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Monitoring of Insecticide Resistance of Anopheles *arabiensis* Patton to DDT 4%, Deltamethrin 0.05%, Permethrin 0.75% and Bendiocarb 0.1% In River Nile State, Sudan, 2015

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Abstract

The study purposed to determine insecticide susceptibility status of An. arabiensis in the three sites in River Nile State of Sudan to DDT 4%, Deltamethrin 0.05%, Permethrin 0.75% and Bendiocarb 0.1%. The insecticide susceptibility tests were implemented using the WHO standard procedure under optimum conditions and by using impregnated papers with recommended diagnostic concentrations. The tests done with three days-old An. arabiensis non-blood fed females. One hundred twenty to one hundred fifty females were tested for each insecticide at the diagnostic concentration with 6 replicates of 20 - 25 mosquitoes per test. Control tests consisted of groups of 50 female mosquitoes exposed to papers impregnated with carrier oil without insecticide under the same conditions. The exposure time was one hour; then mosquitoes were transferred into holding tubes for a 24 hours' recovery period after that the mortality recorded. Results revealed that An. arabiensis was susceptible to Bendiocarb with 100% mortality rate in all three sites in River Nile state; Shendi, Alzidab and Al Bawga. Anophele sarabiensis was also susceptible to Deltamethrin in Al zidab (98.8%), Al bawga (98.7%) and resistant in Shendi (81.2%). Anopheles arabiensis showed resistance to Permethrin and DDT in all three sites with mortality rates ranged 31.4% - 80.1%. For Bendiocarb KDT50 ranged between 18.1 - 38.5 minutes, while KDT95 ranged between 30.6 –94.7 minutes. The fastest KDT50 and KDT95 recorded were in Shendi area. Whereas for Deltamethrin KDT50 ranged between20.5 – 24.2 minutes, while KDT95 ranged between 90.6 – 132.2 minutes. The fastest KDT50 and KDT95 recorded was in Albawga. These results should be taken in consideration by the current vector control interventions in River Nile State.

Keywords: Susceptibility, Anopheles arabiensis, River Nile State

Introduction

Malaria is one of the most common infectious diseases and an enormous public health problem [1]. Globally, an estimated 3.2 billion people are at risk of being infected with malaria and developing disease, and 1.2 billion are at high risk (>1 in 1000 chance of getting malaria in a year). According to the latest estimates, 198 million cases of malaria occurred globally in 2013 and the disease led to 584,000 deaths. The burden is heaviest in the WHO African Region, where an estimated 90% of all malaria deaths occur, and in children aged under five years, who account for 78% of all deaths [1]. A number of 280 million people in Eastern Mediterranean Region where an eight countries were at some risk of malaria with a figure of 104 million at high-risk. From them two countries accounted for >90% of the deaths in 2013 where the Sudan alone represent (67%) and Pakistan (24%) [1]. In African region there are several areas of critical concern because of particularly widespread resistance to

pyrethroids or to multiple insecticide classes [2]. The situation of malaria in north Sudan, malaria represents around 21% of outpatient consultations and around 30% of inpatient admissions. However, the rates are significantly higher in rural when compared to urban populations. In Sudan Malaria causes considerable mortality, mainly among young children and pregnant women. Possible factors leading to this situation include floods, drought, famine, widely extended irrigated schemes without due consideration to the health component as well as population movement (internal displacement and influx refugees) [3]. The situation is further complicated by the spread of insecticide and drug resistance [4]. The current study aimed to investigate insecticide resistance status of *An. arabiensis* in River Nile State as part of the overall mapping of insecticide resistance in the Sudan.

Materials and Methods

Study Area

This study was conducted in River Nile State in northern Sudan during the period March to May 2015 River Nile State is located between Latitudes 16°-22° N and Longitudes 32°-35° E. It's bordered from the north by the Arab Republic of Egypt, from the East by Kassala state and Red Sea States, from the South by Khartoum State and from the West by the Northern State.

Study Sites

The selection of the study sites was based mainly on WHO criteria (accessibility, representation, availability, distribution and productivity of the breeding sites). Three localities representing irrigated and urban areas were chosen as sentinels sites, which were: Shendi site in Shendi locality(16° 40′ 631″ N, 033° 25′ 536″E; Al zidab site in Aldamar locality(033° 51′ 783″ E , 17° 26′ 297″ and Al bawga site in Barbar locality(18° ' 16.103" N, 33° ' 53.857" E): is in the northern sector of the study area.

