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Fauna and spatial distribution of mosquitoes (Diptera: Culicidae) in River Nile State, Sudan

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Abstract

Mosquito-borne diseases (MBDs) are a serious public health problem worldwide. The study was carried out from March 2015 to February 2016 to determine the fauna and distribution of mosquitoes (Diptera: Culicidae) in ten sentinel sites in the River Nile State, Sudan. Mosquitoes were collected as adults from resting places and larvae from aquatic habitats. The location of mosquito collection was marked using a global positioning system (GPS). A total of 4,803 specimens were collected in the area. These comprised 9 mosquito species with *Anopheles arabiensis* (30.7%), and *Culex univittatus* (30.8%) formed the bulk of the collection. Variation was detected in the numbers of mosquitoes collected in different sentinel sites where the higher proportion was recorded in the Albawga site. The spatial distribution and the occurrence of the different species might indicate a high risk of transmission of several parasitic and viral MBDs in this state in the future. Serious planning, management, and decision-making towards vector control are urgently needed.

Keywords: Mosquito-borne diseases, fauna, distribution, River Nile State, Sudan

1. Introduction

Mosquitoes (Diptera: Culicidae) belong to the most important group of disease vectors, as exemplified by the large number of species involved in the transmission of human and animal parasites and pathogens [1]. Mosquitoes are at the center of worldwide entomological research, because of their importance as vectors of a wide-range of diseases [2]. Up to date, there are some 3,530 species of mosquitoes, which are traditionally placed in 43 genera, all contained in the family Culicidae. However, some mosquito experts recognize a different classification that has 113 genera. For example, some mosquitoes previously in the genus *Aedes* have been transferred to genera, such as *Ochlerotatus* and *Stegomyia* [3].

Mosquito borne-diseases (MBDs) cause high morbidity and mortality during the few last years worldwide. Globally, vector-borne diseases (VBDs) account for > 1 billion cases and >1 million people die worldwide every year [4]. However, the majority are due to MBD. Currently, it is estimated that more than half the world's population to be at risk of MBD [4]. These are mainly caused by various mosquito species, such as *Anopheles* species, *Aedes aegypti*, and *Culex* species [5].

According to the distribution maps of the Sudan, 156 species, 2 subspecies of mosquitoes, and 7 varieties of Culicidae have been recorded in Sudan. Of these mosquito species, the following species were recorded in the River Nile state; *An. arabiensis* Patton, *An. rufipes* Gough, *An. pharoensis* Theobald, *Cx. univittatus* Theobald, *Cx. pipiens* SI, *Cx. bitaeniorhynchus* Giles (formerly *Cx. ethiopicus* Edwards) and *Ae. vittatus* Bigot [6, 7]. Currently, several studies were conducted to determine mosquito fauna and their spatial distribution in different regions in Sudan [8-11]; however, no such data is available from the River Nile State. Therefore, the current study was carried out to determine the mosquito fauna and their spatial distribution in River Nile State.

2. Materials and Methods

2.1 Study Areas

The River Nile State is located in northern Sudan (16 – 22' N and 30 – 32 E) (Figure 1). The State covers 124,000 km² with an estimated arable land is about 3,289,600 acres.

The State is bounded by the Arab Republic of Egypt from the north, Kassala and the Red Sea States from the east, Khartoum and the Kassala States from the south, and the North Kordofan States from the west. The state is divided into 7 administrative localities. The population of the state is estimated as 1,120,441.

The State is dominated by desert and semi-desert vegetation, such as *Vallechia flava* (Forssk) (Salam) and *Capparis decidua* (Tondob). The River Nile State represents the arid semi-desert and desert biomes which are characterized by sand and silt soils in many parts, with vertisol soil on the riverine ecosystem. The average rainfall is ranged from 25 mm to 150 mm and the relative humidity (RH%) not exceeding 59%. The temperatures ranging from 47 °C to 8 °C. Water resources, including surface water, are represented by the River Nile and the Atbara River, in addition to the valleys.

