

## Susceptibility Status of The Malaria Vector *Anopheles Arabiensis* To Insecticides in Khartoum State, Sudan

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### ABSTRACT

**Background:** Increasing insecticide resistance in the major anopheline vectors remain the main obstacle for malaria control programmes in African countries including Sudan.

**Objectives:** To assess the susceptibility status of *Anopheles arabiensis* the malaria vector to different classes of insecticides in Khartoum State.

**Materials and Methods:** Using WHO procedure, susceptibility tests were conducted on adults of *An. arabiensis* from nine sentinel sites in Khartoum State. Mortality rates and knockdown times of insecticides for *An. arabiensis* were calculated.

**Results:** A total of 8345 females of *An. arabiensis* were tested against eight insecticides, these were DDT 4%, fenitrothion 1%, malathion 5%, propoxur 0.1%, permethrin 0.75%, deltamethrin 0.05% and lambda cyhalothrin 0.05%. Of these insecticides tested, *An. arabiensis* from Khartoum State was susceptible to only fenitrothion 1% and lambda cyhalothrin 0.05% with overall mean percentage mortalities  $99 \pm 0.12$  and  $100 \pm 0.45$  respectively. In addition, the overall results, revealed a low mean mortality rates in *An. arabiensis* indicating resistance to the other remaining insecticides. Furthermore, all populations of *An. arabiensis* from different sentinel sites showed variation in mortality rates for the tested insecticides. The exception were for fenitrothion 1% and lambda cyhalothrin 0.05% which were resulted in 100% mean mortality in *An. Arabiensis* in each sentinel sites. Similarly, the knock down time (KDT) of all insecticide tested for 50% and 95% of *An. arabiensis* varied between populations of sentinel sites.

**Conclusion:** In conclusion, *An. arabiensis* the main malaria vector in Khartoum State is mainly susceptible to fenitrothion and lambda cyhalothrin. Therefore, these two insecticides could be the more suitable for malaria vector control in Khartoum State.

**Key Words:** *Anopheles arabiensis*; susceptibility; KDT, Khartoum State, Sudan

Malaria represents a major health problem in the tropical and subtropical regions of the World. The disease is caused by a protozoan parasites belonging to the genus Plasmodium and transmitted via a bite of infected female anopheline mosquitoes. Globally, in 2013 it is estimated that 198

million malaria cases occurred with 568000 deaths, 3.3 billion people are at risk of infection and 1.2 billion at high risk<sup>2</sup>. Almost more than 90% of malaria cases occur in sub-Saharan Africa especially among children aged under 5 years<sup>2</sup>. In Sudan, malaria causes high morbidity and mortality. It was estimated 9

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million cases, 44,000 deaths due to malaria infection in 2002 in the country<sup>3</sup>. In Khartoum State, it is estimated that an average of 300 000 malaria cases and 500 deaths occurred annually<sup>4-5</sup>. *Anopheles arabiensis* the member of *An. gambiae* complex is the only malaria vector in the State<sup>6</sup> and in other part of Sudan<sup>7-8</sup>. This species was found with some degree of anthropophilic and endophilic behavior in Khartoum State<sup>9</sup>.

Currently, in Africa, the malaria vector control strategies largely rely on the use of insecticides for the impregnation of bed nets (ITNs/LLINs) and for indoor residual spraying (IRS)<sup>10</sup>. As a result, several malaria vectors has developed resistance to different classes of insecticides throughout the African countries<sup>11-13</sup> including Sudan<sup>14-16</sup>. Likewise, control of malaria vectors in most States of Sudan including Khartoum State mainly depend on ITNs/LINNs and IRS. However, due to a daily influx from other regions of Sudan, the population of the Khartoum State shows a steady increase which resulted in expansion of both residential and agricultural areas in both urban and peri-urban settings. This situation has led to a massive increase in anopheline larval habitats across the State<sup>17</sup>. Therefore, in Khartoum State, the control of *An. arabiensis* mainly rely on weekly treatment of larval habitats by Temephos<sup>®</sup> EC50, environmental management<sup>17</sup> and insecticide space spraying during the emergency situation<sup>15</sup>. Similarly, the malaria vector has developed resistance to different classes of insecticides in this State<sup>16</sup>.

