



Monitoring insecticide resistance in *Anopheles arabiensis* Patton (Diptera: Culicidae) in Karima-Merowe-Eldabba area, northern state, Sudan

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Abstract

Background and objectives: *Anopheles arabiensis* is the main vector of malaria disease which spread all over the Sudan. This study conducted to determine the susceptibility of *Anopheles arabiensis* populations to some insecticides.

Materials and methods: Using standard WHO diagnostic kits and procedures, the tested insecticides were DDT, fenitrothion, permethrin, deltamethrin, and bendiocarb.

Results: it has been found that, *An. arabiensis* is fully susceptible to both deltamethrin and fenitrothion, and tolerant to each of bendiocarb and DDT. Evidence for resistance to permethrin in *An. arabiensis* was clearly found in two areas (Merowe –Nouri, and Eldabba) with percentage mortalities of $67.0 \pm 3.0\%$, and $65.0 \pm 7.2\%$, respectively.

Conclusion: it could be recommended that either fenitrothion or deltamethrin can be used as an alternative insecticide for indoor residual spraying campaign against *An. arabiensis* in the study area. Monitoring insecticide resistance in *Anopheles arabiensis* populations every two years is of paramount importance.

Keywords: monitoring, insecticide, resistance, anopheles arabiensis, susceptibility

Introduction

Insecticide resistance is an increasing problem faced by those who need insecticides to efficiently control medical, veterinary and agricultural insect pests. It has been reported by Karaağaç^[1] that the development of resistance in the fields is affected by many different factors; biological, genetic and operational factors. Biological factors are generation time, number of offspring per generation and migration. Genetic factors are frequency and dominance of the resistance gene, fitness of resistance genotype and number of different resistance alleles. These two factors cannot be influenced by man. However, such as treatment, persistence and insecticide chemistry, all of which may and therefore timing and dosage of insecticide application should be operational factors.

Prevention of disease transmitted by mosquitoes could be achieved through the elimination of disease parasites in the human host and the control of mosquito vectors. The main approach to control anopheline vectors was directed towards the larval stage, which required a detailed knowledge of the bionomics of local vectors^[2]. But generally, control of mosquito vectors includes environmental management, biological control, chemical control and genetic control. Chemical control is the reduction of insect population by means of natural insecticides. Insecticides play a central role in controlling mosquitoes. In 1955 the WHO proposed the global eradication of the most prevalent vector-borne disease, malaria by using residual spraying of DDT. An entomological survey conducted by the malaria pre-eradication program in 1961 showed that there was a reduction in malaria cases with 1.9% malaria prevalence^[3]. And it is still the most important practice in vector control. Synthetic insecticides are separated into several categories based on their chemical structure and mode of action on insects. These categories include organochlorines, organophosphates, carbamates and pyrethroids. Chemical control methods of malaria vector in the Northern state of Sudan started as early as 1958 using Benzenehexachloride for indoor residual house –spraying which was followed by DDT, malathion and deltamethrin (State Malaria Control Program, 2007- unpublished data). The Gambia Control Project is a joint project between Sudan and Egypt was established in 1970 to control the sole malaria vector in the area, *An. arabiensis*. The signed protocol between the two countries adopted chemical larviciding and IRS as key interventions in addition to limited use of space spraying^[4]. Temephos is the only larvicide under use till now and the other insecticides applied now in the project area by the health authorities are permethrin and deltamethrin. Other insecticides used by the agricultural sector include cloquintocet-mexyl (topik®), and dursban. It has been reported that *Anopheles*

arabiensis is the main malaria vector in Northern Sudan where the study area is located [4]. Therefore, the objective of this study is to determine insecticide susceptibility status of *Anopheles arabiensis* and to estimate the time required to knockdown 50 % (=KDT₅₀) and 90 % (=KDT₉₀) of the females exposed to the insecticide under the study.

Materials and methods

Location of the study area

The study was conducted in Merowe and Eldabba localities in the Northern State of Sudan from May to July 2011 (Figures 1). Northern State is bordering Sudan with Egypt and Libya, and it lies between the latitudes 16°-32° N and longitudes 30°-32°E. The total population of the state is 699065 inhabitants, while the total area is 348765 square kilometers. It lies within the scrub vegetation belt of the Nubian Desert. The landscape is a vast plain sloping gently towards the River Nile. The region is characterized by extreme high temperature and very low rainfall, insufficient to produce persistent drainage into the Nile. The ground on either side of the River Nile is rocky with more sand than silt which is brought down by seasonal flood waters⁴. According to the epidemiological stratification of Sudan, Northern State is a hypo-endemic area with unstable malaria. The predominant malaria parasite species is *P. falciparum*.

