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Insecticides susceptibility status in *Anopheles arabiensis* Patton (Diptera: Culicidae) in Ghebeish locality, West Kordofan state, Sudan

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Abstract
Resistance to insecticides is a limiting factor in the use of insecticides in controlling mosquitoes. This cross-sectional study aimed to monitor insecticide susceptibility of *Anopheles arabiensis* Patton (Diptera: Culicidae*)* adults to four commonly used insecticides for indoor residual spraying (deltamethrin 0.05%, bendiocarb 0.1%, DDT 4% and fenitrothion 1%), following WHO protocols. This is one of a series of studies aiming at mapping the resistance of this vector in the Sudan. Two areas (urban and rural) in Ghebeish locality were selected to represent West Kordofan State during the period, July – October 2014. A total of 1,200 females were tested as non-blood-fed using the diagnostic concentrations of the insecticides. The results revealed that the adults were susceptible to bendiocarb 0.1% and fenitrothion 1% in both areas, with mortality rate 100% for bendiocarb, and 100% in rural Ghebeish and 98% in urban Ghebeish for fenitrothion. The adults were also found to be resistant to deltamethrin 0.05% in urban Ghebeish and rural Ghebeish, with 77 and 84% mortalities, respectively. With regard to DDT 4% in the rural Ghebeish 68%mortality was registered, i.e. resistant, and 96% (tolerant) in urban Ghebeish. DDT was not used in the Sudan since 1996.

Keywords: Susceptibility, deltamethrin, fenitrothion, bendiocarb DDT, Anopheles arabiensis, West Kordofan, Sudan

1. Introduction
Malaria is one of the most common infectious diseases in sub-Saharan Africa. There are an estimated 216 million of malaria cases in 2010. In 2012, 80% of the 219 million malaria cases and 90% of the 660,000 malaria deaths worldwide were in Africa [1]. Malaria is the main cause of morbidity and mortality in Sudan, it represent 9.3% of patients frequency to private clinics and about 8.9% of hospitals entry. Malaria morbidity rate is 2.6%, while registered killing rate is 0.64% and annual infection rates 375 cases for each 10,000 from population [2]. The transmission season may last from July/August to November/December, with earlier beginning of June in the southern areas (*e.g*. Kadugli, El Damazin) and later start of August in northern areas (Wad Medani, Kosti, Kassala, El Obeid) [3].

Anopheles arabiensis Patton (Diptera: Culicidae) is the principal vector all-over Sudan, besides *A. gambiae*, *A. funestus*, which are mainly distributed in the southern part of Sudan. *A. arabiensis* is small pool breeder, *A. gambiae* and *A. funestus* are more humid and forested habitats breeders [4]. Malaria vector control (VC) in Sudan has a long history. The main VC interventions include indoor residual house spraying (IRS), the use of insecticide-treated bednets, ITNs (now long lasting insecticidal nets, LLINs), chemical larviciding, environmental management and very limited biological control.

In West Kordofan state western Sudan, malaria represents first disease that causes deaths and absence from work, although other vector-borne diseases (VBD) were found (*e.g*. yellow fever). The State relies on ITNs as the main strategy in controlling malaria and other interventions, *e.g.* space spray, are used intermittently.

Since many of the VC interventions rely on the use of insecticides, entomological monitoring, including monitoring for insecticide resistance (IR) is important. Over the years, these activities have been implemented in several states with evidence of resistance to organophosphates (Ops), DDT and recently to pyrethroids. Resistance to pyrethroids is worrying as this is the group used for treating nets, as well as IRS Evidence of IR in different

settings necessitates surveillance studies to allow prompt detection of resistance, should it arise and, thus, enable its management. Insecticide resistance in *Anopheles* mosquitoes is threatening the success of malaria control programs (MCP). Emergence of insecticide resistance against pyrethroids in *A. arabiensis*, at El Gezira State (Central Sudan), Gadarif State (eastern Sudan), EnNuhud (North Kordofan State, and other agricultural areas, poses a certain threat to the malaria control strategies of Sudan.

The present study aims to investigate insecticide susceptibility status of *A. arabiensis* to different classes of insecticides in Ghebeish locality as part of a project for mapping the resistance of this pest in the country.

2. Materials and Methods

2.1 Study design and study Area

This is a cross-sectional study for susceptibility test for four insecticides belonging to chlorinated hydrocarbons (DDT), pyrethroids (deltamethrin), Ops (fenitrothion), and carbamates (bendiocarb), using WHO standard protocols and guidelines [5].

