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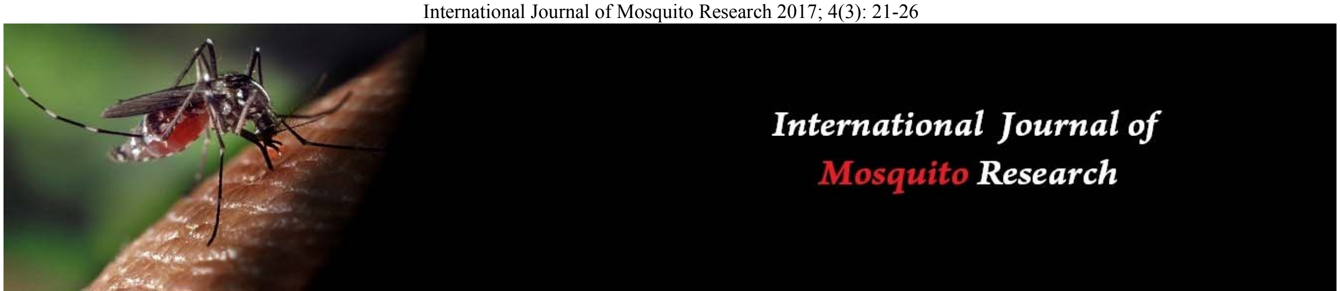

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Monitoring susceptibility of *Anopheles arabiensis* Patton, 1905 (Diptera: Culicidae) to the recommended insecticides in Gedarif state, Sudan

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Abstract
Gedarif state (eastern Sudan) is one of the endemic areas of malaria. The present study aims to monitor and determine the susceptibility of the adult malaria vector *Anopheles arabiensis* Patton, 1905 (Diptera: Culicidae) in two administrative localities in the state, following WHO standard protocols. These were Alfau (far west) and Alfashagha (Far East). The carbamate bendiocarb (1%), the organochlorine DDT (4%), and the two pyrethroids, viz. deltamethrin (0.05%) and permethrin (0.75%) were tested. The mortality results for Alfau and Alfashagha populations were as follows: bendiocarb 100% in both localities (susceptible); permethrin 95 and 96% (tolerant), deltamethrin 99 and 98% (susceptible), and DDT 76 and79% (resistant). DDT populations are still resistant, even though DDT was not introduced to the Sudan since 1996. This can be attributed to the intensive and extensive use of the pyrethroids and endosulfan. The cross-resistance is expected as a result of the shared metabolic pathways and mode of action of DDT and pyrethroids. Monitoring of resistance in yearly basis and searching for more adulticides and larvicides from other chemical groups as alternatives must be considered.

Keywords: *Anopheles arabiensis*, malaria, susceptibility to insecticides, bioassay, DDT, permethrin, deltamethrin, bendiocarb, Sudan, Gedarif state

1. Introduction

Mosquitoes (Diptera: Culicidae) are important vectors of several tropical diseases, including malaria, filariasis, and numerous viral diseases, *e.g*. dengue, Japanese encephalitis and yellow fever. There are about 3,000-3,520 species of mosquito, of which about 100 are vectors of human diseases [1, 2]. There are > 430 species of *Anopheles* world-wide, and of these about 40 species are important vectors of human malaria [3]. The most important vectors in sub-Sahara Africa (SSA; 45 countries) and the most efficient malaria vectors world-wide (109 countries) are *A. funestus*, *A. gambiae* Complex and *A. arabiensis* [3]. In SSA, about 90% of the clinical cases occur (*ca*. 500 million clinical cases worldwide /yr), and 1.1 to 2.7 million people die annually [4]. The Federal Ministry of Health of the Sudan [5] reported that malaria is affecting 52% of outpatients and accounting for 9% of all hospital deaths. Matambo *et al*. [6] have stated that 7.5 million cases and 35, 000 deaths are recorded annually in the Sudan. Malaria costs an estimated $12 billion in lost productivity in Africa. According to WHO [7].

