke, B. D., Mthembu, J., Weeto, M. M., Awolola, T. S. and Coetzee, M. (2003). *Anopheles arabiensis* and *An. quadriannulatus* resistance to DDT in South Africa. *Medical and Veterinary Entomology*, **17:** 417-722.

Hargreaves, K., Koekemoer, L. L., Brooke, B. D., Hunt, R. H., Mthembu, J. and Coetzee, M. (2000). *Anopheles funestus* resistant to pyrethroid insecticides in South Africa. *Medical and Veterinary Entomology*, **14:** 181-189.

Haridi, A. (1972b). Inheritance of DDT resistance in species A and B of the *Anopheles gambi*ae complex. *Bulletin of World Health Organization.* **47:** 619-626.

Haridi, A. M. (1972a). Partial exophily of *Anopheles gambiae* species *B* in the Khashm Elgirba area in eastern Sudan. *Bulletin of World Health Organization*, **46:** 39-46.

Hassan, M. M., Alhag, H, M., Basheer, M. A., Elhaj, H-E. F. and El Sayed, B. B. (2014). Swarming and mating behaviour of male *Anopheles arabiensis* Patton (Diptera: Culicidae) in an area of the Sterile Insect Technique Project in Dongola, northern Sudan. *Acta Tropica,* **32(Suppl):** S64-S69.

Hastings, I. M., Bray, P. G. and Ward, S. A. (2002). A requiem for chloroquine. *Science,* **298:** 74-75.

Helinski, M. E. H., Parker, A. G. and Knols, B. G. J. (2006). Radiation-induced sterility for pupal and adult stages of the malaria mosquito *Anopheles arabiensis*. *Malaria Journal*, **5:** 41.

Hemingway, J. (1983). The genetics of malathion resistance in *Anopheles stephensi* from Pakistan. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **77(1):** 106-108.

Hemingway, J. (2000). Mini review: The molecular basis of two contrasting metabolic mechanisms of insecticide resistance. *Insect Biochemistry and Molecular Biology*, **30:** 1009-1015.

Hemingway, J. and Ranson, H. (2000). Insecticide resistance in insect vectors of human disease. *Annual Review of Entomology*, **45:** 371-391.

Hemingway, J., Hawkes, N. J., McCarroll, L. and Ranson, H. (2004). The molecular basis of insecticide resistance in mosquitoes. *Biochemistry and Molecular Biology*, **34:** 653-665.

Hemingway, J., Penilla, R. P., Rodriguez, A. D., James, B. M., Edge, W., Rogers, H. and Rodriguez, M. H. (1997). Resistance management strategies in malaria vector mosquito control. A large scale field trial in Southern Mexico. *Pesticide Science*, **51(3):** 375-382.

Herrera-Varela, M., Lindh, J., Lindsay, S. W. and Fillinger, U. (2014). Habitat discrimination by gravid *Anopheles gambiae* sensu lato - a push -pull system. *Malaria Journal*, **13:**133.

Himeidan, Y. E., Abdel Hamid, M. M., Jones, C. J. and Ranson, H. (2011a). Extensive permethrin and DDT resistance in *Anopheles arabiensis* from eastern and central Sudan. *Parasites & Vectors*, **4:** 154.

Himeidan, Y. E., Dukeen, M. Y., El-Rayah, E. and Adam, I. (2004). *Anopheles arabiensis*: abundance and insecticide resistance in an irrigated area of eastern Sudan. *East Mediterranean Health Journal*, **13(1):** 17-24.

Himeidan, Y. E., Elzaki, M. M., Kweka, E. J., Ibrahim, M. and Elhassan, I. M. (2011b). Pattern of malaria transmission along the Rahad River basin, eastern Sudan. *Parasites and Vectors*, **4:** 109.

Himeidan, Y. E., Chen, H., Chandre, F., Donnelly, M. J. and Yan, G. (2007). Short report: permethrin and DDT resistance in the malaria vector *Anopheles arabiensis* from eastern Sudan. *American Journal of Tropical Medicine and Hygiene*, **77(6):** 1066-1068.

http://www.tufts.edu/~mcourt01/Documents/Court%20lab%20%20HW%20calculator.xls

Hunt, R. H., Coetzee, M. and Fettene, M. (1998). The *Anopheles gambiae* complex: a new species from Ethiopia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **92:** 231-235.

Ismail, S. M., Nugud, A. D., Jamal, A. E., Abdalmagid, M. A., Bashir, A. I. and Toto, H. K. (2012). Insecticide Resistance in *Anopheles arabiensis* Patton in White Nile State during the dry season. *Sudanese Journal of Public Health*, **7(4):** 136-141.

Jiang, N., Chang, Q., Sun, X., Lu, H., Yin, J., Zhang, Z., et al. (2010). Co-infections with *Plasmodium knowlesi* and other malaria parasites, Myanmar. *Emerging and Infectious Diseases*, **16:**1476-1478.

Jones, C. M., Toé, H. K., Sanou, A., Namountougou, M., Hughes, A., Diabaté, A., Dabiré, R., Simard, F. and Ranson, H. (2012). Additional selection for insecticide resistance in urban malaria vectors: DDT resistance in *Anopheles arabiensis* from Bobo-Dioulasso, Burkina Faso. *PLOS ONE*, **7(9):** e45995.

Kamareddine, L. (2012). The Biological Control of the Malaria Vector. *Toxins*, **4:** 748-767.

Kaya, H. K. and Gaugler, R. (1993). Entomopathogenic nematodes. *Annual Review of Entomology*, **38:** 181-206.