This study was conducted in Ghebeish locality -West Kordofan State, western Sudan, during the rainy-season (July to October 2014 and 2015), in two stations (urban and rural areas).Ghebeish locality lies in the north- west part of the state. Its population is *ca*. 165,905 inhabitants distributed in three administrative units; the area is 8500 km². Ghebeish locality is bordered from South by Elodia locality, from East EnNohud locality, from the North Wad Banda locality and from the West by North Darfur State and East Darfur State. The main economic activities are agriculture and herding. The annual rainfall ranges between 350 – 650 mm and usually commences in June to early November. The annual average temperature reaches 30º C, and 65%R.H., where it increases during autumn (Kharif), due to the rains.

2.2 Sampling Techniques and Sample Size

The study used random sampling technique in seven sentinel sites in Ghebeish locality. Larvae (*A. arabiensis*) were collected from the breeding habitats by using standard larval collection kits including plastic dippers, plastic screened netting, plastic pipettes, plastic buckets, iron dishes and sorted out from other aquatic organisms. Larvae were kept in plastic bottles and buckets, transferred in special containers to the laboratory.

2.3 Specimen rearing and identification

In the insectary larvae were reared and were fed on rice powder. When pupation occurred, pupae were sorted out by pipette and put in beakers and inserted into the cages (75 cm x 75 x 75 cm) with fine meshes (156 meshes/ inch^2) to emerge. Adults emerged from pupation within 2-3 days. They were identified using morphological features according to the keys provided by Abdel-Nour and Nugud [6], Gillies, and Coetzee [7].

2.4 Bioassay

Insecticide susceptibility tests were performed using the WHO standard procedures [5] and test kits for adult mosquitoes under optimum conditions (24- 27ºC and 70-90% R.H). Impregnated papers with recommended diagnostic concentrations of DDT 4%, deltamethrin 0.5%, fenitrothion 1%, and bendiocarb 0.1% were used. Tests were carried out with one to three days-old *A. arabienses*, non-blood-fed (starved) female mosquitoes.

2.5 Data Collection and Analysis

Data were collected using standard WHO susceptibility tests format. The resistant status was determined according to the WHO criteria [5]. The data was analyzed using SPSS version 20 and Computer software Program. Probit analysis during SPSS software was used to calculate the knockdown (KdT) time /min to each insecticide.

3. Results

3.1 Bioassay

3.1.1 Deltamethrin

Table (1) shows the results of susceptibility tests of *A. arabiensis* females to deltamethrin 0.05%in the two study sites. The results gave percentage mortality rates of 77% in Urban Ghebeish and 84% in Rural Ghebeish. According to WHO criteria for determining resistance, *A. arabiensis* was resistant to deltamethrin 0.05% in all two stations.

3.1.2 DDT 4%

A. arabiensis from Rural Ghebeish were resistant to DDT 4% with mortality rate 68%, whereas urban Ghebeish population proved to be tolerant to this insecticide, with mortality rate 96%.

3.1.3 Bendiocarb 0.1%

The females were highly susceptible to bendiocarb in the both areas. The mortality to this dose was recorded 100%.

3.1.4 Fenitrothion 1%

Mortality of 100% in *A. arabiensis* in Rural Ghebeish was detected. However, mortality of 98% was found in Urban Ghebeish. Thus, the populations of the two sites were susceptible to this insecticides.

Table 1: Bioassay result of discriminatory doses of four commonly used adulticides against test female of *A. Arabiensis* at urban and rural Ghebeish locality, West Kordofan State, Sudan during rainy-season.

Area	Insecticide tested	No. of Tested population (Reps)	% Mortality	Resistance status
Urban	DDT 4%	25×(4)	96	T
	Deltamethrin 0.05%	25×(4)	77	R
	Fenitrothion 1%	25×(4)	98	S
	Bendiocarb 0.1%	25×(4)	100	S
Rural	DDT 4%	25×(4)	68	R
	Deltamethrin 0.05%	25×(4)	84	R
	Fenitrothion 1%	25×(4)	100	S
	Bendiocarb 0.1%	25×(4)	100	S

S= Susceptible, R= Resistance T = Tolerant reps = replicates

3.2 Knockdown time (KdT_{50}) and (KdT_{95})

3.2.1 Deltamethrin 0.05% (KdT_{50}) and (KdT_{95})

The KdT50 for deltamethrin was 14.6 min in Urban Ghebeish, whereas it was 17.1 min in the Rural sites. For KdT_{95}, it was 33.8 min in Urban Ghebeish, and 35.7 min in the Rural sites(Table 2). For bendiocarb, The KdT50 was 27.5 min in Urban Ghebeish and 23.4 min in Rural Ghebeish. The KdT_{95}'s was 45.9 min in Urban Ghebeish and 37 min in Rural locations. The values for fenitrothion were 64.4 min in Urban Ghebeish, and 82 min in Rural Ghebeish. As KdT50s, and 98.5 min and 151.2 min, respectively, for KdT95s. For DDT discriminatory dose, KdT50 registered 14.6 and 42.2 min in Rural Ghebeish. KdT_{95}'s for the same populations were 52.7 and 129.9 min, following the same order of sites (Table 2).

