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First evidence of occurrence of East African knock-down resistance mutation (L1014S): Insecticide resistance and *Plasmodium* infection in populations of *Anopheles arabiensis* Patton in Khartoum State, Sudan

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Abstract
Continuous monitoring of the susceptibility/resistance status in malaria vectors is important for implementing an effective control program. This study was conducted to assess the susceptibility status, the occurrence of the knock-down resistance gene (*kdr*) mutation and to detect *Plasmodium* infection in *An. arabiensis* in Khartoum State. WHO Insecticide susceptibility tests were conducted in *An. arabiensis*. The *kdr* and *Plasmodium* infection in *An. arabiensis* were also studied using PCR. *Anopheles arabiensis* from resistant to malathion, dichlorodiphenyltrichloroethane (DDT) and permethrin. Both *kdr* mutant alleles (L1014F and L1014S) and *Plasmodium falciparum* infection were detected in *An. arabiensis* as well. This is the first report on occurrence of L1014S allele in *An. arabiensis* in Khartoum State. The occurrence of the *kdr* mutation has a negative impact on the malaria control in Sudan since pyrethroids are used for vector control.

Keywords: *Anopheles arabiensis*, insecticide resistance, *kdr*, Sudan.

1. Introduction
Malaria still represents a serious public health problem in Sudan, and it causes high morbidity and mortality. Malaria infections *Plasmodium falciparum* and *P. vivax* [1]. The disease is transmitted via females of *Anopheles arabiensis* [2, 3]. *Anopheles arabiensis* has a wide geographical distribution in the country [3, 4, 5]. Although, *An. arabiensis* is more zoophilic and exophilic in northern part of Sudan [6], it showed some degree of anthropophilic and endophilic behaviour in Khartoum State [3].

In Sudan, the current malaria vector control strategy relies on the application of large-scale distribution of insecticide-treated bed nets (ITNs/LLINs), larval source management (LSM) and indoor residual spray (IRS) [7, 8]. However, in Khartoum State, the control of malaria vector has been adopted by weekly treatment of larval habitats by Temephos® EC50 and environmental management [9]. The environmental management includes, intermittent irrigation in the irrigated agricultural schemes as well as rehabilitation and repairing of broken water pipes in urban areas [9]. As a result, resistance to most insecticide classes, especially pyrethroids, has recently emerged in Sudan [7, 10].

Due to continuous and intensive use of insecticides, a common form of resistance in malaria vectors known as knock-down resistance (*kdr*) has occurred. This type of resistance is due to substitution in amino acids in *para*-type voltage-gated sodium ion channel gene [11]. The *kdr* mutation was first described in *An. gambiae* s.s. in coastal Ivory Coast in West Africa [12]. The mutation was due to substitution at amino acid position 1014, resulting in leucine to phenylalanine (TTA to TTT) (L1014F) [13]. Then after, this type of *kdr* allele mutation was also detected in populations *An. gambiae* s.s in Benin, Burkina Faso, Mali and Ghana [14, 15, 16]. Likewise, a second type of *kdr* mutation due to a substitution in amino acid at the same position observed in West African mosquitoes but resulting in leucine to serine (TTA to TCA)

(L1014S) was recorded in *An. gambiae* in Kenya [11]. More recently, due to the scaling up of the recent ITNs/LLINs programmes both types of *kdr* alleles have been reported in *An. arabiensis* and *An. gambiae* s.s. from several countries in East and West Africa [17, 18] including Sudan [7, 10].

Although, the use of dichloro-diphenyl-trichloroethane (DDT) and Pyrethroids have a long history, few studies have been conducted to detect the occurrence and distribution of *kdr* allele in *A. arabiensis* in Sudan [7, 10]. Likewise, such information is scare in Khartoum State where few studies were conducted to elucidate the occurrence of *kdr* mutation in *A. arabiensis* in the state [8]. Therefore, this study was carried out to determine the occurrence of the *kdr* mutations in *An. arabiensis* populations in Khartoum State. Attempts were also made to detect the *kdr* mutation in concomitant with *Plasmodium* infection in *An. arabiensis*. Here is a first report on the occurrence of L1014S-*kdr* mutation in a malaria vector in Khartoum State.

2. Materials and methods

2.1. Khartoum State

Khartoum State is located at the concourse of the Blue Nile and the White Nile (15°30' - 15°45' N and 32°15' - 32° 45' E) (Fig. 1). The state covers a total area of 28,000 km^2. The state is divided into three main administrative areas: Khartoum, Khartoum North and Omdurman. The majority of the area lies within the semi-desert region, with the northern part being mostly desert climate. Vegetation in state consists of dry desert scrub and riverine systems. The state has a cold dry winter between November - March, a hot dry summer between April -July, and a wet rainy season between August - October. The temperature in the winter ranges from 15 to 25 in winter, 25 to 40 ℃ in the summer and 20 to 35 ℃ in the rainy season. The averaged humidity varied between 36%-64%, it reaching its maximum in August and minimum in January.