Site selection and description

The selection of the collection sites was based mainly on previous information about the area including availability, distribution and productivity of the breeding sites. This was ascertained by a general survey of the whole area, after which three areas were chosen to be as collection areas, namely Merowe-Nouri area, Karima area and Eldabba area.

Specimens collection, rearing and identification

Adult susceptibility tests were conducted using 24-48 hours old non-blood fed females as recommended by WHO [5]. Accordingly, wild *Anopheles* larvae were collected from different breeding sites using standard larval collection kits including plastic dippers, plastic screened netting, glass and plastic pipettes, plastic buckets, iron dishes and sorted out from other aquatic organisms. About 200 - 1000 *Anopheles* larvae from one site per day were collected and the period of collection was taken every four days a week for each site. Larvae were kept in plastic bottles and buckets, put in container with wetted towel in the same day of collection and transferred to the insectary. In the insectary *Anopheles* larvae were isolated from other mosquitoes, then were put in dishes and labeled (date, type of breeding sites), after that *Anopheles* larvae were reared and were being fed on rice powder. When pupation of reared larva occurred, pupae were sorted out by wide-mouthed pipettes and put in a plastic beaker and inserted into the cages fitted with fine meshes (156 meshes/ inch²). Two cages were allocated for each site. Emerged females and males were fed on 10% sugar solution until the performance of the tests. When sufficient number were obtained (100 female mosquitoes per test, as recommended by the WHO, the tests were carried out. The morphological identification of the anopheline mosquitoes (both larvae and adults) were carried out according to the identification keys prepared by Abdel Nur and Nugud [6] and Gillies and Coetsee [7]. All emerged males were separated and morphologically identified for confirmation of the species. At the end of the tests, the females were also examined and morphologically identified. At the start of the test, the temperature and humidity inside the insectary were 24.7-27.2°C and 45-69%, and after 24 hours of the test were 23.6-28.1°C and 40-69%, respectively.

Insecticide susceptibility tests

The susceptibility status of *An. arabiensis* was investigated using the WHO procedure and standard kits [5]. The insecticides screened were DDT, fenitrothion, permethrin, deltamethrin, and bendiocarb. Insecticide impregnated papers of the diagnostic dosages of the insecticides were 4% DDT, 0.75% permethrin, 0.05% deltamethrin, 1.0% fenitrothion, and 0.1% bendiocarb that provided by WHO on July 2010.

Data interpretation

The resistant status of mosquito samples was determined according to the WHO criteria [5], which recommends that the percentage of 98-100% mortality indicates susceptibility, 80-97% mortality suggests the possibility of resistance that needs to be further confirmed, and mortality less than 80 % suggests resistance. If the control mortality is between 5-20%, the percentage mortality was corrected by Abbott's formula as follows:

$$\frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}}$$

Data analysis

Data were analyzed using SPSS Program, Probit analysis model to estimate KDT 50% and KDT 95%. A test of significance was conducted to find mean mortality after 24hours.

Results

Species composition

A total of 6245 *Anopheles* larvae that collected from the three sites from the different types of breeding places were shown in table (1). All specimens were identified morphologically as *An. arabiensis* according to Abdel-Nur and Nugud⁶ and Gillies and Coetzee^[7] keys.

Results of susceptibility tests

Table (2) showed the percentage mortality after 24 hours for *An. arabiensis* exposed to the different insecticides under the study. *An. arabiensis* was resistant to permethrin in both Eldabba and Merowe-Nouri areas, in which the mortality rates were found to be $65.0 \pm 7.2\%$ and $67.0 \pm 3.0\%$, respectively, while *An. arabiensis* was found tolerant to permethrin in Karima, in which the mortality rate was found to be $87.0 \pm 1.9\%$. Result regarding to deltamethrin and fenitrothion, *An. arabiensis* was found to be completely susceptible, the mortality recorded was 100% in all the three areas. Furthermore, *An. arabiensis* were found tolerant to bendiocarb at Merowe-Nouri, Karima, and Eldabba areas mortality rates were $95.0 \pm 1.0\%$, $87.0 \pm 2.5\%$, and $92.0 \pm 1.6\%$, respectively. As for DDT, *An. arabiensis* are tolerant at both Eldabba $85.0 \pm 6.0\%$ and Karima $95.0 \pm 1.0\%$, while slight tolerant was detected in Merowe-Nouri $97.0 \pm 1.0\%$.