Due to expansion in agro-development and in build-up of residential areas in both urban and peri-urban areas in Khartoum State, an extensive uses of the same insecticides for agriculture and public health practices become common. Whenever, the same insecticides used for

both practices in the same area, this will greatly enhanced development and accelerate insecticide resistance in malaria vectors<sup>18</sup>. Such situation will also have important implications for the vector control programs, especially when considering the scaling up of ITNs/LLINs, IRS and Larval Source Management LSM<sup>19</sup>. Hence, knowledge on susceptibility status of malaria vectors to the four classes of insecticides approved by the World Health Organization for use in vector control is very crucial for the health authorities program to shift for alternative insecticides in case of tolerance. Therefore, in this study an entomological surveys were carried out during the period March 2011 February 2013, to determine the susceptibility status of populations of *An. arabiensis* to seven different insecticides and their distribution in nine sentinel sites across Khartoum State.

## MATERIALS AND METHODS:

### Study area:

The present study was carried out in Khartoum State which 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 1). The State occupies approximately about 28 000 km<sup>2</sup>. 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 (December to February), a dry hot summer (April-July) and a short rainy season (July to September). The average annual rainfall

is 160mm 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 averaged humidity varied between 36%-64%.

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 peri-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<sup>4</sup> where *P. Falciparum* parasite is the main causative<sup>9</sup>.

#### Study sites:

Nine sentinels were selected to collect immature stages of *An. Arabiensis* according to the 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, 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 1).

#### Anopheline mosquito collection and rearing:

Anopheline mosquito larvae and pupae were collected from nine sentinel sites from possible larval habitats of different type's e.g. wells, pools, broken drinking water pipes and irrigation canals 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 bowls covered with