Table 2: Number of *A. Arabiensis* females knocked-down after exposure for 60 min and (KdT_{50}, KdT_{95} and Slope) in Urban and Rural Ghebeish, West Kordofan State, Sudan.

Site	Insecticide	No. of adults Kd after exposure for 60 min							KdT_{50}% (min.)	KdT_{95}% (min.)	Slope
		10 min	15 min	20 min	30 min	40 min	50 min	60 min			
Urban	deltamethrin	37	61	81	95	99	98	100	14.6	33.8	4.51
	bendiocarb	0	3	12	77	94	97	99	27.5	45.9	7.35
	fenitrothion	6	51	79	92	94	100	100	64.4	98.5	8.90
	DDT	17	51	80	95	96	97	96	14.6	42.2	3.56
Rural	deltamethrin	6	51	79	92	94	100	100	17.1	35.7	5.11
	bendiocarb	0	6	36	85	97	100	100	23.4	37	8.26
	fenitrothion	0	0	0	0	6	7	26	82	151.2	6.2
	DDT	1	1	1	7	20	64	78	52.7	129.9	4.19

Figs 1-8 show the log-time probability lines for the four chemicals and the two locations from which the populations were collected. From the lines the slopes were as follows: for deltamethrin 4.51 and 5.11 for urban and rural location, respectively (Figs 1 and 2). However, for bendiocarb the slopes, following the same order, were 7.35 and 8.26 (Fig.3 and 4). The slopes for the lines of fenitrothion were 8.9 and 6.2 for the urban and rural populations, respectively (Figs.5 and 6), whereas the slopes for DDT, following the same order were 3.56 and 4.19 (Fifs.7 and 8).

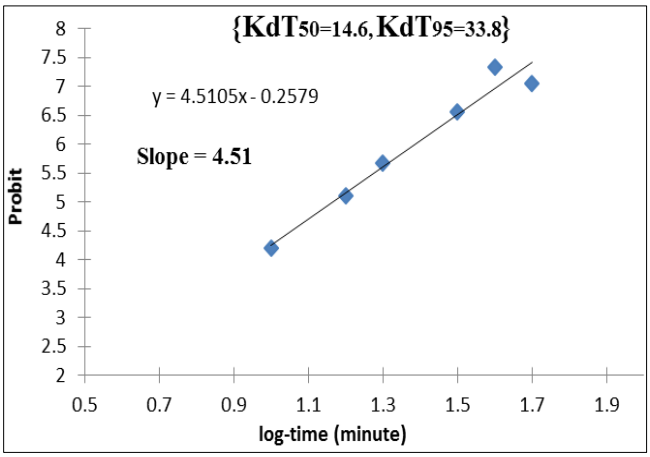

Fig 1: KdT (min) of *A. arabiensis* to deltamethrin in Urban Ghebeish, West Kordofan State, Sudan.

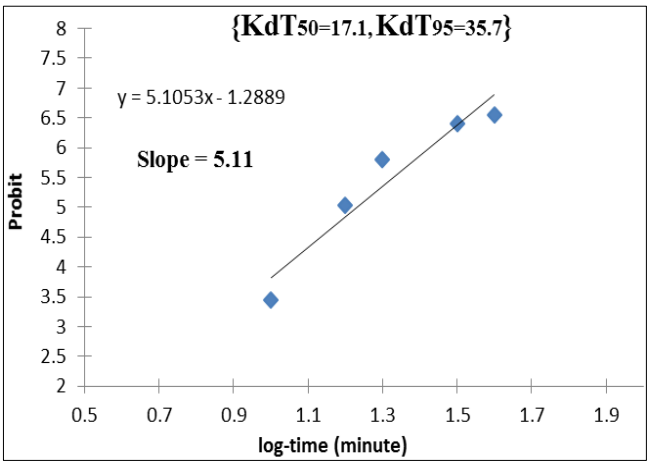

Fig 2: KdT (min) of *A. arabiensis*; to deltamethrin in Rural Ghebeish, West Kordofan State, Sudan.