Table 1: collection No of *Anopheles* larvae collected per sites and total breeding sites

Sites	Type of breeding site	Geographical coordinates		Number of <i>Anopheles</i> larvae collected
		Latitudes N	Longitudes E	
Merowe –Nouri	Broken pipe	18.48380	031.83543	650
	Broken pipe	18.49180	031.83350	150
	Broken pipe	18.49180	031.83350	100
	Broken pipe	18.55584	031.90187	200
	Broken pipe	18.55090	031.89610	100
	Pool	18.64499	032.05876	250
	Basin	18.5769	031.88922	475
Total				1925
Karima	Broken pipe	18.47952	031.300952	850
	Broken pipe	18.47599	031.79807	250
	Broken pipe	18.49135	031.80544	300
	Broken pipe	18.65599	032.05986	450
	pool	18.65499	032.05976	100
Total				1950
Eldabba	Broken pipe	18.08233	030.92958	150
	Broken pipe	18.04121	030.51534	100
	Broken pipe	18.09319	030.90441	150
	Brickworks	18.9983	030.90702	450
	Brickworks	17.98513	031.12808	500
	Brickworks	18.00092	31.10879	800
	Basin	18.07887	030.92758	20
Total				2370
Total				6245

Table 2: Percentage mortality (mean \pm SE) after 24 hours for *An. arabiensis* exposed to different insecticides by sites.

Insecticide	Area	No. of population tested	Mortality mean \pm SE	Resistance status
DDT 4%	Merowe –Nouri	100	97.0 ± 1.0	T
	Karima	100	95.0 ± 1.0	T
	Eldabba	100	85.0 ± 6.0	T
Permethrin 0.75%	Merowe –Nouri	100	67.0 ± 3.0	R
	Karima	100	87.0 ± 1.9	T
	Eldabba	100	65.0 ± 7.2	R
Deltamethrin 0.05%	Merowe –Nouri	100	100.0 ± 0.0	S
	Karima	100	100.0 ± 0.0	S
	Eldabba	100	100.0 ± 0.0	S
Bendiocarb 0.1%	Merowe –Nouri	100	92.0 ± 1.6	T
	Karima	100	87.0 ± 2.5	T
	Eldabba	100	95.0 ± 1.0	T
Fenitrothion 1%	Merowe –Nouri	100	100.0 ± 0.0	S
	Karima	100	100.0 ± 0.0	S
	Eldabba	100	100.0 ± 0.0	S

SE = Standard Error, S= Susceptible, T= Tolerant, R= Resistant

Knockdown results

The estimation of Knockdown times was shown in table (3). The times required for knocking down were 50% (KDT₅₀) and 95% (KDT₉₅) of the exposed *An.arabiensis* females to the insecticides tested were calculated using Probit analysis model. For the non- knockdown group insecticides (bendiocarb, and fenitrothion) the KDT₅₀ values obtained for bendiocarb 0.1% at the three sites of Eldabaa, Merowe –Nouri and Karima were 49.4, 50.6 and 64.7minutes, respectively. Fenitrothion 1.0% produced the most delayed knock-down effects at the three sites with 84.6, 78.0 and 78.1 minutes post exposure at Eldabba, Merowe –Nouri and Karima, respectively. For the knockdown group insecticides (DDT, deltamethrin, and permethrin) the fastest KDT₅₀ obtained were for deltamethrin 0.05% with 13.6, 14.9 and 15.7 minutes at Eldabba, Merowe –Nouri and Karima, respectively, followed by permethrin 0.75% and the KDT₅₀ observed for this insecticide were 21.5, 16.6 and 20.8 minutes at Eldabba, Karima and, Merowe –Nouri areas, respectively. The study observed that, a delayed KDT₅₀ obtained with non-knockdown insecticides was fenitrothion 1% and the fastest obtained one was for deltamethrin in the three study sites. The fastest knockdown time for DDT was obtained at “Merowe –Nouri” (KDT₅₀ = 28.7 minutes), while the most delayed one was obtained at “Eldabba” (32.5 minutes).