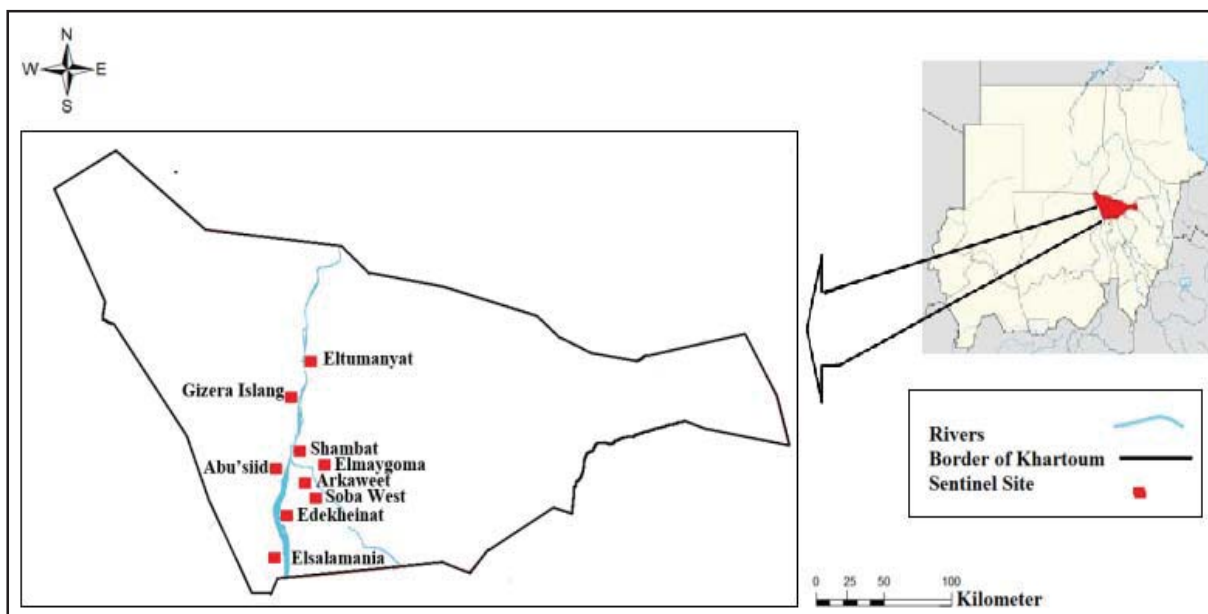


Figure (1): A map of Khartoum State showing the location of the surveyed sentinel sites.

mosquito mesh and transported to the Khartoum Malaria Free Project (KMFP) insectary in Khartoum. In the insectary, the immature stages were transferred in to 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<sup>20</sup>. The larvae in the trays were reared provided with Tetramin1 fish and the pupae were put in plastic cups and then transferred to mosquito cages for emergence. The emerged adults were maintained on a 10% glucose solution on filter-paper until they subsequently used for WHO insecticide susceptibility tests.

**Identification of *Anopheles* mosquitoes:**

The emerged adult *Anopheles* mosquitoes from the colony-reared specimens were identified using proper entomological keys<sup>21</sup>.

Furthermore, a sub-samples (n = 225) of the morphologically identified *Anopheles* mosquitoes were randomly selected and analyzed by PCR using species-specific primers. Genomic DNA was extracted from individual female mosquitoes using LIVAK buffer method<sup>22</sup>. The DNA pellets were then re-suspended in 100 µl of 1X TE-buffer and stored at -20°C for subsequent PCR analysis. DNA samples

were then analyzed by PCR using *An. arabiensis* species-specific primers (Ao: ATGCCTGAACGCCTCTAAGG and A05: CAAGATGGTTAGTTACGCCAA). PCR conditions used in this study were similar to that described by Scott et al.<sup>23</sup>. The PCR condition with an initial step of 10 min at 94°C, followed by 30 cycles each consisting of 30 s denaturation at 94 °C, 30 s annealing at 50 °C and 30 s extension at 72 °C with the final cycle products extended for 7 min at 72 °C.

PCR product amplicons were resolved in 1.5% agarose gel containing ethidium bromide and 100 bp DNA molecular weight ladder were used as a marker. The specific primers gave PCR amplicons of 500 bp band sizes a characteristic for *An. arabiensis*.

**Insecticide susceptibility test using WHO kits:**

In this study, seven insecticides used in public health sector for control of malaria vector recommended by WHO were selected (Table 1) to determine the susceptibility/resistance status and the knock down time for 50 and 95% (*KDT*<sub>50%</sub> and *KDT*<sub>95%</sub>) for *An. arabiensis*. The selected insecticides belong to the classes which are widely used to control public health and agricultural pests in different African countries including Sudan.

Table (1): 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

Insecticide susceptibility tests were conducted according to WHO standard procedures<sup>12</sup> using impregnated papers provided by WHO in March 2010. *Anopheles arabiensis* mosquitoes were tested against insecticides impregnated papers with discriminating doses shown in table 1. For each insecticide different numbers of batches (5-20 replicates) 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 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.

#### **Information on agricultural and public health insecticides uses in Khartoum State:**

Information on the commonly used insecticides in agriculture and public health practices during the last five years in Khartoum State were investigated. For this purpose, farmers in two administrative areas as well public health workers were visited and interviewed using a well-designed questionnaire format. A sample of 60 farmers (30 in each area) and 60 health workers were recruited to answer questions mainly on the type of

insecticides, dosage, the frequency of usage.

#### **Data analysis:**

Data obtained from this study were analyzed using SPSS software version 20 and EXCEL software. The mortality rates after exposure to each insecticide was calculated as the number of dead mosquitoes/total tested for each test replicate using Excel Software. The resistance/susceptibility status of the tested mosquitoes from each sentinel site for each insecticide was determined according WHO criteria<sup>12</sup>: mortality rate  $\geq 98\%$  = susceptible, 90-97% = suspected/potential resistance, and  $< 90\%$  = resistant.