Killeen, G. F., Fillinger, U. and Knols, B. G. J. (2002). Advantages of larval control for African malaria vectors: Low mobility and behavioural responsiveness of immature mosquito stages allow high effective coverage. *Malaria Journal*, **1:** 8.

Killeen, G. F., McKenzie, F. E., Foy, B. D., Bogh, C. and Beier, J. C. (2001). The availability of potential hosts as a determinant of feeding behaviours and malaria transmission by mosquito populations. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **95:** 469-476.

Kirby, M. J. and Lindsay, S. W. (2004). Responses of adult mosquitoes of two sibling species, *Anopheles arabiensis* and *Anopheles gambiae* s.s. (Diptera: Culicidae) to high temperatures. *Bulletin of Entomological Research,* **94:** 441-448.

Knipling, E. F., Laven, H. Craig, G. B., Pal, R., Kitzmiller, J. B., Smith, C. N. and Brown, A. W. (1968). Genetic control of insects of public health importance. *Bulletin of the World Health Organization*, **38:** 421-438.

Koenraadt, C. J. M. and Takken, W. (2003). Cannibalism and predation among larvae of the *Anopheles gambiae* complex. *Medical and Veterinary Entomology*, **17:** 61-66.

Kulkarni, M. A., Rowland, M., Alifrangis, M., Mosha, F. W., Matowo, J., Malima, R., Peter, J., Kweka, E., Lyimo, I., Magesa, S., Salanti, A., Rau, M. E. and Drakeley, C. (2006). Occurrence of the Leucine-to-Phenylalanine Knockdown Resistance (*kdr*) Mutation in *Anopheles arabiensis* Populations in Tanzania, Detected by a Simplified High-Throughput SSOP ELISA Method. *Malaria Journal*, **5:** 56.

Lanzaro, G. C., Narang, S. K. and Seawright, J. A. (1990). Speciation in an anopheline (Diptera: Culicidae) mosquito: enzyme polymorphism and the

genetic structure of populations. *Annals of the Entomological Society of America*, **83:** 578-585.

Legner, E. F. (1995). Biological control of Diptera of medical and veterinary importance. *Journal of Vector Ecology*, **20:** 59-120.

Lewis, D. J. (1956). The anopheline mosquitoes of the Sudan. *Bulletin of Entomological Research*, **47:** 475-494.

Lewis, D. J. (1958). Some mosquitoes of the Blue Nile valley in the Republic of the Sudan. *Bulletin of the World Health Organization*, **49:** 133-155.

Lindblade, K. A., Gimnig, J. E., Kamau, L., Hawley, W. A., Odhiambo, F., Olang, G., ter Kuile, F. O., Vulule, J. M. and Slutskera, L. (2006). Impact of sustained use of insecticidetreated bednets on malaria vector species distribution and Culicine mosquitoes. *Journal of Medical Entomology*, **43:** 428-432.

Lindsay, S. W., Parson, L. and Thomas, C. J. (1998). Mapping the ranges and relative abundance of the two principal African malaria vectors, *Anopheles gambiae* sensu stricto and *An. arabiensis*, using climate data. *Proceedings of the Royal Society B: Biological Sciences*, **265:** 847-854.

Lindsay, S., Kirby, M., Baris, E. and Bos, R. (2004). Environmental management for malaria control in the East Asia and Pacific (EAP) region. The International Bank for Reconstruction and Development. The World Bank, NW Washington, DC 20433.

Lines, J. D. (1988). Do agricultural insecticides select for insecticide resistance in mosquitoes? A look at the evidence. *Parasitology Today*, **4:** S17-S20.

Lines, J. D., Lyimo, E. O. and Curtis, C. F. (1986). Mixing of indoor and outdoor-resting adults of *Anopheles gambiae* and *Anopheles funestus* in coastal Tanzania. *Bulletin of Entomological Research*, **76:** 171-178.

Livak, K. J. (1984). Organization and mapping of a sequence on the *Drosophila melanogaster* X and Y chromosomes that is transcribed during spermatogenesis. *Genetics*, **107:** 611-634.

Lynd, A., Ranson, H., MaCall, P. J., Randle, P. N. Black IV, C. M., Walker, D. E. and Donnelly, M.J. (2005). A simplified high-throughput method for pyrethroid knock-down resistance (kdr) detection in *Anopheles gambiae*. *Malaria Journal*, **4:** 16.

Magesa, S. M., Lengeler, C., Desavigny, D., Miller, J. E., Njau, R. J., Kramer, K., Kitua, A. and Mwita, A. (2005). Creating an 'Enabling Environment' fortaking insecticide-treated nets to national scale: the Tanzanian experience. *Malaria Journal*, **4:** 34.

Maharaj, R., Mthembu, D. J. and Sharp, B. L. (2005). Impact of DDT reintroduction on malaria transmission in KwaZulu Natal. *South African Medical Journal*, **95:** 871-874.

Mahgoub, H., Gasim, G. I., Musa, I. R. and Adam, I. (2012). Severe *Plasmodium vivax* malaria among Sudanese children at New Halfa Hospital, Eastern Sudan. *Parasites and Vectors*, **5:** 154.

Mahon, R. J., Green, C. A. and Hunt, R. H. (1976). Diagnostic allozymes for routine identification of adults of the *Anopheles gambiae* complex (Diptera: Culicidae). *Bulletin of Entomological Research*, **66:** 25-31.

Majambere, S., Fillinger, U., Sayer, D. R., Green, C., Lindsay, S. W. (2008). Spatial Distribution of Mosquito Larvae and the Potential for Targeted Larval Control in The Gambia. *American Journal of Tropical Medicine and Hygiene*, **79(1):**19-27.