The use of insecticides is the main strategy for controlling malaria vectors in the Sudan through indoor residual spraying (IRS), and the insecticide treated nets (ITNs) and recently the long lasting treated nets (LLITNs), in addition to larviciding. The currently used adulticides world –wide are DDT (not used in the Sudan since 1996), bendiocarb, permethrin and deltamethrin [8, 9]. ITNs and IRS interventions rely on the continuing susceptibility of *Anopheles* to a limited number of insecticides. Monitoring insecticides resistance in the main Sudanese malaria vector is essential for planning and implementing an effective vector control (VC) program [9]. However, insecticide resistance, in particular pyrethroid-DDT cross-resistance (CR), is a challenge facing malaria VC in Gedarif and other Sudanese States, because pyrethroids represent the only class of insecticides approved for treating bed nets, and also is commonly used for IRS. Chemical control has always been the main strategy of VC in Gadarif State. The use the insecticides to control mosquitoes and sand flies in Gedarif depends on pyrethroids, *viz*. two rounds /yr by cyfluthrin and permethrin, in addition to the organophosphate malathion from 1996 to 2002 [10]. In 2003 deltamethrin was introduced and used throughout the State until 2007.

From 2008-2013, spraying was limited to areas of the irrigated agricultural schemes in the state, where deltamethrin is also one of the major insecticides against agricultural pests alone or in mixtures [10].

The resistance mechanisms against the insecticides are metabolic, target site, penetration, and behavioral resistance [11]. The main defense against resistance is close surveillance of the susceptibility of vector populations [12]. The intensive use of insecticides in agriculture has caused concern, i.e. increased selection pressure. This may have negative implications for VBDs control [13].

Darriet [14] stated that in 1963, the WHO reported that 32 species of Anophelinae were resistant in Africa to DDT and dieldrin. Later, Elissa [15] reported the first case of pyrethroid resistance in *A. gambiae* in Côte d'Ivoire. In other regions of Africa, numerous cases were documented [15-19]. Malathion was first used against *A. arabinoses* in the irrigated cotton-growing area of Gezira, Sudan, in 1975. Laboratory tests showed that adult *A. arabiensis* were resistant to malathion [20]. Later, In central Sudan, it was found to be resistant to HCH and DDT [21]. In Gezira State and Sinnar (2008) s multiple resistance (MR) to permethrin, DDT and malathion was detected [22]. In Gedarif (2010) the vector was found be resistant to DDT and permethrin. Extensive resistance to permethrin and DDT is now being reported in populations from Kosti (White Nile State), and Damazin and Sinnar (Blue Nile State) [23].

Insecticide resistance in *A. arabiensis* has been reported in various Gedarif administrative localities to commonly used insecticides in VC used in the irrigated -area of Gedarif (e.g. Alfau; *viz*. Elrahad Agricultural Corporation; 300,000 acres). In 2010, the were resistant to DDT (67%), and permethrin (74%), and deltamethrin (91%). In the irrigated area 26 villages vector populations proved to resistant [24]. Himeidan *et al*. [25] study in Alfau detected the resistance to seven insecticides. Sulieman [26] divided resistance in the area into four categories: a) resistant to DDT 4% (88%), deltamethrin 0.05% (72.8%), malathion 5% (59.2%) and propoxur (54.4%); b) highly resistant to bendiocarb (12.8%); c) tolerant to fenitrothion 0.1% (96.8%), and d) susceptible to *lambda*-cyhalothrin 0.05% (99.2%). Abdalla *et al*. [9] conducted WHO susceptibility tests to monitor resistance to insecticides from all four WHO-approved classes of insecticides at four sentinel sites in the neighboring Gezira state over a three years period. Resistance mechanisms were studied using PCR and microarray analysis [27]. The e vector from all sites were fully susceptible to bendiocarb and fenitrothion. However, resistance to DDT and ;pyrethroids was detected at three sites, with strong seasonal variations. The knockdown allele was significantly associated with resistance to pyrethroids and DDT with extremely high effect sizes. Microarray analysis of the permethrin –resistant population from Wad Medani identified a number of metabolic genes that were significantly over-transcribed in the field collected resistant samples, when compared to the susceptible Sudanese *A. arabiensis* Dongola strain. These included two cytochrome P450 monooxygenase, previously implicated in pyrethroid-resistance in *A. gambiae* s.s., and the glutathione-S-transferase.