Fifty and 95% knockdown times ( $KDT_{50}$  and  $KDT_{95}$  respectively) of the mosquitoes were estimated by Probit analysis (logtimeprobit model) using SPSS software version 20. The resistance ratio (RR) were calculated by dividing  $KDT_{50}$  of the tested population/ $KDT_{50}$  of the sentinel site with the shortest time.

#### **Ethical considerations:**

In this study no ethical approval was required because the study was a part of routine surveys 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.

#### **RESULTS:**

##### **WHO susceptibility tests for populations of *Anopheles arabiensis* from nine sentinel sites in Khartoum State:**

##### **Anopheline mosquitoes:**

A total of 8345 adult anopheline mosquitoes were obtained from larval collection from the nine sentinel sites investigated and reared in the insectary. All mosquito specimens were identified morphologically as *An. gambiae* s.l. Furthermore, all the 225 randomly selected

sub-samples analysed by PCR were *An. arabiensis*.

### **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 DDT 4%, fenitrothion 1%, malathion 5%, propoxur 0.1%, permethrin 0.75%, deltamethrin 0.05% and lambda-cyhalothrin 0.05%. 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 used to test mosquitoes from the all sentinel sites investigated. Furthermore, lambda-cyhalothrin 0.05% insecticides were used to test mosquitoes from only three sentinel sites; Soba West, Edekheinat and Elsalamania West.

### **Mortality rates of *Anopheles arabiensis*:**

The overall mean percentage of mortalities in populations of *An. arabiensis* from Khartoum State after 24 hour exposure seven WHO different insecticide impregnated papers are shown in figure 2. A total of 8345 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 *An. arabiensis* in Khartoum State were fully susceptible to fenitrothion 1% and

lambda-cyhalothrin 0.05% and were highly resistance to DDT 4%, malathion 5%, propoxur 0.1%, permethrin 0.75% and deltamethrin 0.05% (see figure 2).

The results on the susceptibility/resistance status of populations of *An. arabiensis* from all sentinel sites are shown in table 2. Populations of *An. arabiensis* were resistant to DDT in most of the sentinel sites investigated. However, specimens of *An. arabiensis* from Soba West and Elsalamania West sites were susceptible ( $99\pm 0.53$  and  $98\pm 2.0$  respectively) and those from Eltumanyat site were suspected/potential resistance ( $96\pm 1.71$ ) to DDT. Moreover, populations of *An. arabiensis* from all sentinel sites were susceptible to fenitrothion. Resistance to malathion was observed in *An. arabiensis* from five sentinel sites; Soba West ( $73\pm 9.36$ ), Shambat ( $80\pm 2.67$ ), Elmaygoma ( $81\pm 2.47$ ), Eltumanyat ( $78\pm 7.39$ ) and Elsalamania West sites ( $89\pm 1.0$ ). Nevertheless, suspected/potential resistance was observed in specimens from Edekheinat ( $92\pm 0.57$ ) and Abuseid ( $94\pm 1.25$ ) sites. Of the seven populations of *An. arabiensis* tested using propoxur two showed resistance and five were suspected/potential resistance to this insecticide (see table 2).

Resistance to permethrin insecticide was observed in three out of five tested populations of *An. arabiensis*; these were from Edekheinat ( $88\pm 5.42$ ), Elmaygoma ( $70\pm 6.4$ ) and Elsalamania West sites ( $89\pm 1.91$ ). However, *An. arabiensis* from Arkaweet site was fully susceptible (100%) whereas, specimens from Soba West were suspected/potential resistance ( $95\pm 2.52$ ) 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 population of *An. arabiensis* tested using lambda cyhalothrin were fully susceptible (100% for all) (table 2).