Majambere, S., Pinder, M., Fillinger, U., Ameh, D., Conway, D. J., Green, C., Jeffries, D., Jawara, M., Milligan, P. J., Hutchinson, R. and Lindsay, S. W. (2010). Is mosquito larval source management appropriate for reducing malaria

in areas of extensive flooding in the Gambia? A cross-over intervention trial. *American Journal of Tropical Medicine and Hygiene*, **82:** 176-184.

Malcolm, C. A., El Sayed, B., Babiker, A., Girod, R., Fontenille, D., Knols, B. G. J., Nugud, A. H. and Benedict, M. Q. (2009). Field site selection: getting it right first time around. *Malaria Journal*, **8(Suppl 2):** S7.

Malik, E. M. and Khalafalla, O. (2006). Malaria in Sudan: past, present and the future, *Gezira Journal of Health Sciences*, **1:** 48-49.

Malik, E. M., Ahmed, E. S., Elkhalifa, S. M., Hussein, M. A. and Sulieman A. M. (2003). Stratification of Khartoum urban area by the risk of malaria transmission. *East Mediterranean Health Journal*, **9:** 559-569.

Manda, H., Gouagna, L. C., Foster, W. A., Jackson, R. R., Beier, J. C., Githure, J. I. and Hassanali, A. (2006). Effect of discriminative plant-sugar feeding on the survival and fecundity of *Anopheles gambiae*. *Malaria Journal*, **6:** 113.

Mandavilli, A. (2006). Health agency backs use of DDT against malaria. *Nature*, **443:** 250-251.

Marks, F., Evans, J., Meyer, C. G., Browne, E. N., Flessner, C., Kalckreuth, V., Eggelte, T. A., Horstmann, R. D. and May, J. (2005). High prevalence of markers for Sulfadoxine and Pyrimethamine resistance in *Plasmodium falciparum* in the absence of drug pressure in the ashanti region of Ghana. *Antimicrobial Agents and Chemotherapy*, **49:** 1101-1105.

Martinez-Torres, D., Chandre, F., Williamson, M. S., Darriet, F., Berge, J. B., Devonshire, A. L., Guillet, P., Pasteur, N. and Pauron, D. (1998). Molecular Characterization of Pyrethroid Knockdown Resistance (*kdr*) in the Major Malaria Vector *Anopheles gambiae* s.s. *Insect Molecular Biology*, **7:** 179-184.

Matambo, T. S., Brooke, B. D., Koekemoer, L. L., Hunt, R. H. and Coetzee, M. (2007). Insecticide resistance in *Anopheles arabiensis* and association with the *kdr* mutation. *Medical and Veterinary Entomology*, **21:** 97-102.

Mathanga, D. P., Campbell, C. H., Taylor, T. E., Barlow, R. and Wilson, M. L. (2005). Reduction of childhood malaria by social marketing of insecticide-treated nets: a case control study of effectiveness in Malawi. *American Journal of Tropical Medicine and Hygiene*, **73:** 622-625.

Mattingly, P. F. and Kinght, K. L. (1956). The mosquitoes of Arabia. *Entomology*, **4:** 91 -141.

Mayr, E. (1942). Systematics and the origin of species. New York, Columbia University Press.

Miles, S. I. (1978). Enzyme variation in the *Anopheles gambiae* Giles group of species (Diptera: Culicidae). *Bulletin of Entomological Research*, **68(1):**85-97.

Miller, L. H. and Greenwood, B. (2002). Malaria-a shadow over Africa. *Science*, **298:** 121-122.

Miller, T. A. and Saldago, V. L. (1985). The mode of action of pyrethroids in insects. In: *The pyrethroid insecticides* (Eds) Leahey, J. P., Tayor and Francis. London and Philadelphia pp 439.

Minakawa, N., Omukunda, E., Zhou, G., Githeko, A. and Yan, G. (2006). Malaria vector productivity in relation to the highland environment in Kenya. *American Journal of Tropical Medicine and Hygiene*, **75(3):** 448-453.

Mohammed, T. S. A., Ahmed, K. A., Zain, H. M., Abdelateef, A. G., Gibreel Y. A. S., Abdalmajed, M. A., Bashir, I. A., Kafy, H. T., Nasir, A. A. and Hassan, M. M. (2015). Susceptibility Status of the Malaria Vector *Anopheles arabiensis* to Insecticides in Khartoum State, Sudan. *Sudan Journal of Medical Science*, **10(2):** 39 - 52.

Molina, R., Benito, A., Blanca, F., Roche, J., Otunga, B. and Alvar, J. (1996). The anophelines of Equatorial Guinea: Ethology and susceptibility studies. *Research and Review of Parasitology*, **56:** 105-110.

Msellem, M. I., Mårtensson, A., Rotllant, G., Bhattarai, A., Strömberg, J., Kahigwa, E., Garcia, M., Petzold, M., Olumese, P., Ali, A. and Björkman, A. (2009). Influence of rapid malaria diagnostic tests on treatment and health outcome in fever patients, Zanzibar: a crossover validation study. *PLOS Medicine*, **6(4):**e1000070.

Muirhead-Thomson, R. C. (1948). Studies on *Anopheles gambiae* and *A. melas* in and around Lagos. *Bulletin of Entomological Research*, **38:** 527-558.

Mukhtar, E. A., Gadalla, N. B., El-Zaki, S. E., Mukhtar, I., Mansour, F. A., Babiker, A. and El-Sayed, B. B. (2007). A comparative study on the efficacy of artesunate plus sulphadoxine/pyrimethamine versus artemether-lumefantrine in eastern Sudan. *Malaria Journal*, **6:** 92.