1.2 Problem Statement

The main goal of this study is to monitor and determine the susceptibility *A. arabiensis* adults in Gadarif State, *viz*. Alfau and Alfashaqa administrative localities, eastern Sudan, to four recommended insecticide, *i.e.* bendiocarb, deltamethrin, permethrin, and DDT using WHO procedures.

1.3 Objectives

1.3.1 General objective

To investigate current *A. arabiensis* adults susceptibility level to four commonly used adulticides in Alfau and Alfashagha administrative localities in Gadarif State, as a tool for vector management by decision-makers in the state and the localities, and as part of the resistance mapping project for mosquitoes and other vectors of the Blue Nile National Institute for Communicable Diseases (BNNICD) and the Ministry of Health.

1.3.2. Specific objectives

1.3.1To determine susceptibility of *A. arabiensis* adults towards DDT 4%, the pyrethroids deltamethrin 0.05% and permethrin 0.75%, and the carbamate bendiocarb 0.1% in Alfau (irrigated) and Alfashagha (rainfed) localities in Gedarif state (map), adopting WHO discriminating doses.

1.3.2. To estimate the time required to knock-down 50% (KdT50) and 95% (KdT95) of the adults exposed to the same above-mentioned concentrations of these adulticides.

2. Materials and Methods

2.1 Study area

Gadarif (75,000 km²) is an agricultural state; population is 1.8 million. The state is surrounded by the borders of Kassala state and the Ethiopian western borders from the east, the Gezira state from the west, Sennar state from the south, and Khartoum state (the capital) from the north. The state lies between longitudes 30◦ 20 and 30° 36 E, and between latitudes 40° 12 and 40°15 N. The climate is tropical continental with an estimated annual rainfall of approximately 636 mm, occurring mainly between June and mid-October. The mean annual temperature is 28.8 °C. January, is the coldest month (Mean: 25.8 °C) and April is the warmest month of the year (Mean: 32 °C) [28].

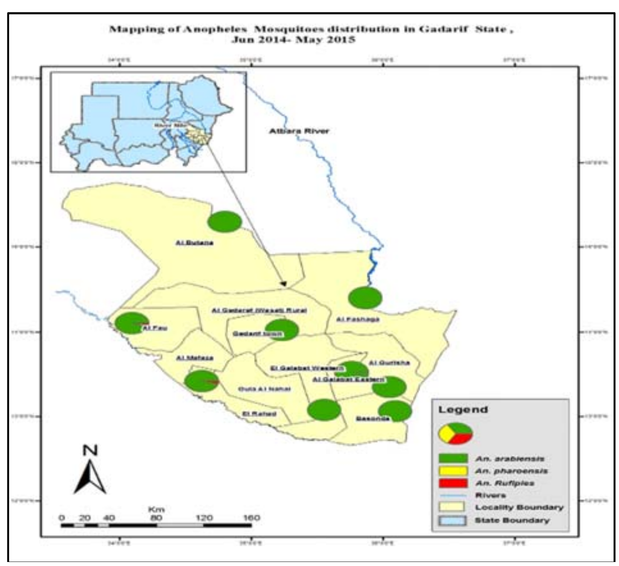

Fig 1: Map by GIS for Gedarif State showing all the administrative localities (Alfashagha far east and Alfau far west) in addition to species distribution, Cobani *et al*. [28]

2.2 Study design
This study design was cross-sectional and carried out by collection of the immatures, *i.e*. the aquatic stages from the breeding sites.

2.3 Sampling technique and sample size
The samples were collected from Alfau and Alfashagha as immatures. Different sites from each area were represented in the sampling (total of nine villages). The larvae were collected weekly for the period January to March. Sampling was conducted by using standard larval collection procedures, employing dippers screened nettings, plastic pipettes, and metal dishes /bowls. Larvae collected from each site were pooled together in plastic buckets and transferred to the laboratory in Gedarif town. Larvae were transferred to rearing trays and fed on fish powder. The pupae were collected from the trays using wide-mouthed pipettes, placed in plastic cups containing clean tab- water and placed inside adult cages. Emerging adults were fed only on 10% sugar solution (sucrose) and used for the bioassay.