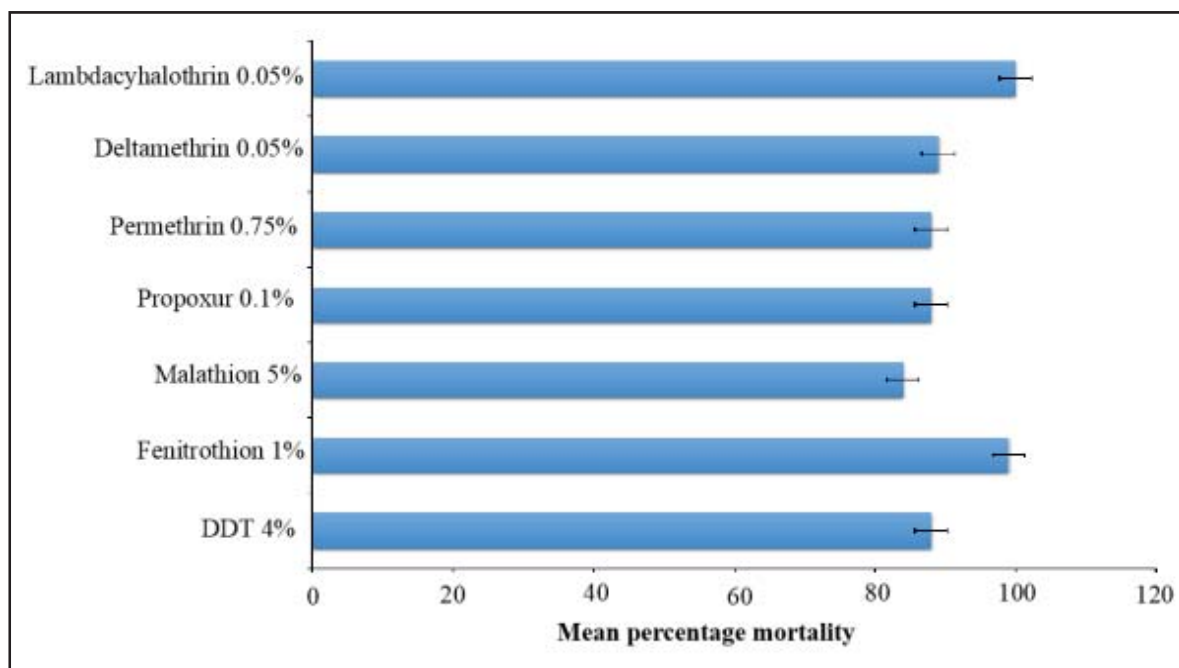


Figure (2): Overall mean mortality rates after 24 hours exposure to insecticides for *Anopheles arabiensis* populations from different sentinel sites in Khartoum State

#### Knockdown time for *Anopheles arabiensis*:

The knockdown effects of the tested insecticides to the *An. arabiensis* population collected from nine sentinel sites in Khartoum State are presented in Table 2. 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 compared to other sentinel sites ( $X^2=2454.36$ ,  $df = 576$ ,  $P = 0.00$ ). 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 ( $X^2=2157$ ,  $df = 495$ ,  $P = 0.00$ ). Moreover, the population of the vector from Edekheinat site showed the lowest  $KDT_{50}$  and  $KDT_{95}$  for malathion (36.08 and 92.77 minutes respectively;  $X^2=1868.41$ ,  $df = 468$ ,  $P = 0.00$ ) and propoxur (44.79 and 94.15 minutes respectively;  $X^2=$

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 permethrin (21.20 and 47.49 minutes respectively;  $X^2=289.42$ ,  $df = 133$ ,  $P = 0.00$ ) and deltamethrin (21.67 and 45.19 minutes respectively;  $X^2=658.88$ ,  $df = 276$ ,  $P = 0.00$ ) than other sites. Lambdacyhalothrin was tested against only three population of *An. arabiensis* with the highest and lowest knock down ( $KDT_{50}$  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 ( $X^2 = 118.22$ ,  $df = 79$ ,  $P = 0.003$ ).