Murray, C. J., Rosenfeld, L. C., Lim, S. S., Andrews, K. G., Foreman, K. J., Haring, D., Fullman, N., Naghavi, M., Lozano, R., Lopez, A. D. (2012). Global malaria mortality between 1980 and 2010: a systematic analysis. *Lancet*, **379:** 413-431.

Mutabingwa, T. K. (2005). Artemisinin-based combination therapies (ACTs): Best hope for malaria treatment but inaccessible to the needy! *Acta Tropica*, **95:** 305-315.

Mutuku, F. M., Bayoh, M. N., Gimnig, J. E., Vulule, J. M., Kamau, L., Walker, E. D., Kabiru, E. and Hawley, W. A. (2006). Pupal habitat productivity of *Anopheles gambiae* complex mosquitoes in a rural village in western Kenya. *American Journal of Tropical Medicine and Hygiene*, **74:** 54-61.

N'Guessan, R., Corbel, V., Akogbeto, M. and Rowland, M. (2007). Reduced Efficacy of Insecticide-Treated Nets and Indoor Residual Spraying for Malaria Control in Pyrethroid Resistance Area, Benin. *Emerging Infectious Diseases*, **13:** 199-206.

Najera J. A. and Zaim, M. (2003). Malaria vector control, decision making criteria and procedures for judicious use of insecticides. Geneva: World Health Organization 2002. WHO/CDS/WHOPES/2002.5, Rev.1.

Namountougou, M. Diabate, A., Etang, J., Bass, C., Sawadogo, S. P., Gnankinié, O., Baldet, T., Martin, T., Chandre, F., Simard, F. and Dabiré, R. K. (2013). First report of the L1014S kdr mutation in wild populations of *Anopheles gambiae* M and S molecular forms in Burkina Faso (West Africa). *Acta Tropica*, **125:** 123-127.

Narahashi, T. (1992). Nerve membrane Na^+ channels as targets of insecticides. *Trends in Pharmacology Science*, **13:** 236-241.

Nardini, L., Christian, R. N., Coetzer, N. and Koekemoer, L. L. (2013). DDT and pyrethroid resistance in *Anopheles arabiensis* from South Africa. *Parasites and Vectors*, **6(1):** 229.

Nasci, R. S. and Miller, B. R. (1996). Culicine mosquitoes and the agents they transmit. In: The biology of disease vectors. Niwot, CO: University Press of Colorado 85-97 Beaty, B. J. Marquardt, WC. editors.

Nauen, R. (2006). Insecticide resistance. *Bayer Environmental Science Journal*, **18:** 10-15.

Ndjemai, H. N., Patchoke, S., Atangana, J., Etang, J., Simard, F., Bilong, C. F., Reimer, L., Cornel, A., Lanzaro, G. C. and Fondjo, E. (2009). The distribution of insecticide resistance in *Anopheles gambiae* s.l. populations from Cameroon: an update. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **103:** 1127-1138.

Newman, R. D., Hailemariam, A., Jimma, D., Degifie, A., Kebede, D., Rietveld, A. E., Nahlen, B. L., Barnwell, J. W., Steketee, R. W. and Parise, M. E. (2003). Burden of malaria during pregnancy in areas of stable and unstable transmission

in Ethiopia during a nonepidemic year. *Journal of Infectious Diseases*, **187:** 1765-1772.

Nieto, T., Mendez, F. and Carrasquilla, G. (1999). Knowledge, beliefs and practices relevant for malaria control in an endemic urban area of the Colombian Pacific. *Social Science and Medicine*, **49(5):** 601-609.

Nkya, T. E., Akhouayri, I., Kisinza, W. and David, J. P. (2013). Impact of environment on mosquito response to pyrethroid insecticides: Facts, evidences and prospects. *Insect Biochemistry and Molecular Biology*, **43(4):** 407-416.

Nkya, T.E., Akhouayri, I., Poupardin, R., Batengana, B., Mosha, F., Magesa, S., Kisinza, W. and David, J. P. (2014). Insecticide Resistance Mechanisms Associated with Different Environments in the Malaria Vector *Anopheles gambiae*: A Case Study in Tanzania. *Malaria Journal*, **13:** 28.

NMCP, (2007). National Malaria Control Programme, Sudan Report on: National Strategic Plan for RBM (2007-2012).

NMCP, (2010). National Malaria Control Programme, Sudan Report on: Five years Strategic Plan for the National (2011-2015).

NMCP, (2014). National Malaria Control Programme, Sudan Report on: National Strategic Plan for RBM (2104-2016).

Noor, A. M., Mutheu, J. J., Tatem, A. J., Hay, S. I. and Snow, R. W. (2009). Insecticide-treated net coverage in Africa: mapping progress in 2000-07. *Lancet*, **373:** 58-67.

Norris, D. E. (2002). Genetic markers for study of the anopheline vectors of human malaria. *International Journal for Parasitology*, **32:** 1607-1615.

Nourein, A. B. Abass, M. A., Nugud, A. D., El Hassan, I., Snow, R. W. and Noor. A. M. (2011). Identifying Residual Foci of *Plasmodium falciparum* Infections for Malaria Elimination: The Urban Context of Khartoum, Sudan. *PLOS ONE*, **6(2):** e16948.

Nugud, A. D. and Elsayed, B. B. (2001). Malaria and mosquito in Sudan unpublished report.