Farming activities, water pipe leakage and rain water represent the main sources of mosquito larval habitat in the state.

2.2. Study sites

Nine sites were selected in Khartoum State as fixed sites for larval collection. These sites were categorized as urban and periurban areas. The urban areas are; Arkaweet, Shambat, Abu'siid. The periurban areas are; Soba West, Edekheinat, Elmaygoma, Eltumanyat, Elsalamania and Gizera Islang (Fig. 1). Also, additional sites were also selected to collect wild adult anopheline mosquitoes. The study sites cover almost about 20,140 km^2 and occupied the area between 15°.10'-16°.30' N and (31°.35'-34°.20' E (Fig. 1). The collection sites were also categorized into urban and periurban areas.

2.3. Sampling of Anopheline mosquito, rearing and morphological identification

Mosquitoes were collected as larvae from larval habitats in selected sites during June - December 2014. Larvae were collected using standard collection methods including scoops, pipettes and collection nets. Larvae and pupae were then kept in plastic bottles (2 litres) and transported to the insectary at Khartoum Malaria Free Project (KMFP). In the insectary, the larvae of anopheline mosquitoes were put in trays and provided with Tetramin1 fish. The pupae were transferred into glass beakers (250 cm^3) and put in mosquito rearing cages. The emerged adults were maintained on a 10% sucrose solution on filter-paper wicks until they subsequently used in WHO insecticide susceptibility tests.

Wild adult female mosquitoes were sampled during June - December 2014 and November - December 2015. The wild mosquitoes were using mouth aspirators from resting sites at outdoors and indoor sites. The sampled adult females were put individually in 1.5 ml eppendorf tubes containing silica gel. The preserved samples were then kept at room temperature until being analyzed by PCR to detect *Plasmodium* parasites (sporozoites) and *kdr* mutations. Based on morphological features, the sampled mosquitoes were first identified using standard entomological keys [19].

2.4. Insecticide susceptibility test using WHO kits

Five insecticides were selected to determine the susceptibility/resistance status of *An. arabiensis* collected from nine sentinel sites in Khartoum State. These insecticides have been used for mosquito control in Sudan. Using WHO standard procedures, non-blood fed females, 2-3 days old were exposed to discriminating dosages of DDT 4%, fenitrothion 1%, malathion 5%, permethrin 0.75% and deltamethrin 0.05% for 1 hour [20]. The females used were colony-reared mosquitoes and morphologically identified as *An. gambiae* s. l. The WHO impregnated papers of these insecticides were obtained from KMFP, Khartoum State Ministry of Health. For each insecticide, batches of 25 sugar fed females were used with controls included a similar number of mosquitoes exposed to untreated papers. Numbers of knocked down and dead mosquitoes during exposure time were recorded at intervals of 10 minutes. After one-hour exposure time, the knock-down and the alive individuals were put in a new WHO clean tubes. The alive female mosquitoes were provided with a sucrose solution (10% w/v) on cotton. Final mortalities were then recorded after 24 hours of exposure to each insecticide.

The efficiency of impregnated papers used in this study was assessed using a proven susceptible strain of colony-reared *An. arabiensis* which maintained at the Tropical Medicine Research Institute, National Centre of Research, Khartoum since 2004.

All insecticide susceptibility tests were conducted at KMFP insectary under optimum conditions (temperature 26℃ and 70 - 80% relative humidity).

2.5. Molecular analysis

2.5.1. DNA extraction

Genomic DNA was extracted from individual female mosquitoes using the LIVAK buffer method [21]. The DNA pellets were then suspended in100-µl of 1X TE-buffer. The extracted DNA solutions were then kept at -20℃ for subsequent PCR analysis.

2.5.2. Molecular identification and detection of the *kdr* mutation

The genomic DNA of individual females *An. arabiensis* were tested using species-specific primers (A^o: ATG CCT GAA CGC CTC TAA GG and A^{05}: CAA GAT GGT TAG TTA CGC CAA). PCR conditions used in this study were similar to those described by Scott *et al*. [22]. These species-specific primers gave PCR amplicons of 500 bp band sizes a characteristic for *An. arabiensis*.

A total of 751 (661 from 2014 to 90 from 2015) samples t in

the two years were assayed for *kdr* mutation. In these PCR reactions, primers Agd1 (5'-ATA GAT TCC CCG ACC ATG-3') and Agd3 (5'-AAT TTG CAT TAC TTA CGA CA-3') were used to amplify the L1014F *kdr* mutation. The primers Agd1 and Agd5 (5'-TTT GCA TTA CTT ACG ACT G -3') were also used to amplify the L1014S *kdr* mutation. In each of the above multiple PCR reaction, the wild allele was analyzed using primers Agd2 (5'-AGA CAA GGA TGAT GA ACC-3') and Agd4 (5'-CTG TAG TGA TAG GAA ATT TA-3').