#### Insecticides used in Agriculture and public health practices in Khartoum State

The results of the questionnaire showed that a total of 12 agricultural pesticides and

10 public health insecticides have been used during the last 5 years. These pesticides used in both practices mainly represented the four classes of insecticides; organochlorines, organophosphates, carbamates and pyrethroids. In addition, deltamethrin, DDT and malathion have been used extensively in agricultural practices. Although, DDT was banned some decade ago, the farmers obtain this insecticide from illegal markets.

#### DISCUSSION:

Control of malaria vectors remains a subject of interest to many scientists because malaria is threatening the life of millions of people especially in Africa<sup>12</sup>. However, insecticide resistance is a growing problem affecting the sustainability of any malaria vector control programmes. The resistance managements are needed so as to delay or prevent insecticide resistance in these vectors. An important part in the resistance management strategies is identifying the resistance strains and mechanisms involved. Therefore, this study was designed to obtain baseline data and distribution of *An. arabiensis* susceptibility/resistance strains across Khartoum State. Such studies will help our understanding to set appropriate control strategies against malaria and other mosquito borne-diseases in Sudan and other endemic areas.

Assessment of resistance to seven insecticides to *An. arabiensis* was carried out in nine sentinel sites in Khartoum State. In this study, field populations of *An. arabiensis* from only three out of nine sentinel sites were tested against the seven insecticides used. Nevertheless, *An. arabiensis* specimens from other six sentinel sites were tested with varied numbers of insecticides (3- 6 insecticides) (see table 2). This variation was due to the availability of larval habitats and/or the

density of larvae in the habitats investigated during months of collection. The reasons of that were the differences in the types of larval habitats between the surveyed areas. The larval habitats in urban areas are mainly formed of leakage of water pipes whereas in the peri-urban (agricultural) areas are formed of irrigation canal seepage. Often, larval habitats formed of canal seepage are more likely suitable for breeding of *An. arabiensis* than those by the broken drinking water pipes<sup>8</sup>.

*Anopheles arabiensis* was the only anopheline mosquitoes recorded in Khartoum State<sup>6</sup>. 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 lambda-cyhalothrin. The results obtained in this study are consistent with a previous study in Khartoum State which also showed that *An. arabiensis* is fully susceptible to fenitrothion and lambda-cyhalothrin<sup>16</sup>. Those authors found that *An. arabiensis* was resistance to only malathion and suspected/resistance to DDT. Furthermore, the resistance of *An. arabiensis* to DDT, malathion, permethrin and deltamethrin observed in populations of most sentinel sites investigated in this study agree with several previous and recent studies in other part of Sudan<sup>15,24-25</sup>.

The results revealed that populations of *An. arabiensis* from three sites were fully resistant to DDT, malathion and permethrin and to propoxur in two sites (see table 2). Likewise, specimens of *An. arabiensis* collected from the nine sites were resistant to deltamethrin with except of those from Arkawet and Elsalamania West sites which were fully susceptible (100% for each). The tolerance of the malaria vector most insecticides used in urban and peri-urban areas in Khartoum



Table (2): Mean mortality rates and Knock down time of *Anopheles arabiensis* exposed to insecticides for a period of 60 (or 80) min; A: DDT4%; B: Fenitrothion 1% and malathion 5%; C: Propoxur 0.1%; D: Permethrin 0.75%, Deltamethrin 0.05% and Lambdacyhalothrin 0.05%.