Nugud, A. D., ELtayeb, R. A. and Abd-EL Nur, O. M. (1997). Vectors of malaria in Sudan. Jount workshop on scientific cooperation, the Federal Ministry of Agricultural, and Forestry, 1997. Sudan, ICIPE, Kenya, Khartoum 6-7 Dec. 1997.

Obala, A. A., Kutima, H. L., Nyamogoba, H. D. N., Mwangi, A. W., Simiyu, C. J., Magak, G. N., Khwa-Otsyula, B. O. and Ouma, J. H. (2012). *Anopheles gambiae* and *Anopheles arabiensis* population densities and infectivity in Kopere village, Western Kenya**.** *The Journal Infection Developing Countries*, **6(8):** 637-643.

Paaijmans, K. P. and Thomas, M. B. (2011). The influence of mosquito resting behaviour and associated microclimate for malaria risk. *Malaria Journal*, **10**:183.

Paskewitz, S. M. and Collins, F. H. (1990). Use of the polymerase chain reaction to identify mosquito species of the *Anopheles gambiae* complex. *Medical and Veterinary Entomology*, **4:** 367-373.

Pasteur, N., Pasteur, G., Bonhomme, F., Catalan, J. and Britton-Davidian, J. (1988). Practical isozyme genetics. Ellis Horwood Ltd., Chichester, UK.

Paterson, H. E. (1962). On the status of the East African salt-water breeding variant of *Anopheles gambiae* Giles. Natzcre (Land.). 195. 469.

Pates, H. V., Takken, W., Curtis, C. F. and Jamet, H. (2006). Zoophilic *Anopheles quadriannulatus* species B found in a human habitation in Ethiopia. *Annals of Tropical Medicine and Parasitology*, **100:** 177-179.

Petrarca, V., Nugud, A. D., Ahmed, M. A., Haridi, A. M., Di Deco, M. A. and Coluzzi, M. (2000). Cytogenetics of the Anopheles gambiae complex in Sudan,

with special reference to An. arabiensis: relationships with East and West African populations. *Medical and Veterinary Entomology*, **14:** 149-164.

Prato, M., Khadjavi, A., Mandili, G., Minero, V. G. and Giribaldi, G. (2012). Insecticides as Strategic Weapons for Malaria Vector Control. INTECH Publisher 2012, ISBN: 9533077808, 9789533077802.

Protopopoff, N., Verhaeghen, K., Van Bortel, W., Roelants, P., Marcotty, T., Baza, D., D'Alessandro, U. and Coosemans, M. (2008). A Significant Increase in kdr in *Anopheles gambiae* Is Associated with an Intensive Vector Control Intervention in Burundi Highlands. *Tropical Medicine and International Health*, **13:** 1479-1487.

Qin, Q., Li, Y., Zhong, D., Zhou, N., Chang, X., Li, C., Cui, L., Yan, G. and Chen, X-G. (2014). Insecticide resistance of *Anopheles sinensis* and *An. vagus* in Hainan Island, a malaria-endemic area of China. *Parasites and Vectors*, **7:** 92.

Ranson, H., Abdallah, H., Badolo, A., Guelbeogo, W.M., Kerah-Hinzoumbé, C., Yangalbé-Kalnoné, E., Sagnon, N., Simard, F. and Coetzee, M. (2009). Insecticide Resistance in *Anopheles gambiae*: Data from the First Year of a Multi-Country Study Highlight the Extent of the Problem. *Malaria Journal*, **8:** 299.

Ranson, H., Jensen, B., Vulule, J. M., Wang, X., Hemingway, J. and Collins, F. H. (2000). Identification of a Point Mutation in the Voltage-Gated Sodium Channel Gene of Kenyan *Anopheles gambiae* Associated with Resistance to DDT and Pyrethroids. *Insect Molecular Biology*, **9:** 491-497.

Ranson, H., N'Guessan, R., Lines, J., Moiroux, N., Nkuni, Z. and Corbel, V. (2011). Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? *Trends in Parasitology*, **27(2):** 91-98.

Roberts, D. R., Chareonviriyaphap, T., Harlan, H. H. and Hshieh, P. (1997). Methods of testing and analyzing excito-repellency responses of malaria vectors to insecticides. *Journal of American Mosquito Control Association*, **13(1):** 13-17.

Rogerson, S. J., Mwapasa, V. and Meshnick, S. R. (2007). Malaria in pregnancy: linking immunity and pathogenesis to prevention. *American Journal Tropical Medicine and Hygiene*, **77(suppl 6):** 14-22.

Rongnoparut, P., Boonsuepsakul, S., Chareonviriyaphap, T. and Thanomsing, N. (2003). Cloning of cytochrome P450, CYP6P5, and CYP6AA2 from *Anopheles minimus* resistant to deltamethrin. *Journal of Vector Ecology*, **28(2):** 150-158.

Russell, T. L., Govella, N. J., Azizi, S., Drakeley, C. J., Kachur, S. P. and Killeen, G. F. (2011). Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. *Malaria Journal*, **10:** 80.

Sachs, J. and Malaney, P. (2002). The economic and social burden of malaria. *Nature*, **415:** 680-685.

Sachs, J. and Malaney, P. (2002). The economic and social burden of malaria. Nature, **415(6872):** 680-685.

Sadasivaiah, S., Tozan, Y. and Breman, J. G. (2007). Dichlorodiphenyltrichloroethane (DDT) for indoor residual spraying in Africa: how can it be used for malaria control? *American Journal of Tropical Medicine and Hygiene*, **77(6-suppl.):** 249-263.

Saddler, A., Burda, P-C. and Koella, J. C. (2015). Resisting infection by *Plasmodium berghei* increases the sensitivity of the malaria vector *Anopheles gambiae* to DDT. *Malaria Journal*, **14:** 134.

Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A. and Arnheim, N. (1985). Enzymatic amplification of beta-globin genomic

sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science*, **230(4732):** 1350-1354.

Santolamazza, F., Calzetta, M., Etang, J., Barrese, E., Dia, I., Caccone, A., Donnelly, M. J., Petrarca, V., Simard, F., Pinto, J. anddella Torre, A. (2008). Distribution of knock-down resistance mutations in *Anopheles gambiae* molecular forms in west and west-central Africa. *Malaria Journal*, **7(1):** 74.

Schneider, P., Takken, W. and McCall, P. J. (2000). Interspecific competition between sibling species larvae of *Anopheles arabiensis* and *An. gambiae*. *Medical and Veterinary Entomology*, **14:** 165-170.

Scholte, E-J., Knols, B. G. J., Samson, R. A. and Takken, W. (2004). Entomopathogenic fungi for mosquito control: A review. *Journal of Insect Science*, **4:** 1-24.

Scott, J. A., Brogdon, W. G. and Collins, F. H. (1993). Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene*, **49:** 520-529.

Scott, J. G. and Wen, Z. (2001). Cytochrome P450 of insects: the tip of the iceberg. *Pest Management Science*, **57:** 958-967.

Seidahmed, O. M., Abdelmajed, M. A., Mustafa, M. S. and Mnzava, A. P. (2012). Insecticide susceptibility status of the malaria vector *Anopheles arabiensis* in Khartoum city, Sudan: differences between urban and periurban areas. *East Mediterranean Health Journal*, **18:** 769-776.

Service, M. W. (2008). Medical Entomology for students. Third edition, Cambridge University Press. P: 91-98.

Sharma, J., Dutta, P., Khan, S. A., Soni, M. and Mahanta, J. (2014). Detection of point mutation in *Plasmodium falciparum* ATPase6 gene associated with artemisinin resistance from Assam and Arunachal Pradesh. *Journal of Vector Borne Diseases*, **51(4):** 282-285.

Sinka, M. E., Rubio-Palis, Y., Manguin, S., Patil, A. P., Temperley, W. H., Gething, P. W., Van Boeckel, T., Kabaria, C. W., Harbach, R. E. and Hay, S. I. (2010). The dominant *Anopheles* vectors of human malaria in the Americas: occurrence data, distribution maps and bionomic précis. *Parasites and Vectors*, **3:** 72.

Skovmand, O., Bonnet, J., Pigeon, O. and Corbel, V. (2008). Median knock-down time as a new method for evaluating insecticide-treated textiles for mosquito control. *Malaria Journal*, **7:** 114.

Slinger, R., Giulivi, A., Bodie-Collins, M., Hindieh, F., John, R. St., Sher, G., Goldman, M., Ricketts, M. and Kevin C. Kain. K. C. (2001). Transfusion-transmitted malaria in Canada. *Canadian Medical Association Journal*, **164(3):** 377-379.

Snounou, G. I., Viriyakoso, S., Zhu, X. P., Jarra, W., Pinheiro, L., Rosario, V. E., Thaithong, S. and Brown, K. N. (1993). High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. *Molecular and Biochemical Parasitology*, **61(2):** 315-230.

Snow, W. F. (1983). The attractiveness of some birds and mammals for mosquitoes in The Gambia, West Africa. *Annals of Tropical Medicine and Parasitology*, **77(6):** 641-651.

Sogoba, N., Vounatsou, P., Bagayoko, M. M., Doumbia, S., Dolo, G., Gosoniu, L., Traore, S. F., Toure, Y. T. and Smith, T. (2007). The spatial distribution of *Anopheles gambiae* sensu stricto and *An. arabiensis* (Diptera: Culicidae) in Mali. *Geospatial Health*, **2:** 213-222.

Sotimehin S. A., Runsewe-Abiodun, T. I. and Oladapo, O. T. (2008). Possible risk factors for congenital malaria at a tertiary care hospital in Sagamu, Ogun State, South-West Nigeria. *Journal of Tropical Pediatrics*, **54(5):** 313-320.

Sougoufara, S., Diédhiou, S. M., Souleymane Doucouré, S., Diagne, N., Sembène, P. M., Harry, M., Trape, J-F., Sokhna, C. and Ndiath, M. O. (2014). Biting by *Anopheles funestus* in broad daylight after use of long-lasting insecticidal nets: a new challenge to malaria elimination. *Malaria Journal*, **13:** 125.

Stilwell, G. E., Rocheleau, T. and Ffrench-Constant, R. H. (1995). GABA receptor minigene rescues insecticide resistance phenotypes in *Drosophila*. *Journal of Molecular Biology*, **253:** 223-227.

Stump, A. D., Atieli, F. K., Vulule, J. M. and Besansky, N. J. (2004). Dynamics of the Pyrethroid Knockdown Resistance Allele in Western Kenyan Populations of *Anopheles gambiae* in Response to Insecticide-Treated Bed Net Trials. *American Journal of Tropical Medicine and Hygiene*, **70:** 591-596.

Sutherland, C. J., Ord, R., Dunyo, S., Jawara, M., Drakeley, C. J., Alexander, N., Coleman, R., Pinder, M., Walraven, G. and Targett, G. A. T. (2005). Reduction of Malaria Transmission to *Anopheles* Mosquitoes with a Six-Dose Regimen of Co-Artemether. *PLOS Medicine*, **2(4):** e92.

Takken, W. and Knols, B. G. J. (1999). Odor-mediated behaviour of Afrotropical malaria mosquitoes. *Annual Review of Entomology*, **44:** 131-157.