2.2 Study sites

Ten sentinel sites; 4 constant sites (Albawga, Alzidab, Soola, and Gandato), and 6 cross-check (Almikharif, New Manaseer, Abusleem, Alfadlab, Alsyalla, and Alsheriq) sites were

selected to conduct the entomological surveillance in this study (Figure 1). The sites were selected on the basis that they are part of the national stations, have intensive agricultural activities, environmental change is expected, due to the establishment of Merowe Dam, other human activities (*i.e.* gold mining activities), the availability of the breeding sites for *Anopheles*, *Culex*, and *Aedes* Mosquitoes, and the high mosquito density in some areas recorded in preliminary entomological surveys conducted during 2014.

2.3 Sampling

2.3.1 Adult collection

Adult mosquitoes resting indoor were collected from each of the constant and cross-check sentinel sites from March 2015 to February 2016. The constant sentinel sites were surveyed monthly, and the cross-check sentinel sites were surveyed twice during the study. In constant and cross-check, adults were collected from possible outdoor resting places. Using suction tubes between 7:00 – 11:00 am in outdoor sites. In each survey, adults were collected for 5 consecutive days.

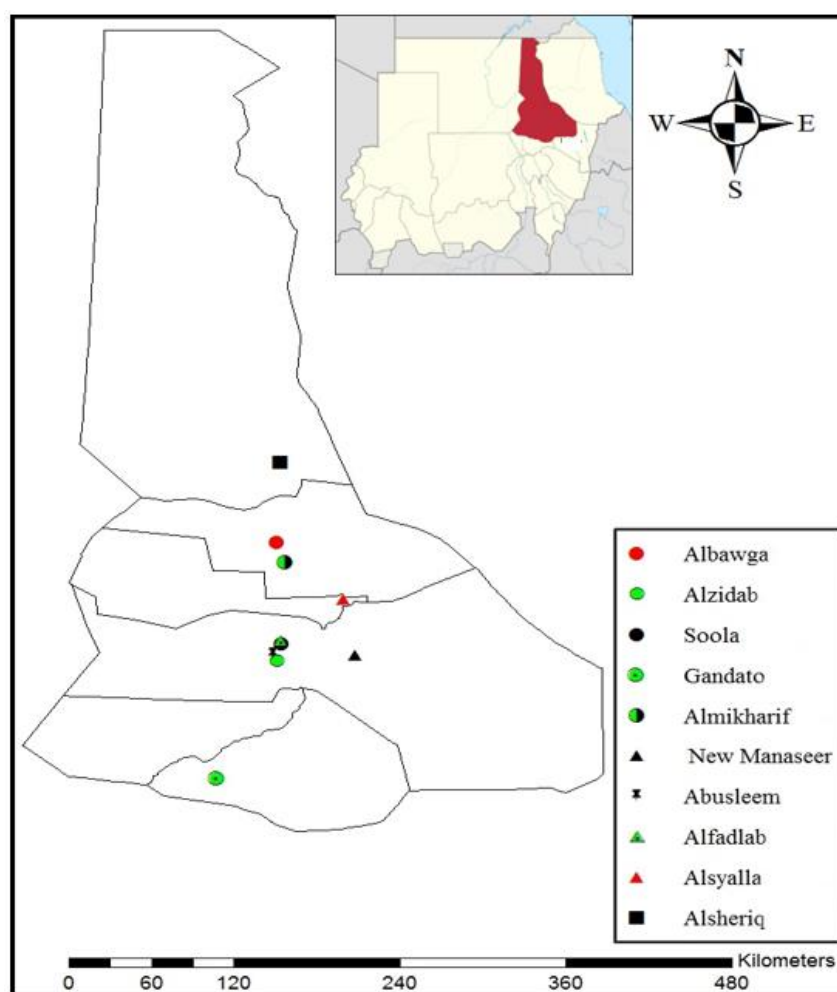


Fig 1: A map of the River Nile State showing constant and cross-check sentinel sites surveyed in this study.

2.3.2 Larval collection

Larvae and pupae were collected from aquatic habitats in all sentinel sites by using scoops, larval net, and Pasteur pipettes on monthly basis from each of the constant, and twice a year from each cross-checks sentinel. In each sentinel site, the larvae and pupae were collected between 7:00 am – 9:00 am

for 5 consecutive days in each larval survey. The collected larvae and pupae were then maintained and reared using the standard method for mosquito rearing [12].