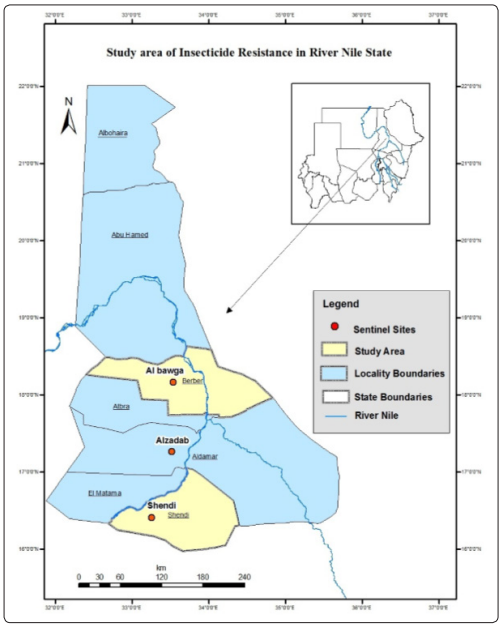

Figure 1: Map of the River Nile State showing sentinels sites

Specimen Collection, Identification and Rearing

Anopheles larvae were collected from different breeding sites using standard larval collection kits including plastic dippers, plastic screened netting, plastic pipettes, plastic buckets, dishes and sorted out from other aquatic organisms. Larvae were kept in plastic buckets, and transferred to the laboratory. In the laboratory, larvae were reared and fed on rice powder. When pupation of reared larva occurred, pupae were sorted out by pipette and replaced in paper cups and inserted into the cages to emerge. Adults emerged from pupation during a period of two to three days. The emerging adults were fed only on 10% sugar solution (sucrose) and kept until tests started. Larvae were identified using morphological features according to the keys [5]. When sufficient number was obtained as recommended by the WHO, the bioassay tests were carried out.

Insecticides Susceptibility Tests

Insecticide susceptibility tests were conducted using the WHO standard procedures using test kits for adult mosquitoes Bioassay tests were done under optimum conditions (temperature 25Co± 2 and 80% ±10 R.H) [6]. Impregnated papers with recommended diagnostic concentrations of 4% DDT, 0.5% Deltamethrin, 0.75% Permthrin, and 0.1% bendiocarb were used, Tests were carried out with one to three days-old *An. arabiensis*, non-blood-fed female mosquitoes. One hundred twenty to one hundred fifty female mosquitoes were tested for each insecticide at the diagnostic concentration, with 6 replicates of 20 - 25 mosquitoes per test. Control tests consisted of a group of fifty mosquitoes exposed to papers impregnated with carrier oil without insecticide under the same conditions. At the end of exposure time (one hour), mosquitoes were transferred into holding tubes for recovery period during which time provided 10% sucrose solution and allowed a 24-hour recovery period after which mortality was recorded. During the exposure time, numbers of knocked down mosquitoes were recorded after (10, 15, 20, 30, 40, 50) and 60 minutes of exposure.

Statistical Analysis

Data was analyzed using SPSS statistical and EXCEL programme. Descriptive statistics as percentages was used to describe mortality rates and Probit statistical analysis was used to determine KdT_{50}, KdT_{95}. The resistant status of mosquito samples was determined according to the WHO criteria as follows: mortality rates between 98%-100% indicate full susceptibility; mortality rates between 90%-97% require further investigation and mortality rates < 90%, the population is considered resistant to the tested insecticides [6]. If the control mortality is between 5-20%, the percentage mortality should be corrected by Abbott's formula.

Results

Species Composition

A total of 6400 Anopheles larvae were collected from the three sites from different types of larval habitats (Table 1). All specimens were identified morphologically as An. *gambiae* complex. As may studies showed that *An. arabiensis* is the only member of the *An. gambiae* complex in northern Sudan we accordingly assigned *An. arabiensis*to all larvae specimens found during this study.

Table 1: Total number of *Anopheles* larvae collected during March–May 2015, River Nile State

Sites	Total Number of larvae
Shendi	2145
Al zidab	2060
Al bawga	2195
Total	6400

Susceptibility Status of An. arabiensis in River Nile State

A total of 1640 females *An. arabiensis* were exposed to the diagnostic concentrations of DDT (4%), Deltamethrin (0.05%), Permethrin (0.75%), and Bendiocarb (0.1%). A total of 72 replicates were conducted, in each replicate 25 females *An. arabiensis* were used. Control tests included 600 *An. arabiensis* females in 24 replicates.