Takken, W. and Lindsay, S. W. (2003). Factors affecting the vectorial competence of *Anopheles gambiae*: a question of scale, pp. 75-90. *In* W. Takken, and T. W. Scott (eds.), Ecological aspects for application of genetically modified mosquitoes. Frontis, Springer, Dordrecht.

Takken, W., Klowden, M.J. and Chambers, G. M. (1998). Effect of body size on host seeking and blood meal utilization in Anopheles gambiae sensu stricto (Diptera: Culicidae): the disadvantage of being small. *Journal of Medical Entomology*, **35(5):** 639-645.

Tirados, I., Costantini, C., Gibson, G. and Torr, S. J. (2006). Blood-feeding behaviour of the malarial mosquito *Anopheles arabiensis*: implications for vector control. *Medical and Veterinary Entomology*, **20:** 425-437.

Toé, K. H., N'Falé, S., Dabiré, R. K., Ranson, H. and Jones, C. M. (2015). The recent escalation in strength of pyrethroid resistance in *Anopheles coluzzi* in West Africa is linked to increased expression of multiple gene families. *BMC Genomics*, **16:** 146.

Torr, S. J., Torre, A., Calzetta, M., Costantin, C. and Vale G. A. (2008). Towards a fuller understanding of mosquito behaviour: use of electrocuting grids to compare the odour-oriented responses of *Anopheles arabiensis* and *An. quadriannulatus* in the field. *Medical and Veterinary Entomology*, **22:** 93-108.

Touré, Y. T., Petrarca, V., Traoré, S. F., Coulibaly, A., Maiga, H. M., Sankaré, O. M., DI Deco, M. A. and Coluzzi, M. (1998). The distribution and inversion polymorphism of chromosomally recognized taxa of the *Anopheles gambiae* complex in Mali, West Africa. *Parassitologia*, **40:** 477-511.

Townson, H., Nathan, M. B., Zaim, M Guillet, P., Manga, L., Bos, R. and Kindhauser, M. (2005). Exploiting the potential of vector control for disease prevention. *Bulletin of the World Health Organization*, **83:** 942-947.

UN, (2006) L'ame´lioration de la durabilite´ de la production du coton eb afrique de l'ouest et du centre. In Confe´rence des Nations Unies Sur le Commerce et le De´veloppement: Fonds Commun pour les Produits de Base Consultatif Internationnal du Coton, Nations Unies.

Valecha, N., Bhatia, S., Mehta, S., Biswas, S. and Aditya P Dash, A. P. (2007). Congenital malaria with atypical presentation: A case report from low transmission area in India. *Malaria Journal*, **6:** 43.

Verhaeghen, K., Bortel, W. V., Roelants, P., Okello, P. E., Talisuna, A. and Coosemans, M. (2010). Spatiotemporal patterns in *kdr* frequency in permethrin

and DDT resistant *Anopheles gambiae* s.s. from Uganda. *American Journal of Tropical Medicine and Hygiene*, **82(4):** 566-573.

Verhaeghen, K., Van Bortel, W., Roelants, P., Backeljau, T. and Coosemans, M. (2006). Detection of the East and West African kdr Mutation in *Anopheles gambiae* and *Anopheles arabiensis* from Uganda Using a New Assay Based on FRET/Melt Curve Analysis. *Malaria Journal*, **5:** 16.

Vijayakumar, K. N., Gunasekaran, K., Sahu, S. S. and Jambulingam, P. (2009). Knowledge, attitude and practice on malaria: a study in a tribal belt of Orissa state, India with reference to use of long lasting treated mosquito nets. *Acta Tropica*, **112(2):** 137-142.

Vijverberg, H. P. M., Ringt, G. S. T. and Van den Bercken, J. (1982). Structure related effect of pyrethroid insecticides on the lateral-line sense organ and peripheral nerves of clawed frog (*Xenopus laevis*). *Pesticide Biochemistry and Physiology*, **18:** 315-324.

Vontas, J. G., Hejazi, M. J., Hawkes, N. J., Cosmidis, N., Loukas, M. and Hemingway, J. (2002). Resistance-associated point mutation of organophosphate insensitive acetylcholinesterase, in olive fruit fly *Barctrocera oleae*. *Insect Molecular Biology*, **11:** 329-336.

Walsh, S. B., Dolden, T. A., Moores, G. D., Kristensen, T., Devonshire, A. L. and Williamson, M. S. (2001). Identification and characterization of mutation in housefly (*Musca domestica*) acetylcholinesterase involved in insecticide resistance. *Biochemistry Journal*, **359:** 175-181.

White, G. B. (1972). The *Anopheles gambiae* complex and malaria transmission around Kisumu, Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **66:** 572-581.

White, G. B. (1974). *Anopheles gambiae* complex and disease transmission in Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **68:** 278-301.

White, G. B. (1985). *Anopheles bwambae* sp.n., a malaria vector in the Semliki Valley, Uganda, and its relationships with other sibling species of the *An. gambiae* complex (Diptera: Culicidae). *Systematic Entomology*, **10:** 501-522.

White, G. B. and Rosen, P. (1973). Comparative studies on sibling species of the *Anopheles gambiae* Giles complex (Diptera: Culicidae). II. Ecology of species A and B in savanna around Kaduna, Nigeria during transition from wet to dry season. *Bulletin of Entomological Research*, **62:** 613-625.

White, M. T., Griffin, J. T., Churcher, T. S., ferguson, N. M. and Ghani, A. C. (2011). Modeling the impact of vector control interventions on *Anopheles gambiae* population dynamics. *Parasites & Vectors*, **4:** 153.