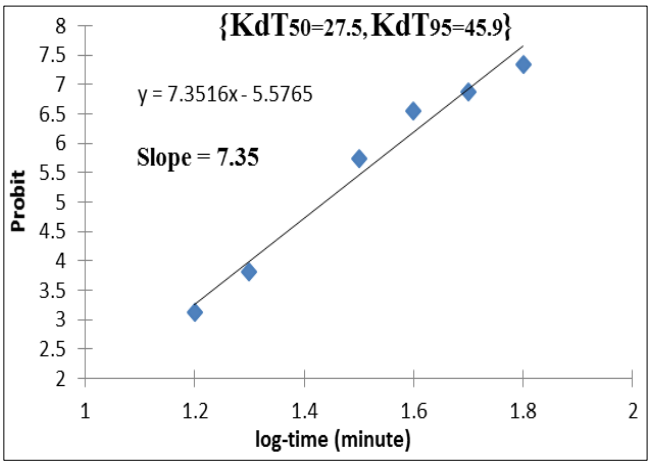

Fig 3: KdT (minutes) of *A. arabiensis* to bendiocarb in Urban Ghebeish, West Kordofan State, Sudan.

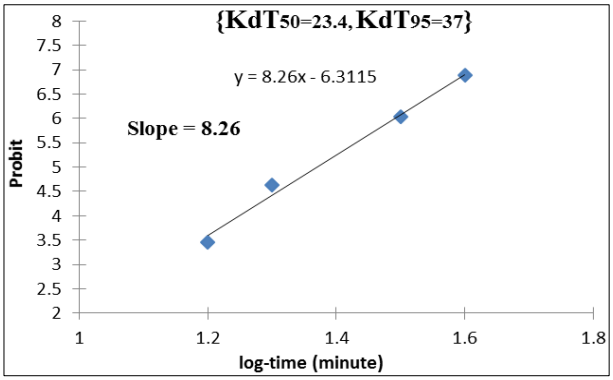

Fig 4: KdT (min) of *A. arabiensis*; to bendiocarb in Rural Ghebeish, West Kordofan State, Sudan.

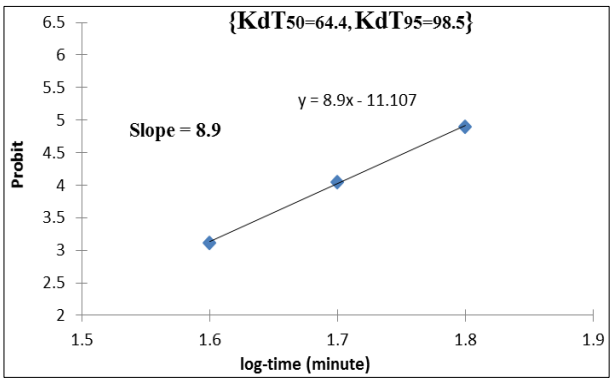

Fig 5: KdT (min) of *A. arabiensis*; to fenitrothion in Urban Ghebeish, West Kordofan State, Sudan.

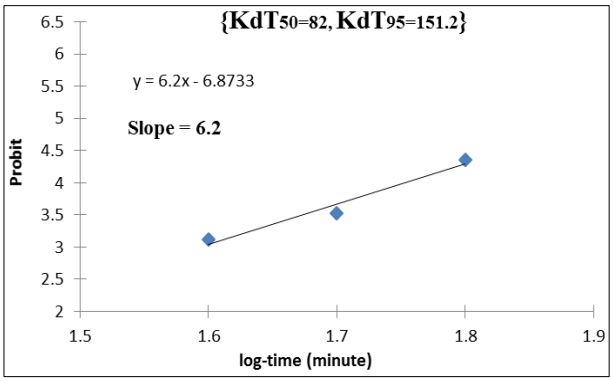

Fig 6: KdT (min) of *A. arabiensis*; to fenitrothion in Rural Ghebeish, West Kordofan State, Sudan.

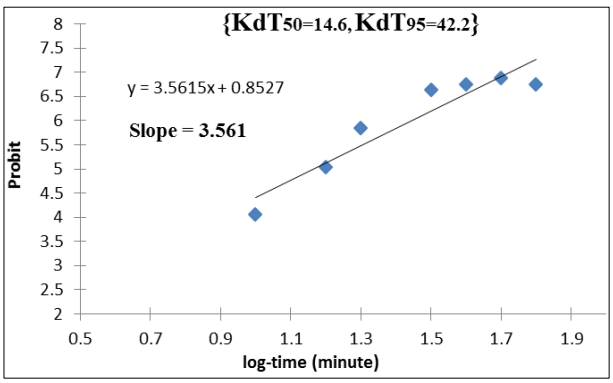

Fig 7: KdT (minutes) of *A. arabiensis*; to DDT in Urban Ghebeish, West Kordofan State, Sudan.