Table 3: Estimations of Knockdown times (KDT₅₀ and KDT₉₅) with confidence limits of *An. arabiensis* exposed to five insecticides at the three monitored sites

Insecticide tested	Area (site)	Knockdown times		Test of significance
		KDT50 (95% CI)	KDT95 (95% CI)	
DDT 4%	Merowe – Nouri	28.7 (27.4 - 30.0)	49.9 (46.5 - 54.2)	Chi-Square = 16.66 df =5, P= 0. 483
	Karima	29.6(28.0 - 31.2)	59.6 (54.7 -66.2)	Chi-Square= 4.832, df =5, p= 0.437
	Eldabba	32.5(30.0 - 34.4)	69.5(63.0 - 78.3)	Chi-Square= 9.359 df =5, p= 0.096
Permethrin 0.75%	Merowe – Nouri	20.8(19.8 - 21.8)	33.7(31.3- 36.8)	Chi-Square= 7.554, df =5, p= 0.183
	Karima	16.6(15.8 - 17.5)	29.4(27.1 - 32.6)	Chi-Square= 7.855, df =5, p= 0.164
	Eldabba	21.5(20.4 - 22.6)	37.1(34.4 - 34.4)	Chi-Square= 5.063, df =5, p= 0.408
Deltamethrin 0.05%	Merowe – Nouri	14.9(14.3 - 15.4)	19.2(18.2 - 20.8)	Chi-Square= 3.931, df =5, p= 0.559
	Karima	15.7(14.8 - 16.9)	31.1(28.4 - 34.9)	Chi-Square= 3.252, df =5, p= 0. .661
	Eldabba	13.6(12.9 - 14.3)	21.9(20.4 - 24.3)	Chi-Square= 6.547, df =5, p= 0.257
Bendiocarb 0.1%	Merowe – Nouri	49.4(45.7 - 54.2)	125.9(105.0 - 161.1)	Chi-Square= 7.337, df =5, p= 0.197
	Karima	50.6(46.9 - 55.5)	133.4(110.3 - 174.6)	Chi-Square 6.360, df =5, p= 0.274
	Eldabba	64.7(59.1 -73.6)	141.8(113.5- 201.7)	Chi-Square= 4.066, df =5, p= 0.540
Fenitrothion 1%	Merowe – Nouri	78.0(75.8 - 80.2)	120.4(114.7 - 127.5)	Chi-Square= 11.472, df =11, p= 0.118
	Karima	78.1(76.0 - 80.2)	115.6(110.6 - 121.9)	Chi-Square= 11.472, df =11, p= 0.405
	Eldabba	84.6(82.3 - 87.0)	131.2(124.4 - 140.0)	Chi-Square= 17.040, df =11, p= 0.107

95% CI =confidence limit, KDT₅₀ = knockdown time 50%, KDT₉₅= knockdown time 95%, df = degree of freedom

Discussion

During the observation period of this study, all of the collected anopheline species was identified to be only *An. Arabiensis*, which means that the *An. Arabiensis* is prevailed in the study area. Similar result has been recently recorded by a study conducted by Ageep *et al.*⁸ and Azrag⁹. At earlier time, a study done by Zahar^[10] reported that there were other species found to be capable of transmitting malaria in the study area. Based on the WHO criteria for characterizing insecticide resistance/susceptibility (susceptibility is defined by mortality rates greater than 98%, and resistance is confirmed by a mortality rate of less than 80% 24 hours post-exposure), there was evidence for resistance to permethrin in two areas of the study (Merowe –Nouri, and Eldabba) with percentage mortalities 67.0 ±3.0%, and 65.0 ± 7.2%, respectively (Table 2). Tolerance to permethrin insecticide was observed in Karima area as well, where this insecticide recorded 87.0±1.9 % mortality. On contrast, a study conducted by Mbepera *et al*^[11]. in Northern Tanzania showed that *An. arabiensis* were full susceptible to DDT. Resistance of *An .arabiensis* to permethrin may be associated with the wide use of insecticides in both public health and in agriculture for mosquito control in the study area. In Sudan, the Malaria Control programme depends largely on the use of pyrethroid insecticides, mainly permethrin, which is being used for space spraying, indoor residual house spraying, and for insecticide-treated bed nets. Furthermore, there is a considerable usage of