2.4 Bioassay
Susceptibility tests were conducted according to the standard WHO procedures [2]. Females (1-2 days-old) were tested as 25 mosquitoes / exposure (1hr) and control tube (4 replicates each) /each insecticide in each locality. During the exposure time (1hr), numbers of knocked- down (Kd) mosquitoes were recorded after 10, 15, 20,30,40,50 and 60 min. Following the exposure period, mosquitoes were transferred into the holding tubes lined with untreated papers, provided with 10% sucrose solution and allowed a 24 hr recovery period after which mortality was recorded (%M). Control groups were exposed to the control papers (impregnated only with silicone oil) for 1 hr. From these experiments, the LC50s and LC90s of were calculated.
The *Kd* data is expected to reflect the effect of exposure time on Kd (temporary paralysis). Their mortality after 24 hr is based on the exposure time (T) and the concentration (C) (T x C) of the insecticide absorbed by the treated individuals. Comparing these results with the previous results obtained by former investigators might reflect the occurrence/ level of resistance in the field [2].
The treatments were as follows (all insecticides provided by WHO):
DDT 4%, permethrin 0.75%, deltamethrin 0.5%, bendiocarb 1%, and untreated control.
Data was presented in tables and histogram. The resistance / susceptibility status of the tested populations was determined for each insecticide according to WHO [30] criteria. A resistant population is defined by mortality rates < 80% after the 24 hr observation period, while mortality rates > 98% are indicative of susceptible populations. Mortality rates between 80–98% suggest a possibility of resistance (suspected resistance).

3.7 Statistical Analysis
Data was subjected to SPSS software and Probit analysis to determine KdT_{50}, KdT_{95},. and % mortality after 24 hr.

4. Results
4.1 Knock-down (Kd)
In table (1) the time (T) required for knocking down 50% (KdT_{50}) and 95% (KdT_{95}) of the exposed *A. arabiensis* females to the insecticides tested were calculated. For bendiocarb 0.1%, the KdT50 in Alfashaqa locality was found to be 7.8 min., and in Alfau locality 3.19 min. However, $KdT_{95's}$ were 60.8 and 48.12 min, respectively. Deltamethrin KdT 50% was 6.9 min in Alfashaqa, and 7.7 min in Alfau locality. Regarding the KdT_{95}, the respective values were 66.10 and 57.06 min. Permethrin KdT50s were 6.9 and 6.13 min in Alfashaqa and Alfau localities, respectively. The KdT_{95s} values, for the same areas were 56.6 and 23.7 min, following the same order. DDT KdT 50 in Alfashaqa and Alfau localities were 3.6 in and 3.8 min., respectively, whereas the KdT_{95}s were 226 and 341.8 min, following the same order of localities.

Table 1: KdT50 and KdT95 for *A. arabiensis* collected from Alfashagha and Alfau localities.

No.	Insecticides	KdT50 (min) (95% CL)	KdT95 (min) (95% CL)	%Mortality (24hr)
1	Bendiocarb AlFash Alfau	7.8 (1.1- 13.0) 3.19 (2.14-2.26)	60.6 (38.9- 289.5) 48.12 (41.36-58.89)	100 100
2	Deltamethrin Alfash. Alfau	6.9 (4.6- 9.05) 7.7 (5.20-9.9)	66.10 (53.10- 92.6) 57.06 (46.5-77.10)	99 98
3	Permethrin Alfash. Alfau	6.9 (5.6- 8.1) 6.13 (2.40-9.51)	58.6 (51.8- 68.4) 58.7 (74.6-52.4)	95 96
4	DDT Alfash. Alfau	61.6 (0.665-7.1) 3.8 (2.1-5.6)	226 (114.0-128.0) 341.8 (213.2-715.2)	76 79

4.2 Mortality
From table (1) and figs. (1 and 2) data showed that bendiocarb was very effective in both localities, killed 100% of the adults with 24hr. deltamethrin ranked second and killed 99% of Alfashagha population and 98% of Alfau population. The other pyrethroid permethrin mortalities were 95% and 96%, respectively. However, DDT killed 76% of Alfashagha population and 79% of Alfau population.