Mortality Rates

Based on the WHO criteria, bioassay results showed the An. arabiensis was susceptible to Bendiocarb (0.1%) in Shendi, Albawga and Alzidab with 100% mortality rates in three sites. For Deltamethrin 0.05% Al bawga and Alzidab were susceptible 98.7% and 98.8% mortality rates respectively. However *An. arabiensis* population from Shendi was resistance with 80% mortality rate. High rates of insecticide resistance were reported for DDT and Permithrin in all three study sites with mortality rates ranged between 80.1% - 33.2% mortality rates for DDT. However, higher resistance rates with mortality rates ranged 55.9% - 31.4% were reported using Permithrin shown in tables (2, 4).

Table 2: Mortality percentages of *An. arabiensis* female exposed to different insecticides in Shendi area, River Nile State, 2015

Insecticide Tested	No. of Tested population (Replicates)	%Mortality test	%Mortality control	Classification according To WHO Criteria (2013)
Deltamethrin 0.05	80 (4)	80 %	6%	R
Bendiocarb 0.1%	100 (4)	100%	16%	S
DDT 4%	80 (4)	33.2%	12%	R
Permethrin 0.75%	90 (4)	40.8%	8%	R
Control	50 (2)			

S= Susceptible (98-100%); T= Tolerant (90-97%); R=; Resistance (<90%)

Table 3: Mortality percentage for female of *An. arabiensis* exposed to different insecticides in Al bawga, River Nile State 2015

Insecticide Tested	No. of Tested population (Replicates)	%Mortality test	%Mortality control	Classification according To WHO Criteria (2013)
Deltamethrin 0.05	80 (4)	98.7 %	8 %	S
Bendiocarb 0.1%	100(4)	100 %	8 %	S
DDT 4%	80 (4)	80.1 %	12 %	R
Permethrin 0.75%	100 (4)	55.9 %	16 %	R
Control	50 (2)			

S= Susceptible (98-100%); T= Tolerant (90-97%); R=; Resistance (<90%)

Table 4: Mortality percentage for female of *An. arabiensis* exposed to different insecticides in Alzidab, River Nile State 2015

Insecticide Tested	No. of Tested population (Replicates)	%Mortality test	%Mortality control	Classification according To WHO Criteria (2013)
Deltamethrin 0.05%	85(4)	98.8%	2%	S
Bendiocarb 0.1%	100(4)	100%	4%	S
DDT 4%	85(4)	69.4%	0%	R
Permethrin 0.75%	80(4)	31.4 %	16%	R
Control	50 (2)			

S= Susceptible (98-100%); T= Tolerant (90-97%); R=; Resistance (<90%)

Results of KDT_{50} and KDT_{95}

Bendiocarb 0.1% (KDT_{50}) and (KDT_{95})

The fastest KDT_{50} of *An. arabiensis* against Bendiocarb 0.1 was reported in Shendi area 30.6minutes (95% 28.3-33.8) followed by Albawga area 36.6minutes (95% 34.3-39.2) and Alzidab area 38.5minutes (95% 36.4- 40.7). Fastest KDT_{95} was reported in Shendi area 30.6 minutes (95% 28.3-33.8), followed by Al zidab 81.5minutes (95% 73.1-93.7) and Albawga area 94.7minutes (95% 82.6-112.9) shown in table (5). Probit transformed responses of *An. arabiensis* exposed to Bendiocarb 0.1% shown in table 5.

Table 5: KDT_{50} and KDT_{95} of *Anopheles arabiensis* exposed to Bendiocarb 0.1% after 60 minutes' exposure in the selected sentinel sites of River Nile State, 2015

Sentinel Site	95% CI (Lower –Upper)	95% CI (Lower -Upper)	Significance level
	KDT_{50}	KDT_{95}	
Shendi	18.1 (17.2-19.0)	30.6 (28.3-33.8)	0.429
Al bawga	36.6 (34.3-39.2)	94.7 (82.6-112.9)	0.146
Alzidab	38.5 (36.4-40.7)	81.5 (73.1-93.7)	0.059