aerosols against household pests, most of which contain permethrin. Insecticide Resistance Action Committee reported in 2010 that poor application, such as under dosing, may accelerate the rate of resistance development as the vector population will be exposed to sub-lethal doses of insecticides. The fact that resistance in this area has developed after only six years of application of permethrin in the study area. In normal situations, insecticide resistance requires a long time to develop especially when the insecticide is properly applied. An example for this was reported by a study conducted in South African. There, the vector, *An. arabiensis* was found fully susceptible to deltamethrin in 2002 ^[12], and in 2009 it was found still susceptible to deltamethrin ^[13]. In Central Sudan *An. arabiensis* was found to be resistant to permethrin ^[14] as well as to other insecticides including DDT. Probable causes for this multiple resistance may be attributed to the prolonged usage of agricultural spraying. Based on surveys carried out in three sites of Kassala State of Eastern Sudan, Himeidan *et al* ^[15], reported that *An. arabiensis* was susceptible to permethrin and the mortalities were ranged from 98.4% to 100%. However, recent surveys conducted by Himeidan *et al* ^[16], in towns of Kosti, Damazin, and Sennar (middle of Sudan) reported that *An. arabiensis* populations was found to be resistant to permethrin. More recently, surveys done in Kassala town showed very high resistance to permethrin, as reported across the country (Himeidan, personal communication). With view to the current results from Northern Sudan, it is obvious that resistance to permethrin has become widespread in the populations of *An. arabiensis*. The study showed that *An. arabiensis* was found to be fully susceptible to deltamethrin and fenitrothion in all sites under the study. Results of susceptibility tests were similarly conducted by Dukeen ^[17] and Seidahmed ^[18] in the Northern State of Sudan which reported 100 % mortality of *An. arabiensis* to fenitrothion and deltamethrin. These findings are also similar to that obtained in the South African by Gerickea *et al.* ^[12], which showed that *An. arabiensis* is fully susceptible to fenitrothion and deltamethrin. As well as the study conducted in Central Kenya by Kamau and Vulule ^[19], where found that the 24 hours post-exposure mortality for fenitrothion was to be 100%. Although the usage of DDT in the Northern State was stopped more than twenty years ago, a slight resistance (tolerance) to DDT was found in all sites under the study. This tolerance of *An. arabiensis* is probably due to agricultural pesticides which might be exerting selective pressure on malaria vectors by leaching into breeding habitats so that the immature stages are continuously exposed ^[20]. A study carried out in Eastern Sudan reported similar results of slightly reduced mortalities in agricultural areas which no longer using DDT ^[15]. Some tolerance was observed in the carbamate compound, bendiocarb, in all sites. The result obtained with bendiocarb is a strange one since bendiocarb has not been previously used in the study area for both indoors or even in agriculture. Therefore, more investigation regarding insecticide resistance mechanisms should be conducted in the near future. The KDT50 and KDT95 values obtained for both the knockdown and non-knockdown insecticides (Table 3) can be considered as a base line data for insecticide resistance studies in the Northern State. This is because there were no previous insecticide studies in the current study area, and accordingly no records of knockdown responses towards any insecticide in use in the area.

Conclusions and recommendations

It could be concluded that *An. arabiensis* in Northern State is susceptible to deltamethrin and fenitrothion and tolerant to DDT and bendiocarb. The study has determined insecticide susceptibility status of *Anopheles arabiensis* to all groups of insecticides studied, in addition, the study estimated the time required to knockdown 50 % (=KDT₅₀) and 95 % (=KDT₉₅) of the females exposed to DDT, deltamethrin, permethrin, fenitrothion and bendiocarb. According to the study findings, either fenitrothion or deltamethrin can be used as an alternative for indoor residual spraying campaign against *An. arabiensis* in the study area. The resistance status to the DDT and bendiocarb needs to be confirmed by using other procedures, methodologies and techniques, *e.g.* Polymerase Chain Reaction.

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