Table (2) shows the average for both localities. For bendiocarb, the average was 100% (susceptible). For deltamethrin the average was 98.5%, which is also susceptible. However, for permethrin the average was 95.5%, indicating that it is tolerant. Finally, for DDT, the average was 77.5 (resistant).

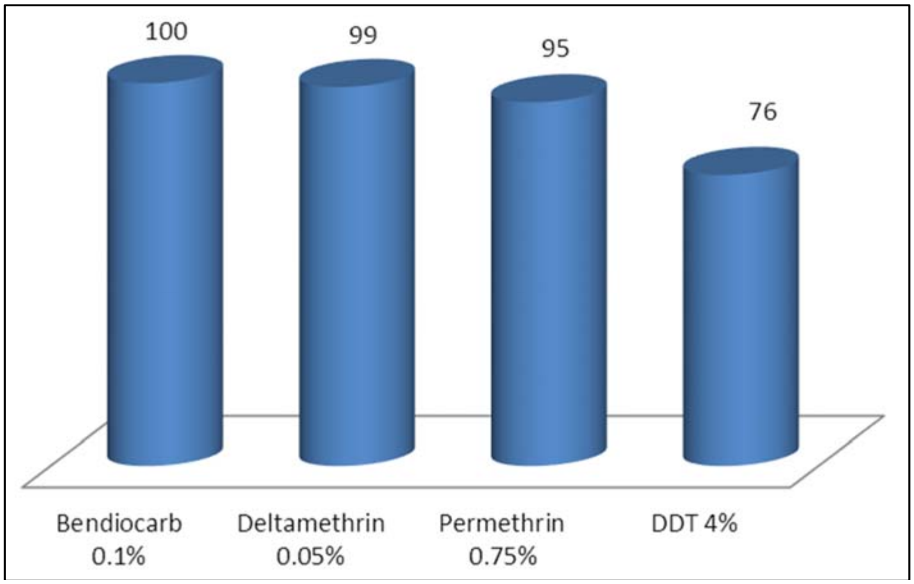

Fig 2: Percentage mortality for female *A. Arabiensis* after 24 hr to four insecticides in Alfashagha locality (resistant for DDT, tolerant for permethrin and susceptible for deltamethrin and bendiocarb)

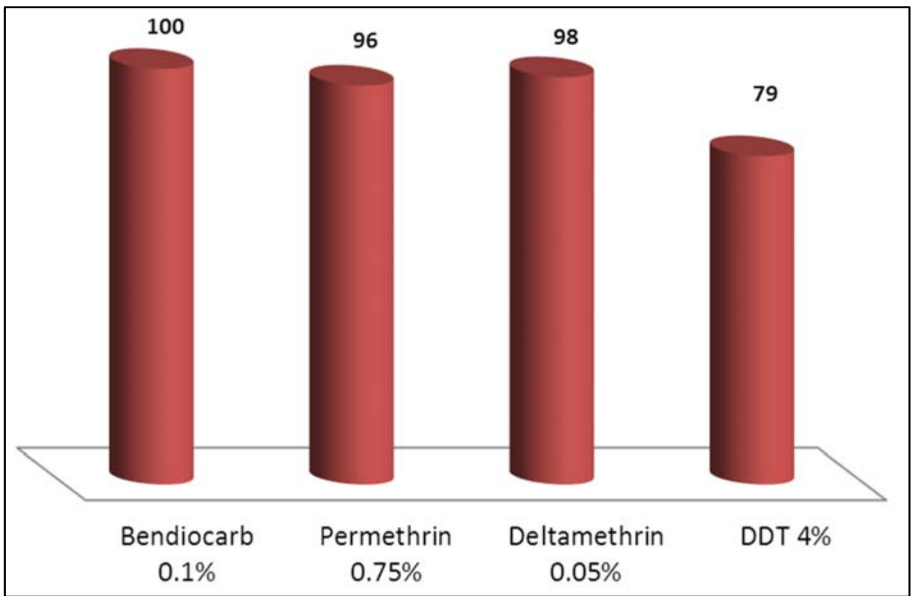

Fig 3: Percentage mortality for *A. arabinoses* after 24 hr to four tested insecticides in Alfau locality (Resistant for DDT, tolerant for permethrin, susceptible for both deltamethrin and bendiocarb).