2.5.3. Sporozoites detection by nested PCR

Samples *An. arabiensis* (n = 751) collected in the years 2014 and 2015 were subjected to nested PCR for detection of *Plasmodium* sporozoites. In this assay, two amplification reactions were carried out as described by Snounou *et al*. [23]. The PCR condition for each amplification reaction was 95℃ for 5 min followed by 25 cycles of: 94℃ for 30 sec, 58℃ for 2 min, 72℃ for 2 min, final extension at 72℃ for 5 min. *Plasmodium* genus specific SSUr DNA primers rPLU5 (5′-CCTGTTGTTGCCTTAAACTTC-3′) and rPLU 6 (5′-TTAAAATTGTTGCAGTTAAAACG-3′) were used in the first amplification reaction (Nest 1). In the second amplification reaction (Nest 2), species-specific primers were used to detect infection with *P. falciparum* (rFAL1; 5′TTAAACTGGTTTGGGAAAACCAAATATATT-3′ and rFAL2: (5′ACACAATGAACTCAATCATGACTACCCGTC3′) and *P. vivax* (rVIV1: 5′CGCTTCTAGCTTAATCCACATAACTGATAC3′ and rVIV2: 5′ACTTCCAAGCCGAAGCAAAGAAAGTCCTTA-3′).

Reference strains of malaria parasites (i.e. *Plasmodium falciparum* and *P. vivax*) and sterile water (PCR-water) were employed in these assays as positive and negative controls, respectively. In the Nest 2, DNA amplicons of 120 bp and 205 bp were regarded as positive for *P. falciparum* and *P. vivax*, respectively.

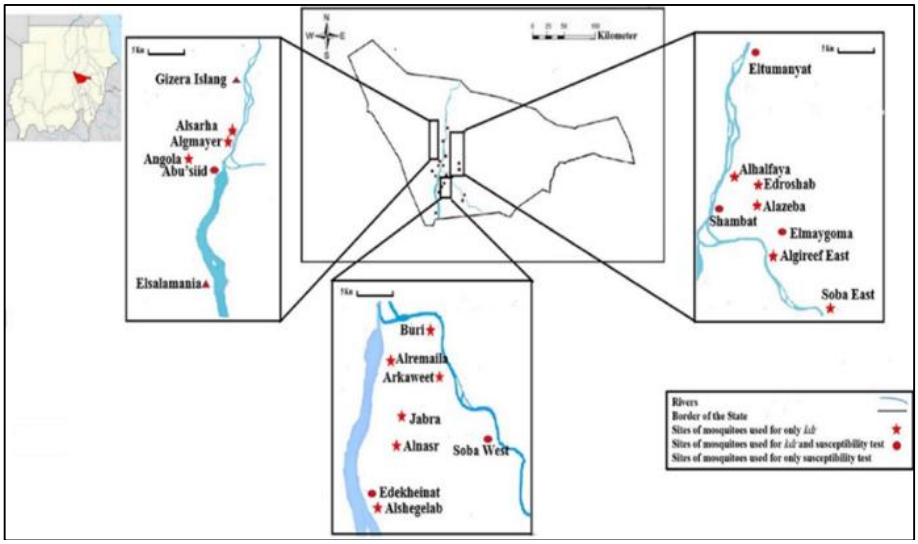


Fig 1: A map of Khartoum State showing; sentinel sites of mosquito larvae and pupae collection to conducted insecticide susceptibility and, wild anopheline adult sampling for *kdr* and *Plasmodium* parasites infection.

2.6. Statistical analysis

The mortality rates after 24 hours of exposure to insecticide were calculated as the number of dead females/the total tested mosquitoes. The resistance/susceptibility status of the tested *An. arabiensis* due to each insecticide was also determined according WHO criteria [25]. The mortality rate ≥ 98%, 90-97% and < 90% indicates susceptible, suspected resistance and resistant individual mosquitoes [25]. Using SPSS software version 20, Kruskal–Wallis and Mann–Whitney tests were employed to assess the variation in the mortality among *An. arabiensis* between sentinel sites and between urban and periurban areas, respectively. The knock-down times (minutes) of 50% and 95% (KDT_{50} and KDT_{95}) exposed *An. arabiensis* were recorded by Probit analysis. The knock-down resistance ratios (KRR) were calculated by dividing KDT_{50} of the tested population/KDT_{50} of the area with the shortest time.