***P-value considered significant at .05 levels

Deltamethrin 0.05% (KDT_{50}) and (KDT_{95})

The fastest KDT_{50} of *An. arabiensis* against Deltamethrin 0.05% was reported in AlBawga area 20.5minutes (95% 11.4-28.9) followed by Shendi area 24.0minutes (95% 18.8-29.6) and Alzidab area 24.2minutes (95% 21.8-26.7), The fastest KDT_{95} was found in Albawga area 90.6 minutes (95% 53.2-547.9) followed by Alzidab area 123.2 minutes (95% 97.2—170.8) and Shendi area 132.2minutes (95%84.2-327.8) shown in table(6). Probit transformed responses of *An.arabiensis* exposed Deltamethrin 0.05% shown in table 6.

Table 6: KDT_{50} and KDT_{95} of *An. arabiensis* exposed to Deltamethrin 0.05% in selected sentinel sites after 60 minutes' exposure of River Nile State, 2015

Sentinel Site	95% CI (Lower –Upper)	95% CI (Lower -Upper)	Significance level
	KDT_{50}	KDT_{95}	
Shendi	24.0 (18.8-29.6)	132.2 (84.2-327.8)	0.038***
Al bawga	20.5 (11.4-28.9)	90.6 (53.2-547.9)	0.000**
Alzidab	24.2 (21.8-26.7)	123.2 (97.2-170.8)	0.753

***P-value considered significant at .05 levels

Permethrin 0.75% (KDT_{50}) and (KDT_{95})

The fastest KDT_{50} of *An. arabiensis* against Permethrin was found in Al Bawga area 20.7 minutes (95% 17.2-24.7) followed by Shendi area 39.5minutes (95% 36.9-42.2) and Alzidab area 45.1minutes (95% 42.2-48.7), The fastest KDT_{95} was found in Al- bawga area 36.4 minutes (95% 29.3-56.8), followed by Shendi 103.2minutes (95% 89.0-125.1) and Al zidabarea 112.8minutes (95%94.3-146.3) shown in table 7.

Table 7: KDT50 and KDT95 of Anopheles arabiensis exposed to Permethrin 0.75% after 60 minutes' exposure in the selected sentinel sites, River Nile State, 2015

Sentinel Site	95% CI (Lower –Upper)	95% CI (Lower -Upper)	Significance level
	KDT_{50}	KDT_{95}	
Shendi	39.5 (36.9-42.4)	103.2 (89.0-125.1)	0.081***
Al bawga	20.7 (17.2-24.7)	36.4 (29.3-56.8)	0.000***
Alzidab	45.1 (42.2-48.7)	112.8 (94.3-146.3)	0.147

***P-value considered significant at .05 levels

DDT 4% KDT_{50} and (KDT_{95})

Shendi area also was registered the fastest KDT_{50} of An. arabiensis against DDT 4% 24.67minutes (95% 18.8-29.6) followed by Albawga area 40.7minutes (95% 34.5-50.3) and Al zidab area 61.47minutes (95% 55.2- 72.1). The fasts KDT_{95} was reported in Shendi area 449.9 minutes (95% 274.0-988.0) followed by Al zidab 184.1 minutes (95% 135.5-305.5) and Al-bawga area 131.8minutes (95% 91.4-261.1) shown in table 8, Probit transformed responses of *An. Arabiensis* exposed DDT 4%. A comparison of Knockdown time 50%and Knock down 95.

Table 8: KDT_{50} and KDT_{95} of *Anopheles arabiensis* exposed to DDT 4% after 60 minutes' exposure in the selected sentinel sites of River Nile State, 2015

Sentinel Site	95% CI (Lower –Upper)	95% CI (Lower -Upper)	Significance level
	KDT_{50}	KDT_{95}	
Shendi	24.6 (64.3-102.8)	449.9 (274.0-988.4)	.929
Al bawga	40.7 (34.5-50.3)	131.8 (91.4-261.1)	.021***
Alzidab	61.4 (55.2-72.1)	184.1 (135.5-305.5)	.817

***P-value considered significant at .05 levels

KDR Result

Knockdown Resistance Gene Detection

Samples were tested for mosquitoes of state stations in the microbiology laboratory of the National Malaria Research & Training Center in Sinner area to compare the results in the method of testing sensitivity and genetic testing of the samples for detection of kdr mutations, genomic DNA was isolated from 136 mosquitoes according to the method [7]. Two separate PCR reactions were run, one to detect alleles of the leucine-phenylalanine substi- tution, the other, to detect wild-type susceptible alleles following the methods described [8,9]. The occurrence of kdr was con- firmed by direct sequencing of the 293 base pair fragment of the sodium channel gene amplified using Agd1 and Agd2 primers.

kdr Gene Detection

One hundred thirty six *An. arabiensis* specimens from River Nile State were examined for the occurrence of 1014 F kdr mutation. The frequency of genotypes of tested mosquitoes on each site depicted in Table (9) the allelic and genotypic Genotypic frequency of kdr.