Table 2: Average (of the 2 locations) of the percent mortality for *A. arabiensis* 24 hr after exposure to the tested insecticides.

Treatments	Average % mortality (treatments)	Remarks
DDT 4%	77.5	Resistant
Deltamethrin 0.05%	98.5	Susceptible
Permethrin 0.75%	95.5	Tolerant
Bendiocarb 0.1%	100	Susceptible
Control	0	

3. Discussion

The study revealed that the populations of the two localities are susceptible to the carbamate bendiocarb causing 100%mortality. However, the pyrethroids permethrin (95% and 96, Alfashagha and Alfau, respectively), and deltamethrin (99% and 98%, following the same order), did not behave similarly. Permethrin results proved that the populations of the two localities are not susceptible to it, whereas the deltamethrin proved to be more effective, *i.e.* resistance started to develop against the former that can be attributed to its date of introduction to Gedarif and to the Sudan, in addition to that it was one of the substitute of the organochlorines in agriculture where it was used alone or in mixtures with some Ops, namely in cotton and vegetable crops by the end of 1970's. Alfau is the headquarters of Elrahad Agricultural Corporation (300,000 acres), which is shared between Gedarif State and Gezira State. The field crops in the rain-fed areas of the Sudan are not usually sprayed/treated with pesticides, especially sorghum, groundnuts and sesame.

DDT resulted in 76% and79% mortality for Alfashagha and Alfau, respectively. This indicates, according to WHO criteria that both populations of both localities still resistant to DDT. As mentioned earlier, all organochlorines were banned in agriculture in the Sudan since 1980/81 season. The last batch received for public health was in the year 1996. The only remaining of these pesticide, which is intensively and extensively used now is the cyclodiene endosulfan in controlling field crop pests, mainly cotton pests. Currently, Gedarif State became one of the major rainfed cotton producing states. This necessitated the use of insecticides and herbicides, especially the broad-spectrum endosulfan. Therefore, the resistance to DDT might be attributed to the use of endosulfan in agriculture and the dependence on permethrin and deltamethrin in controlling the adults whether as IRS or ITNs or as LLTNs. Continuous exposure, leads to continuous selection- pressure, induction of specific enzymes, *viz*. hydrolases, oxidases and reductases, glutathione-S-transferases, ending up by developing metabolic resistance in terms of cross- and/or multiple- resistance [10, 16, 20, 31-36]. As mentioned earlier, the bioassay conducted in 2010 showed that adults were resistant to DDT (67%), and permethrin (74%), and deltamethrin (91%). In the present work these percentages have changed where DDT mortality changed from 67% to an average of 77.5%; permethrin from 74% in 2010 to 95.5%. For deltamethrin, the % mortality changed from91% to 98.5%. This indicates that the level of resistance for the three insecticides is decreasing. Himeidan *et al*. [25] study in Alfau detected the resistance to seven insecticides. Sulieman [26] in the same area reported different results and showed that DDT 4%killed 88%, deltamethrin 0.05% (72.8%), malathion 5% (59.2%) and propoxur (54.4%). Moreover, surprisingly, the population was highly resistant to bendiocarb (12.8%). The author reported tolerance to fenitrothion 0.1% (96.8%), and susceptibility to *lambda*-cyhalothrin 0.05% (99.2%).

To prevent or delay the development of resistance in mosquitoes, especially the malaria vectors, the decision-makers must set programs for ecologically-sound and practical use of the very limited arsenal of insecticides suitable for adults and larval control. Depending mainly and continuously on pyrethroids will lead to devastating crisis (MR, CR, larger doses, frequent application, contamination, and destruction of non-targets). It is obvious that the agricultural and health authorities of the state must have harmonized activities during the seasons, especially when these activities involve pesticides. Timing, active ingredient, formulation and the application techniques must be planned in advance, especially during the rainy-season for Ministry of Health authorities. Mosquitoes have fast generations, consequently, this must be taken into consideration when planning. Continuous monitoring for resistance is not a luxury, it is a necessity.

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