The frequencies of *kdr* genotypes in *An. arabiensis* from different sites were compared by Chi^2 tests using SPSS software version 20. The frequencies of L1014F and L1014S genotypes were compared to Hardy-Weinberg expectations using the Court's online calculator. L1014F and L1014S mutations were considered as independent bi-allelic loci because these alleles do not target the same base at the DNA level in codon 1014.

2.7 Ethical considerations

The protocol of this study was reviewed and approved by the review board of the College of Medical Laboratory Sciences, Sudan University of Science & Technology, Ministry of Higher Education and Scientific Research, Sudan (Approval No: MLS-IEC-03-11).

3. Results

3.1. Mosquito identification

All specimens of anopheline mosquitoes randomly selected from the samples were identified morphologically and by PCR as *An. arabiensis*.

3.2. Results of insecticide susceptibility tests

The overall results of susceptibility test revealed that *An. arabiensis* was susceptible to fenitrothion (100%), suspected resistance to DDT (90.0%) and deltamethrin (94.0%), and confirmed resistance to malathion (84.0%) and permethrin (89.0%). Significant differences were observed in mortality

rates in *An. arabiensis* between the sentinel sites due to insecticides (For DDT χ^2 = 41.108, df = 7, P = 0.00; for permethrin χ^2 = 14.566, df = 4, P = 0.006 and for deltamethrin χ^2 = 20.897, df = 8, P = 0.007).

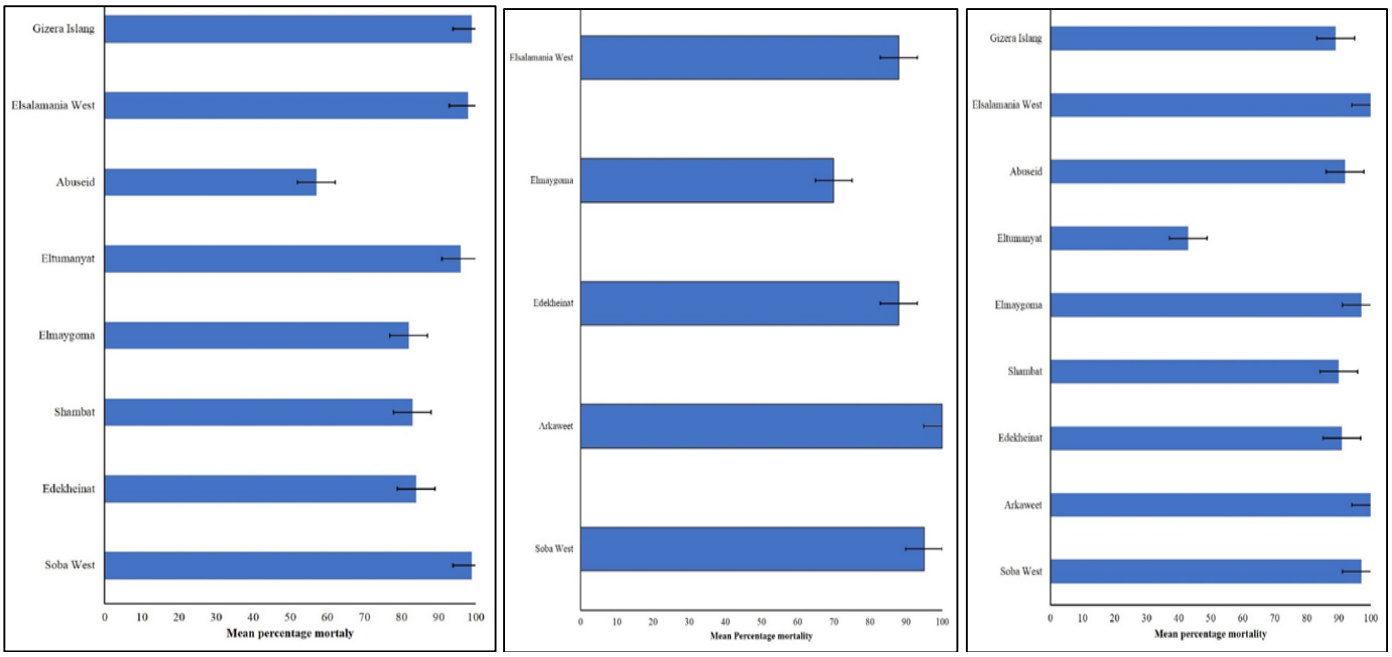
The mortality rates in *An. arabiensis* due to DDT, permethrin and deltamethrin insecticides in the nine sentinel sites are presented in figure *2* (A, B and C). The results showed that *An. arabiensis* from four sentinel sites were resistant to DDT with the lowest mortality rates in periurban areas of Elmaygoma (82.0%) and Edekheinat (83.0%). Mortality due to the permethrin was low in *An. arabiensis* from Elmaygoma (71.0%), Edekheinat (88.0%) and Elsalamania (89.0%). *Anopheles arabiensis* from the periurban areas of Eltumanyat (76.0%) and Gizera Islang (88.0%) were resistant to deltamethrin.