Table 9: Genotypic frequency of kdr from bioassayed *An. arabiensis* from River Nile State

sentinel site	Latit	Longit	total	SS	RR	kdr freq
Albawga	17.7	33.98	40	40	0	0
El Zedab	17.59	33.96	48	36	12	0.25
Shendi	16.74	33.57	48	42	6	0.125

Table 10: Camper lower 95%CL and upper 95% CL in site of study

sentinel site	Latit	Longit	total	Full allele	kdr freq	lower 95% CI	upper 95% CI
Albawga	17.7	33.98	48	3	0.063	0.0130792	0.0130792
El Zedab	17.59	33.96	48	12	0.25	0.1363723	0.1363723
Shendi	16.74	33.57	48	6	0.125	0.0472838	0.0472838

Discussion

Control of *Anopheline* mosquito vectors of malaria by the use of insecticides has been shown to have a significant impact on both

morbidity and mortality of this disease. This positive impact can, under certain circumstances, be compromised. Some of these events that lead to a negative, or less than desirable, impact is poor coverage, application with sub-lethal dosages and development of resistance by the vectors to the applied insecticides. Evidence of insecticide resistance in different settings necessitates surveillance studies to allow prompt detection of resistance should it arise and thus enable its management [10]. Concurrently, studies on the prevalence, distribution and relative densities of the vectors are very essential.

The results obtained in this study, regarding the *Anopheline* species composition showed that only *An.arabiensis* prevailed in the study area during the observation period where a total of 6400 *Anopheles* larvae were collected from the three sites from different types of breeding places and were all identified as *An. gambia* complex previous studies done on River Nile State showed that *An. Arabiensis* is only members of the *gambia* complex present in that area [11,12] Accordingly we considered that Anopheles species found on all *Anopheles arabiensis*. Based on WHO criteria for characterizing insecticide resistance susceptibility, where susceptibility is defined by Mortality rates between 98% -100% indicate full susceptibility. Mortality rates between 90%-97% require further investigation. Mortality rates < 90%, the population is considered resistant to the tested insecticides [6]. The findings of this study showed that *An. arabiensis* from the River Nile State was resistant to Permethrin 0.75% in Shendi, Albawga and Alzidab localities. However, *An. arabiensis* was full susceptible to Bendiocarb 0. 1%, in all three sites with recorded 100% mortality rates. Bendiocarb was not used for vector control in River Nile state and that may explain the sensitivity to this insecticide. The Gambia Control Project was extended to River Nile State in 2001 Thelarvicide Temephos was used; concurrently, Malathion was first used, followed by Deltamethrinin 2003 upto now. Deltamethrin 0.05% was resistance in Shendi (80%) while it was susceptible in Al bawgaand Alzidab. The low efficacy of Permethrin in all sites can be explained in terms of the extensive domestic and agricultural use of this pesticides in the locality and used this insecticide in the long time for control major. Also, the high resistance to DDT can be explained by the long use of DDT especially in the agricultural sector. The first record for DDT resistance in Sudan was from El Gunaid sugar cane area in 1970 [13] This is in agreement with studies done in Central Sudan in Gezira and Sinnar States [9,14].