The surveyed habitats for larvae and resting places for adult mosquitoes in all sentinel sites were marked using GPS 12 XL (Garmin, U.S.A) with an accuracy of 1-5 m.

2.4 Identification

2.4.1 Morphological

The collected larvae and adults were identified based on morphological features [13, 14]. Briefly, adults were pinned on card points and examined under a dissecting microscope. The morphological characteristics used to differentiate between Anopheline and Culicine species were wings, legs, thorax, abdomen, head, and mouthparts.

2.4.2 Molecular

Genomic DNA was extracted individually from adults *An. gambiae* s.l. emerged from field-collected anopheline mosquito larvae as described by Livak [15]. The extracted genomic DNA solutions were kept at -20°C until being analyzed by PCR. The genomic DNA samples from individual females *An. gambiae* s.l. were analyzed by PCR using *An. arabiensis* species-specific primers (A⁰: ATG CCT GAA CGC CTC TAA GG and A⁰⁵: CAA GAT GGT TAG TTA CGC CAA). PCR reaction conditions were conducted as described by Scott *et al.* [16]. The reaction gave PCR amplicons of 500 bp band sizes which is a characteristic for *An. arabiensis*.

2.6 Statistical analysis

The data obtained from different parts of this study were analyzed using the computer software SPSS ver. 22. Data from all parts of this study were analyzed using descriptive

analysis.

3. Results

3.1 Mosquito fauna in the River Nile State

The morphological identification of the field-collected adult and the colony-reared mosquitoes from the larvae indicated that the mosquito fauna in the study area comprised 5 genera, viz *Anopheles*, *Culex*, *Aedes*, *Lutzia*, and *Ochlerotatus*. The morphologically identified mosquitoes included 9 species; *An. gambiae* s.l., *An. pharoensis*, *Cx. quinquefasciatus*, *Cx. univittatus*, *Cx. poicilipes*, *Cx. bitaeniorhynchus*, *Ae. vexans*, *L. tigripes* and *Oc. Caspius*. Furthermore, using PCR, the morphologically determined specimens of *An. gambiae* s.l. were identified as *An. arabiensis*.

3.2 Relative abundance of mosquitoes in the study area

A total of 4,803 specimens were collected and identified in this study. *Culex* spp. was found more abundant than members of other mosquito genera (Figure 2). Of the total mosquito collection, *An. arabiensis* (30.7%), *Cx. quinquefasciatus* (31.5%), and *Cx. univittatus* (30.7%) were the most dominant species in the area (Table 1). Moreover, *An. arabiensis* formed 99.3% of the total collection of anopheline mosquitoes. In contrast, *Cx. quinquefasciatus* and *Cx. univittatus* constituted > 90% of the total *Culex* mosquitoes recorded in the area.