Knock-down times for *An. arabiensis* from the three administrative areas in Khartoum State are depicted in table 1. Significant differences in knock-down times in *An. arabiensis* were observed between the three administrative areas for all insecticides tested; DDT 4% (χ^2 = 244.42, df =58, P = 0.00), fenitrothion 1% (χ^2 = 9307.84, df= 500. P= 0.00), malathion 5% (χ^2 = 2413.78, df = 472, P= 0.00), permethrin 0.75% (χ^2 = 814.11, df = 135, P = 0.00) and deltamethrin 0.05% (χ^2 = 802.65, df = 282, P = 0.00).

The knock-down resistance ratios (KRR) for *An. arabiensis* tested against the five insecticides were relatively similar between the populations of the three administrative areas (Table 1). The exception was for DDT 4% which showed KRR in *An. arabiensis* from Khartoum area that was highest by 1.4-fold than those in the two other areas.

The mortality rates in *An. arabiensis* due DDT, permethrin and deltamethrin insecticides in the urban and periurban area are shown in figure *3*. Significant differences in the mortality rates were observed between the *An. arabiensis* from urban and periurban areas due to DDT, malathion and permethrin (P = 0.042, 0.005 and 0.003, respectively). In the urban area, *An. arabiensis* was resistant to malathion and suspected resistance to DDT, permethrin and deltamethrin (Fig. *3*). In the periurban areas, *An. arabiensis* was tolerant to DDT, malathion and permethrin, and suspected resistant to deltamethrin (Fig. *3*).

Variation in proportions of *An. arabiensis* from the urban periurban area was knocked down after a 24-hours exposure to DDT, fenitrothion, malathion, permethrin and deltamethrin insecticides (60 minutes for all insecticides expect 80 minutes for fenitrothion) (Table 2). A significant difference in knock-down time in *An. arabiensis* for only DDT was observed between the two areas (t = 3.435, df = 82 and P = 0.001) (Table 2). The knock-down resistance ratio (KRR) for DDT, malathion, permethrin and deltamethrin in the periurban areas were higher by 1.4, 1.1, 1.3 and 1.4-fold than in urban areas. In contrast, KRR for fenitrothion in urban areas was a 1.1-fold of that in the periurban areas.



(A) DDT 4%; **(B)**; Permethrin; **(C)**; deltamethrin 0.05%

Fig 2: Mean percentages mortality rates of *Anopheles arabiensis* from nine sentinel sites in Khartoum State (Sudan) exposed to the insecticides: **(A)** DDT 4%; **(B)**; permethrin; **(C)**; deltamethrin 0.05%.

Overall, L101F and L1014S point mutations detected in *An. arabiensis* were found at very low allelic frequencies. The allelic frequencies of L1014F and L1014S in *An. arabiensis* specimens collected from different sites ranged between 0-0.268 and 0-0.250, respectively (Tables 3 and 4). For the L101F point mutation, the allelic frequency was significantly different between the sentinel sites (χ^2 = 163.2, df = 19, P = 0.00) where the highest values were observed in the Alshegelab (0.268) and Edroshab (0.260) sites in Khartoum and Khartoum North areas, respectively. However, out of the four populations of *An. arabiensis* recorded with no L101F point mutation, three were collected from Omdurman area (Abu'siid, Alsarha and Algmayer sites). In contrast, the highest allelic frequency for the L1014S allele was observed in Edekheinat site (0.250). The genotype frequencies of *kdr*-mutation observed in *An. arabiensis* populations was found to be deviated from the expected frequencies that predicted by the Hardy-Weinberg equilibrium (χ^2 =137.53, P = 0.00) with

0.08 variation in the allelic frequency. However, when considering only L1014F, no deviation was observed between the genotype frequencies detected in mosquito populations and the expected frequencies that predicted by the Hardy-Weinberg equilibrium (χ^2 = 2.82, P = 0.092) with 0.04 variation in allelic frequency. In contrast, when considering L1014S only, the frequency of the genotype in the populations is deviated from the expected frequencies that predicted by the Hardy-Weinberg equilibrium (χ^2 = 118.68, P = 0.00) with 0.07 variation in allelic frequency.

3.4. PCR detection of *Plasmodium* parasites in *Anopheles arabiensis*

No *Plasmodium* parasites were detected in all samples of *An. arabiensis* (n = 661) collected during the year 2014 However, 6 out of 90 (6.7%) of females collected during 2015 were infected with *P. falciparum*. Of the 6 infected females of *An. arabiensis*; 3 (6.8%; 3/44) were from Soba West, 2 (6.5%; 2/31) from Elmaygoma and 1 (14.3%; 1/7) from Alazeba sites.