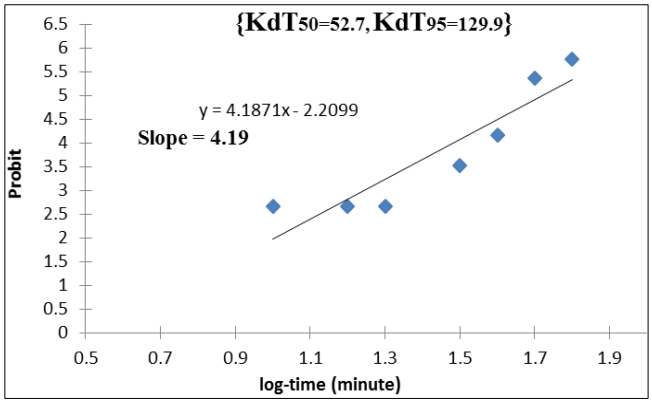

Fig 8: KdT (min) of *A. arabiensis*; to DDT in Rural Ghebeish, West Kordofan State, Sudan

4. Discussion

In this study *A. arabiensis* females were found to be resistant to deltamethrin 0.05% in Urban and Rural Ghebeish (77% and 84% mortality, respectively). Also, the rural population DDT-resistant (68% mortality), whereas it was found to be tolerant (T) in Urban Ghebeish (96%).The results are in line with the other studies in East Darfur State, where the population was found to be resistant to DDT 4% and deltamethrin 0.05%, in Abu Matarig, Elferdus, Asslaia and Eldeaein localities [4]. In Damazin (Blue Nile State), resistance to DDT was found with mortality rate 78.4%, whereas tolerance to deltamethrin was reported in Rosaries and Damazin [2]. It is worth-mentioning that DDT was not used in the Sudan since 1996.

West Kordofan State relies on ITNs as main strategy for controlling malaria, in addition to space spray, using pyrethroid insecticides (namely permethrin). Therefore, the level of resistance might be due to the ITN coverage achieved in recent years in Ghebeish locality since in 2010. The covered population was 65%, in 2013, it was 50%, and in 2014, the percentage covered was 75% [9]. In addition, resistance in the present study might happen, because of indiscriminate use of the insecticides and inaccuracy of application techniques and dosages.

According to WHO criteria, the susceptibility test results indicated that resistance in *A. arabiensis* to DDT and deltamethrin, might be due to these populations were subjected to insecticide selection pressure during the present decade against malaria vector or agricultural purposes against locust and grasshoppers or due to extensive use of pyrethroids in homes against domestic pests, especially pyrethroids, which is reflecting the cross-resistance to DDT [5]. Moreover, globally, several studies showed that the selection of resistance to DDT in populations of malaria vectors was due to the longstanding and extensive use of DDT in the IRS programs [8].

In present study *A. arabiensis* females were highly susceptible to bendiocarb 0.1% with mortality rate 100% in urban and rural areas; also *A. arabiensis* females were susceptible to fenitrothion1% in Rural Ghebeish 100% and Urban Ghebeish 98%. Evidence in East Darfur state, showed that *A. arabiensis* was recorded as highly susceptible to bendiocarb 0.1% with 100% mortality and susceptible to malathion with 99% in Asslaia locality [3]. Also, in Blue Nile State, the population was susceptible to bendiocarb; this was reported in Rosaries, Damazin and Geissan localities with mortality percentages ranged between 98%- 100% [2].

The KdT_{50} and KdT_{95} values are usually considered as indicators for imminent development of resistance to insecticides and can be considered as base-line data for future observations on the development of resistance by the vector to the insecticides used in Ghebeish locality and other localities and states. In this study, it is observed that Log-time line slope in deltamethrin and DDT was the lowest in Urban and Rural Ghebeish (4.51, 3.56 and 5.11, 4.19, respectively), compared with bendiocarb and fenitrothion, in Urban and Rural Ghebeish was (7.35, 8.9 and 8.26, 6.2, respectively). Therefore, the results showed that *A. arabiensis* population proved to be more homogeneous towards bendiocarb and fenitrothion in their response than deltamethrin and DDT, to which the populations proved to be heterogeneous.

However, specifically, there is no single published report about West Kordofan or Ghebeish Locality. Also, the differences between rural and urban populations in all states must be investigated. This will contribute in the success of the control programs and in mapping of the resistance project lead by the BNNICD and the NMCP, Ministry of Health. This work conclusion is to substitute deltamethrin by another IRS insecticide to be used in alternation with bendiocarb, in addition to others from the Ops, carbamates and pyrethroids to avoid resistance.

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