**A: DDT 4%**

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

**B: Fenitrothion 1% and Malathion 5%.**

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

<b>Malathion 5%</b> Site	Number exposed (N) (Replicates)	Mean Mortality (%) $\pm$ SE	<i>KDT</i> <sub>50</sub> (in min) (95% CI)	<i>KDT</i> <sub>95</sub> (in min) (95% CI)	<i>KDT</i> <sub>50</sub> ratio(RR)
Soba West	300 (12)	73 $\pm$ 9.36	59.48 (53.60-66.29)	152.86 (133.36-178.00)	1.7
Edekheinat	400 (16)	92 $\pm$ .57	36.08 (33.51-38.92)	92.77 (83.73-104.06)	1.0
Shambat	300 (12)	80 $\pm$ 2.67	42.51 (39.41-45.94)	109.25 (98.10-123.28)	1.2
Elmaygoma	500 (20)	81 $\pm$ 2.47	45.96 (43.27-48.94)	118.13 (106.86-132.40)	1.3
Eltumanyat	100(4)	78 $\pm$ 7.39	120.44 (93.68-156.48)	309.53 (235.31-416.26)	3.3
Abuseid	200 (8)	94 $\pm$ 1.25	43.43 (39.47-47.88)	111.63 (98.84-127.75)	1.2
Elsalamanyia West	100 (4)	89 $\pm$ 1.0	42.71 (37.35-49)	109.78 (94.28-129.51)	1.2

**C: Propoxur 0.1%.**

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

**D: Permethrin 0.75%, Deltamethrin 0.05% and Lambdacyhalothrin 0.05%.**

<b>Permethrin 0.75%</b> Site	Number exposed (N) (Replicates)	Mean Mortality (%) $\pm$ SE	<i>KDT</i> <sub>50</sub> (in min) (95% CI)	<i>KDT</i> <sub>95</sub> (in min) (95% CI)	<i>KDT</i> <sub>50</sub> ratio(RR)
Soba West	100(4)	95 $\pm$ 2.52	41.46 (37.95-45.38)	93.07 (82.74-106.60)	2.0
Arkewit	100(4)	100 $\pm$ 0	21.20 (19.36-23.16)	47.59 (42.82-53.63)	1.0
Edekheinat	100(4)	88 $\pm$ 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
Elsalamanyia West	100 (4)	89 ±1.91	44.48 (40.75-48.71)	99.84 (88.56-114.70)	2.1
<b>Deltamethrin 0.05%</b>	Number exposed (N) (Replicates)	Mean Mortality (%) ±SE	<i>KDT</i> <sub>50</sub> (in min) (95% CI)	<i>KDT</i> <sub>95</sub> (in min) (95% CI)	<i>KDT</i> <sub>50</sub> ratio(RR)
Soba West	100 (4)	97 ± 1.91	29.09 (26.69-31.70)	60.68 (55.07-66.89)	1.3
Arkewit	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
Eltomanyat	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
Elsalamanyia 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
<b>Lambdacyhalothrin 0.05%</b>	Number exposed (N) (Replicates)	Mean Mortality (%) ±SE	<i>KDT</i> <sub>50</sub> (in min) (95% CI)	<i>KDT</i> <sub>95</sub> (in min) (95% CI)	<i>KDT</i> <sub>50</sub> ratio(RR)
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
Elsalamanyia West	100 (4)	99 ±1.0	44.00 (41.65-46.54)	77.17 (71.38-84.59)	13

state has previously been reported<sup>16</sup>. 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.

The results of the surveys revealed that almost about 10 insecticides belongs to the four major classes have been used during the last five years for both purposes. Moreover, further support also can be drawn from the significant differences in knockdown rates (*KDT*<sub>50</sub> & *KDT*<sub>95</sub>) for *An. arabiensis* for the seven insecticides tested between the nine sentinel sites. Similar results have also been observed previously

in studies conducted using the same insecticides on *An. arabiensis* from different areas in Sudan<sup>16,24,26</sup>.

### CONCLUSION:

In conclusion, the results reported here reduce the possibility of the use of most of the insecticide tested except for fenitrothion and lambda-cyhalothrin. These insecticides could be used in malaria vector control in Khartoum State and other regions in Sudan with similar situation. Furthermore, 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.

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