Table 1: Mean mortality rates and knock-down time of *Anopheles arabiensis* from Khartoum State exposed to insecticides.

Insecticide used	Site	Number exposed (Replicates)	*KDT50* (in min) (95% Cl)	*KDT95* (in min) (95% Cl)	*KDT50* ratio (RR)
DDT 4%	Khartoum	475 (19)	36.81 (35.24 ± 38.46)	84.10 (78.45 ± 90.89)	1.1
	Khartoum North	825 (33)	53.65 (51.29 ± 56.23)	122.56 (113.23 ± 133.97)	1.5
	Omdurman	400 (16)	34.80 (32.76 ± 36.97)	79.50 (73.42 ± 86.76)	1.0
Fenitrothion 1%	Khartoum	800 (32)	69.40 (61.02 ± 83.04)	156.30 (120.8 ± 235.5)	1.1
	Khartoum North	650 (26)	60.94 (53.88 ± 71.75)	137.24 (107.58 ± 201.88)	1.0
	Omdurman	350 (14)	80.72 (66.0 ± 105.27)	181.80 (133.45 ± 292.42)	1.3
Malathion 5%	Khartoum	700 (28)	43.87 (40.88 ± 47.21)	119.18 (105.97 ± 136.58)	1.0
	Khartoum North	900 (36)	48.40 (45.78 ± 51.37)	131.50 (117.40 ± 150.23)	1.1
	Omdurman	300 (12)	43.55 (39.70 ± 47.89)	118.31 (103.89 ± 137.25)	1.0
Permethrin 0.75%	Khartoum	400 (16)	48.62 (45.83 ± 51.73)	89.23 (81.23 ± 100.06)	1.1
	Khartoum North	100 (4)	46.72 (39.32 ± 56.15)	125.33 (99.24 ± 169.02)	1.0
	Omdurman	150 (6)	45.86 (38.57 ± 55.15)	123.03 (97.53 ± 165.56)	1.0
Deltamethrin 0.05%	Khartoum	300 (12)	36.45 (33.14 ± 40.31)	97.79 (82.00 ± 123.74)	1.1
	Khartoum North	475 (19)	31.42 (29.68 ± -33.21)	67.09 (62.53-72.43)	1.0
	Omdurman	400 (16)	31.97 (30.50 ± 33.52)	68.28 (64.04 ± 73.26)	1.0

4. Discussion

Anopheles arabiensis is the only malaria vector in Sudan [3, 4]. Several studies revealed that population of *An. arabiensis* in Sudan resistant to major insecticide classes [7, 8, 9, 10]. However, population *An. arabiensis* from different geographic regions in Sudan was susceptible to fenitrothion (organophosphate) and bendiocarb (carbamates) [9]. Bendiocarb has been used for IRS in Sudan since 2007 because the malaria vector, *An. arabiensis* has developed a strong resistance to the permethrin [7, 8, 9, 10].

In Khartoum State, the use of pesticides for agriculture and insecticides for vector control might have an impact on development of high resistance in populations of malaria vectors. This might be the reason that the populations of *An. arabiensis* from the urban and periurban areas were not completely susceptible to each of the insecticides tested in this study. The results of overall mean mortality revealed that *An. arabiensis* was fully susceptible to fenitrothion, resistance to Malathion and permethrin and suspected resistance to DDT and deltamethrin. In the urban area, *An. arabiensis* was resistant to Malathion; whereas, in the periurban areas, it was tolerant to DDT, malathion and permethrin. Moreover, significant differences in the knock-down rates between urban and periurban areas were observed for DDT (P = 0.001).

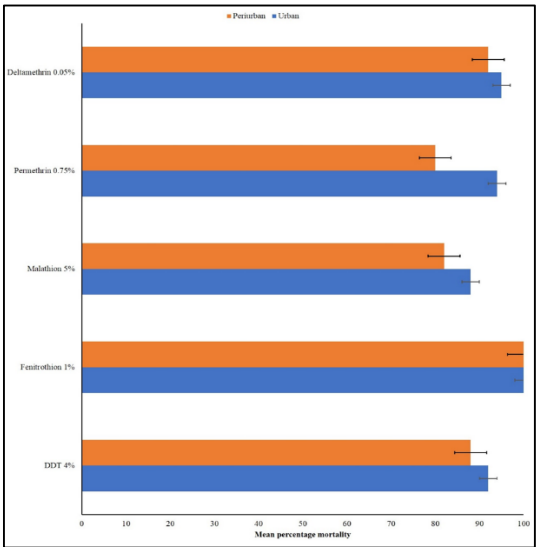


Fig 3: Mean percentages mortality rates of *Anopheles arabiensis* from urban and periurban areas in Khartoum State (Sudan) exposed to the insecticides DDT 4%, Fenitrothion 1%, malathion 5%, permethrin 0.75% and deltamethrin 0.05%.