White, N. J. (2008a). Editorial Commentary: *Plasmodium knowlesi*: The fifth human malaria parasite. *Clinical Infectious Diseases*, **46:** 172-173.

White, N. J. (2008b). Qinghaosu (Artemisinin): The price of success. *Science*, **320:** 330-334.

Whiteacre, D. M. and Ware, G. W. (2004). The pesticides book 6th edition, Meister Media Worldwide, Welloughby, Ohio.

WHO, (1976). Resistance of Vectors and Reservoirs of Disease to Pesticides. *WHO technical report Series*, **585:** 8-16.

WHO, (1982). Manual on environmental management for mosquito control, with special emphasis on malaria vectors. WHO Offset Publication No. 66.

WHO, (1992).Vector Resistance To Pesticides. *WHO Technical Report Series*, **818**: 5-42.

WHO, (1993). Implementation of the global malaria control strategy, In: Technical Report Series 839, World Health Organization, ISBN 9-24120-839-2, Geneva, Switzerland.

WHO, (1997). Chemical Methods for Control of Vectors and Pests of Public Health Importance. WHO/CTD/WHOPES/97.2:12.

WHO, (2001). Malaria vector control, insecticides for indoor residual spraying. WHO/CDS/WHOPES/2001.3.

WHO, (2005).Scaling-up insecticide treated netting programmers' in Africa, a strategic framework for coordinated national action. *WHO/CDS/RBM/2002.43,Geneva.*

WHO, (2006). Malaria Vector Control and Personal Protection. *WHO Technical Report Series*, **936:** 7-8.

WHO, (2008). World malaria report. WHO/HTM/GMP/2008.1. Geneva, Switzerland.

WHO, (2010). Global report on antimalarial drug efficacy and drug resistance: 2000–2010. World Health Organization, Geneva, Switzerland.

WHO, (2011). The malaria world report 2011. World Health Organization, Geneva, Switzerland.

WHO, (2012). Global Plan for Insecticide Resistance Management in Malaria Vectors (GPIRM). In: WHO/HTM/GMP/20125. Edited by Organization WH. Geneva, Switzerland: World Health Organization; 2012: 130.

WHO, (2013a). World Malaria Report 2013. WHO Global Malaria Programme. World Health Organization, Geneva, Switzerland.

WHO, (2013b). Test Procedures for Insecticide Resistance Monitoring in Malaria Vector Mosquitoes. World Health Organization, Geneva, Switzerland.

WHO, (2014). World Malaria Report. World Health Organization, Geneva, Switzerland.

WHO/ANVAR, (2005). Atlas of insecticide resistance in malaria vectors of the WHO African region. World Health Organization & African Network for Vector Resistance. Harare, October 2005.

WHO/EMRO, (2004). Responsiveness of the RBM Programme to country needs in the WHO Eastern Mediterranean Region. WHO-EM/MAL/307/E/08.04/500.

Wilkins, E. E., Howell, P.I. and Benedict, M. Q. (2006). IMP PCR primers detect single nucleotide polymorphisms for *Anopheles gambiae* species identification, Mopti and Savanna rDNA types, and resistance to dieldrin in *Anopheles arabiensis*. *Malaria Journal*, **5:** 125.

Wondji, C., Simard, F., Petrarca, V., Etang, J., Santolamazza, F., della Torre, A. and Fontenille, D. (2005). Species and populations of the *Anopheles gambiae* complex in Cameroon with special emphasis on chromosomal and molecular forms of *Anopheles gambiae* s.s. *Journal of Medical Entomology*, **42:** 998-1005.

Yadouleton, A., Martin, T., Padonou, G., Chandre, F., Asidi, A., Djogbenou, L., Dabiré, R., Aïkpon, R., Boko, M., Glitho, I. and Akogbeto, M. (2011). Cotton Pest Management Practices and the Selection of Pyrethroid Resistance in *Anopheles gambiae* Population in Northern Benin. *Parasites and Vectors*, **4:** 60.

Yadouleton, A.W., Asidi, A., Djouaka, R.F., Braima, J., Agossou, C.D. and Akogbeto, M. C. (2009) Development of Vegetable Farming: A Cause of the Emergence of Insecticide Resistance in Populations of *Anopheles gambiae* in Urban Areas of Benin. *Malaria Journal*, **8**: 103.

Yagoop, J. E. S., Bashir, N. H. H. and Assad, Y. O. H. M. (2013). Susceptibility of *Anopheles arabiensis* (Diptera: Culicidae) adults to some commonly used agricultural insecticides in El Rahad Agricultural Corporation, Central Sudan. Scholarly *Journal of Agricultural Science,* **3(1):** 10-20.

Yewhalaw, D., Wassie, F., Steurbaut, W., Spanoghe, P., Van Borte, W., Denis, L., Tessema, D. A., Getachew, Y., Coosemans, M., Duchateau, L. and Speybroeck, N. (2011). Multiple Insecticide Resistance: An Impediment to Insecticide-Based Malaria Vector Control Program. *PLOS ONE*, **6(1):** e16066.

Yohannes, M. and Boelee, E. (2012). Early biting rhythm in the Afro-tropical vector of malaria, *Anopheles arabiensis* and challenges for its control in Ethiopia. *Medical and Veterinary Entomology*, **26:**103-105.

Zahar, A. R. (1985). Vector bionomics in the epidemiology and control of malaria. P1. WHO African Region and Southern WHO Eastern Mediterranean Region. VBC/ 85.3_MAP / 85.3.