Similar study was carried out in 2007 in the Northern State, which neighboring the River Nile State, against eight insecticides three Pyrethroids (Deltamethrin, Permethrin, Lambdacyhalothrin), Organochlorines (DDT), two Organophosphates (Malathion and Fenitrothion) and two Carbamates (Bendiocarb, Propoxur) and the results showed no evidence of resistance in *An.arabiensis* to seven out of eight insecticides tested [15]. Another study carried out in Khartoum town, which bordering the state from the south, in 2007 showed that: *Anarabiensis* was susceptible to (Bendiocarb, Propoxur, Fenitrothion, Deltamethrin and Lambda-Cyhalothrin) with the exception of suspected resistance to DDT (total mortality rate 96.9%) and Permethrin (97.4%), and confirmed resistance to Malathion (69.1%) [15]. Agricultural use of pesticides considered to be one of the major factors of insecticide resistance in malaria vectors. Considering the expansion of urban agriculture, which is often resulted to intensive use of regulated insecticide which, had serious impact on the ecology and resistance levels in *An. arabiensis* population [15]. 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 the state. The results obtained in this study have serious implications for the malaria control programme not only in River Nile State, but also the national level, since the control depends largely on the use of pyrethroids insecticides for indoor residual spraying and for insecticide-treated bed nets. At present only pyrethroids are used in the treating of bed nets. It is not known however, how this finding will affect the impact of ITNs since killing the vectors is not the only method of protection with this strategy. It can be concluded that; *An. arabiensis* in River Nile State is only susceptible to Bendiocarb with recorded 100% mortality rates in all three sites of study: Shendi, Al zidab, Al bawga, while Deltamethrin was reported resistance in Shendi with mortality rate (80 %) and susceptible in Al damar, and Barbar localities. In addition, evidence of insecticides resistance to DDT and Permethrin was observed in all three site shedi, Al bawga and Al zidab. The current study suggested that the Gambiae Control Project could adopt using Deltamethrin in the River Nile State except in Shendi area. Hence, further coordination is needed between integrated vector control and management of plant protection and pesticides for better management of insecticide resistance.

References

1. WHO (2014).World Malaria Report .World Health Organization
2. Ranson H, Jensen B, Vulule JM, Wang X, Hemingway J, et al. (2000) Identification of a novel mutation in the voltage-gated sodium channel gene of Anopheles gambiae associated with resistance to pyrethroid insecticides. Insect Molecular Biology 9: 491-497.
3. Malik EM, Mohamed TA, Elmardi KA, Mowien MM, Elhassan AH, et al. (2006) From chloroquine to artemisinin-based combination therapy: The Sudanese experience. Malaria Journal 5: 65.
4. Himeidan YE, Dukeen MY, El-Rayah AI (2004) Anopheles arabiensis: abundance and insecticide resistance in an irrigated area of eastern Sudan. East Mediterranean Journal 10: 167-174.
5. Abdel Nur, Nugud AD (1994) Key of identification of mosquitoes. Department of Medical Entomology. National Health Laboratory, Ministry of Health, Sudan (unpublished report).
6. WHO (2013) Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. Geneva, World Health Organization.
7. Collins FH, Mendez MA, Rasmussen MO, Mehaffey PC, Besansky NJ, et al. (1987) A ribosomal RNA gene probe differentiates member species of Anopheles gambiae complex. Am J Trop Med Hyg 37: 37-41.
8. Matambo TS, Abdalla H, Brooke BD (2007) Insecticide resistance in Anopheles arbiensis and association with the kdr mutation. Medical and Veterinary Entomology 21: 97-102.
9. Abdalla H, Matambo TS, Koekemoer LL, Mnzava AP, Hunt RH, et al. (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.
10. Kamau and Vulule (2006) Status of insecticide susceptibility in Anopheles arabiensis from Mwea rice irrigation scheme, Central Kenya. Malaria Journal 5:46.
11. Ageep TB, Cox J, Hassan MM, Knols BG, Benedict MQ, et al. (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.
12. Azrag RS (2008) Microsatellite and mitochondrial genetic differentiation of Anopheles arabiensis Patton (Diptera: Culicidae) along the river Nile.
13. Haridi AM (1972) Partial exophily of Anopheles gambiae B in Khashm Elgirba area in eastern Sudan. Bulletin of the World Health Organization 47: 39-46.
14. Elsyed EM (2013) Resistance of Anopheles.arabiensis Patton Mosquito to DDT, Fenitrothion, Deltamethrin, Permethrin and Bendiocarb in Shendi, Aldamar,Atbara and Abu Hamad localities, River Nile State, Sudan. Partial dissertation of Master of Science. University of Gezira.
15. Seidahmed OM (2007) Insecticides Susceptibility Status of the Malaria Vector Anopheles. arabiensis Patton at the African Continental Fringe in Sudan. Department of Medical Entomology. Notional health laboratory, Sudan (unpublished report).