Malathion was banned more than ten-years ago by the Malaria Control Program of Khartoum State due to development of resistance in *An. arabiensis* (KMFP, personal communication). Currently, tolerance of *An. arabiensis* to malathion has become widely spread in Sudan such as Khartoum, Gizera, White Nile and Gedarif States [7, 8, 9, 10]. A survey conducted in Khartoum State showed that carbamates and organophosphates were the most common pesticides that used in agriculture practice in the area [9]. Hence, resistance of *An. arabiensis* to malathion might indicate that this insecticide is still used in agriculture practice in Sudan. However, it has previously been suggested that the resistance to malathion in *An. arabiensis* in Gizera State due to house spraying rather than crop spraying [24].

The detection of confirmed resistance to permethrin and DDT in *An. arabiensis* is inconsistent with previous studies that conducted in Khartoum State [9]. These authors found that *An. arabiensis* is fully susceptible to DDT. Although, DDT was banned in Sudan since late1970s, it has been illegally used in agriculture [9] where it could be purchased from illegal markets. Abuelmaali *et al*. [9] found that this species is fully susceptible to permethrin in this state. However, these findings are consistent with previous studies from other regions in Sudan [7, 9, 10]. The confirmed resistance to permethrin and suspected resistance to deltamethrin is of concern to the Integrated Vector Management that depends on ITNs/LLINs as a protection measure against malaria vector.

In this study, L1014F and L1014S-*kdr* mutant alleles were recorded in *An. arabiensis* collected from urban and periurban areas. The L1014F mutant alleles were detected in *An. arabiensis* collected from 16 out of 20 sites surveyed. In contrast, L1014S *kdr* mutations were detected in *An, arabiensis* from three periurban areas. The occurrence of L1014F *kdr* mutation has been reported in *An. arabiensis* from Khartoum State and in other geographic regions in Sudan [7, 8, 10, 25]. The L1014S *kdr* mutation has previously been reported in a very low frequency in *An. arabiensis* from Kassala State in eastern Sudan [25]. The results on the L1014S *kdr* mutation from this study suggested that this type of mutation is so limited; however, more future investigations are needed.

The *kdr* (L1014F and L1014S) mutation in *Anopheles* mosquitoes is known mainly to be associated with resistance to pyrethroids and organochlorines (i.e. DDT). Although, DDT was stopped for uses in agricultural and public health practices some decades ago, this insecticide has illegally been used in agricultural practice [9]. In contrast, pyrethroid insecticides are currently in use in Sudan including Khartoum State where permethrin and deltamethrin are used in ITNs/LLINs and in a very low scale in IRS, respectively (personal comm. Department of IVM unit, FMOH, Sudan). Therefore, the *kdr* mutation detected in populations of *An. arabiensis* in Khartoum State in this study may be due to the resistance to pyrethroids rather than to organochlorines. However, the role of the organochlorines DDT cannot be neglected since it has been used in agriculture practice. The L1014F *kdr* mutation recorded in this study was lower than that previously reported in *An. arabiensis* from Khartoum State and different regions in Sudan [7, 8, 9, 10]. The L1014S *kdr* allele was detected for the first time in Khartoum State. This type of mutation is scare in *An. arabiensis* population in most of East African countries including Sudan although it is originally reported from Kenya but in *An. gambiae* [11]. In this study the L1014S *kdr* mutation was observed in a relatively high frequency compared to that previously reported by Himeidan *et al*. [25] who recorded mutation in *An. arabiensis* from eastern Sudan.

Previous studies on *Plasmodium* sporozoites infection in *An. arabiensis* were conducted using the ELISA technique [2, 26]. All infected females detected here were with *P. falciparum*, the most common and widely spread malaria parasites in Sudan [27]. Nevertheless, no *Plasmodium* parasites infection was detected in concomitant with a *kdr* allele mutation in *An. arabiensis* in this study. Despite epidemiological importance, the effect of insecticide resistance on vector-parasite interactions and the transmission of malaria parasites is poorly understood. Few studies have been conducted to elucidate the impact of insecticide resistance in *Plasmodium* sporozoites infection and the competence of malaria vectors [27, 28]. Saddler *et al*. [28] suggest that the continuous application of insecticides against already resistant mosquito vectors might select mosquitoes to be more susceptible to *Plasmodium* infection. In another study, the sensitivity to DDT was found to be higher in mosquitoes infected by *Plasmodium* [27].

Table 2: Knock-down times in populations of *Anopheles arabiensis* from urban and periurban areas in Khartoum State (Sudan) after exposure to seven insecticides of three different classes.

Insecticide used	Land use	No. fem. tested (Replicates)	KDT_{50} (50% Cl)	KDT_{95} (95% Cl)	KDT_{50} Ratio
DDT 4%	Urban	1300(52)	36.16 (34.67±37.74)	84.54 (78.70±91.62)	1.0
	Periurban	400(16)	49.21 (47.25±51.36)	115.04 (106.36±125.73)	1.4
Fenitrothion 1%	Urban	1600(64)	70.57 (62.89±82.79)	161.00 (126.25±234.26)	1.1
	Periurban	200(8)	65.22 (58.18±76.05)	148.81 (117.44±213.99)	1.0
Malathion 5%	Urban	1700(68)	43.87 (41.29±46.77)	119.16 (106.57±135.76)	1.0
	Periurban	200(8)	47.84 (45.38±50.62)	129.99 (116.28-148.17)	1.1
Permethrin 0.75%	Urban	400(16)	36.45 (36.45±33.15)	97.78 (82.04±123.60)	1.0
	Periurban	150(6)	46.29 (40.96±52.90)	124.18 (101.52±162.05)	1.3
Deltamethrin 0.05%	Urban	650(26)	28.08 (26.88±29.31)	59.70 (56.28±63.69)	1.0
	Periurban	375(15)	32.38 (31.16±33.65)	68.85 (65.04±73.32)	1.1

Table 3: Genotype and allelic frequency of L101F mutation in *Anopheles arabiensis* collected from in Khartoum State, Sudan

Study Sites	*No.	Genotype			Allelic frequency		Study Sites	*No.	Genotype			Allelic frequency	
		SS	RS	RR	S	R			SS	RS	RR	S	R
Soba West	30	28	2	0	0.970	0.030	Edroshab	27	20	0	7	0.740	0.260
Arkaweet	29	26	3	0	0.948	0.052	Eltumanyat	28	25	3	0	0.946	0.054
Edekheinat	24	20	3	1	0.895	0.105	Alhalfaya	32	32	0	0	1.00	0.00

Alshegelab	28	15	11	2	0.732	0.268	Alazeba	36	32	4	0	0.944	0.056
Alnasr	29	28	1	0	0.983	0.017	Algireef East	38	32	6	0	0.921	0.079
Alremaila	30	29	1	0	0.983	0.017	Soba East	38	32	6	0	0.921	0.079
Jabra	33	32	1	0	0.985	0.015	Abu'siid	30	30	0	0	1.00	0.00
Buri	36	35	1	0	0.986	0.014	Alsarha	31	31	0	0	1.00	0.00
Shambat	34	33	1	0	0.985	0.015	Algmayer	38	33	5	0	0.934	0.066
Elmaygoma	31	30	1	0	0.983	0.017	Angola	38	38	0	0	1.00	0.00

Table 4: Genotype and allelic frequency of L101S mutation in *Anopheles arabiensis* collected from in Khartoum State, Sudan.

Study Sites	*No.	Genotype			Allelic frequency		Study Sites	*No.	Genotype			Allelic frequency	
		SS	RS	RR	S	R			SS	RS	RR	S	R
Soba West	30	30	0	0	1.00	0.00	Edroshab	27	27	0	0	1.00	0.00
Arkaweet	29	29	0	0	1.00	0.00	Eltumanyat	28	24	0	4	0.857	0.143
Edekheinat	24	18	0	6	0.750	0.250	Alhalfaya	32	32	0	0	1.00	0.00
Alshegelab	28	23	0	5	0.821	0.176	Alazeba	36	32	0	0	1.00	0.00
Alnasr	29	29	0	0	1.00	0.00	Algireef East	38	32	0	0	1.00	0.00
Alremaila	30	30	0	0	1.00	0.00	Soba East	38	32	0	0	1.00	0.00
Jabra	33	33	0	0	1.00	0.00	Abu'siid	30	30	0	0	1.00	0.00
Buri	36	36	0	0	1.00	0.00	Alsarha	31	31	0	0	1.00	0.00
Shambat	34	34	0	0	1.00	0.00	Algmayer	38	33	0	0	1.00	0.00
Elmaygoma	31	31	0	0	1.00	0.00	Angola	38	38	0	0	1.00	0.00

*No. = Number of mosquitoes examined.
SS, RS and RR are three different kdr genotypes;
R = resistant L101S allele and S = susceptible wild-type allele.

Conclusions

This study reports the first evidence of the occurrence of L014S-*kdr* allele mutation in *An. arabiensis* in Khartoum State. Moreover, *An. arabiensis* has developed resistance to DDT and permethrin, and *kdr* (L014F and L014S) mutation that could significantly affect the malaria vector control in this State. Therefore, more attention is needed to monitor the ongoing vector control strategies in Khartoum State as well as in the country.

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