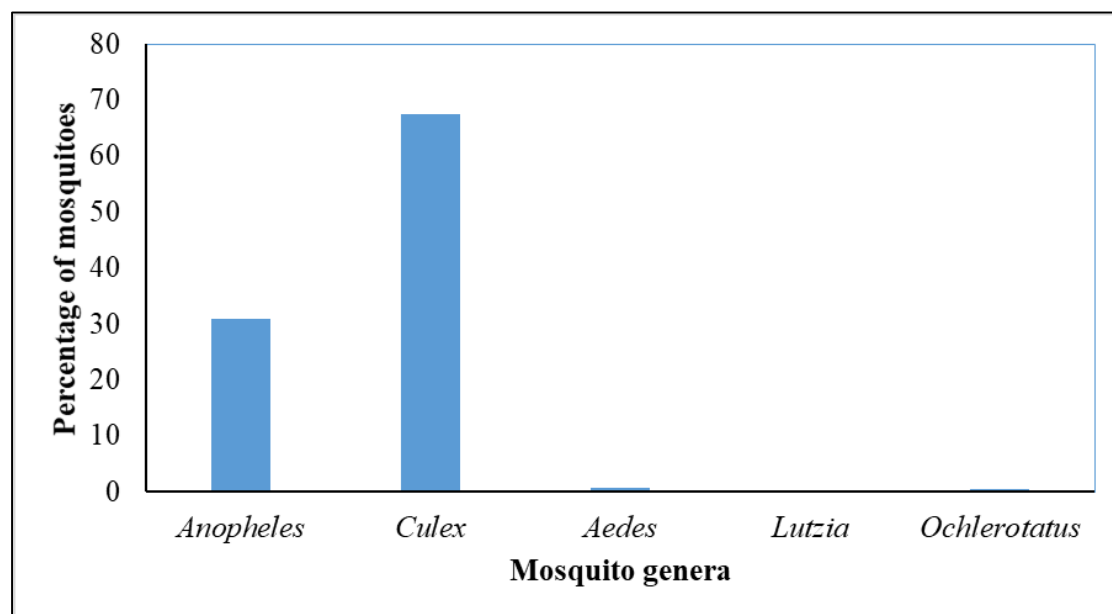


Fig 2: Percentages of the mosquitoes of different genera recorded in the River Nile State, Sudan.

Table 1: Numbers and percentages of mosquito species recorded in the River Nile State, Sudan.

Species	Number	%
<i>Anopheles arabiensis</i>	1473	30.7
<i>An. pharoensis</i>	9	0.2
<i>Culex quinquefasciatus</i>	1512	31.5
<i>Cx. univittatus</i>	1477	30.7
<i>Cx. poicilipes</i>	252	5.2
<i>Cx. bitaeniorhynchus</i>	1	0.02
<i>Aedes vexans</i>	38	0.8
<i>Lutzia tigripes</i>	17	0.3
<i>Ochlerotatus Caspius</i>	24	0.5
Overall total	4803	

3.3 Relative abundance and species diversity of mosquitoes in the constant and cross-check sentinel sites

Table 2 shows the occurrence of different mosquito species in the surveyed constant and cross-check sentinel sites in the River Nile State during this study. A greater diversity of mosquito species was observed in Soola and Alzidab than the other sites, and the least in Alsheriq site. Soola and Alzidab sites showed the presence of 7 out of the 9 species recorded in the study area. However, Albawga site showed the presence of 6 species; Gandato and Alfadlab sites had 5 species each. Most of the cross-check sentinel sites showed the existence of 3 to 4 species of mosquitoes; except the Alsheriq site, which had only 2 species (Table 2).

The numbers and percentages of the species in different constant and cross-check sentinel sites are depicted in table 3. Out of 4,803 specimens collected, 89.7% (4,310 specimens) were from the constant sentinels and 10.3% (493 specimens) cross-check. Out of 4,310 mosquitoes collected from the constant sentinel sites, the highest proportion was from Albawga site (45.2%; 1984) and the lowest was from Soola site (Figure 3). As shown in the table, most species were collected in higher numbers from the Albawga than other surveyed sites. The exception was *Cx. poicilipes* and *Oc. Caspius*, which were collected in higher numbers from Alzidab and Abosleem, respectively. However, *An. pharoensis*, *Cx. bitaeniorhynchus*, and *L. tigripes* were collected in very few numbers in the area.

3.4 Spatial distribution of adult mosquitoes

The data obtained on species of mosquitoes from different methods of collection in the constant and cross-check sentinel sites during the study period, coupled with that of locations

(GPS) were overlaid on maps of the River Nile State (Figure 4 - 6). The maps showed the distribution of mosquito species in the 4 constant and 6 cross-check sentinel sites in the State. For *Anopheles* species, *An. arabiensis* occurred in all the sites; whereas, *An. pharoensis* was recorded only in 3 sites; viz. Alzidab, Soola, and Gandato (Figure 4). In contrast, the distribution map of *Culex* spp. showed that, *Cx. quinquefasciatus* occurred in all surveyed sites, while *Cx. univittatus* was absent only from New Manaseer, Abusleem, and Alsheriq sites (Figure 5). *Culex poicilipes* was recorded in the 4 constants (Albawga, Alzidab, Soola, and Gandato sites) and 2 cross-check sites (New Manaseer and Alsyalla) whereas, *Cx. bitaeniorhynchus* was recorded in the Alzidab site only (Figure 5). The distribution map of *Aedes* mosquitoes showed that *Ae. vexans* occurred in 3 sites (viz. Albawga, Alzidab, and Soola), *L. tigripes* in Soola, and Almikharif sites, whereas *Oc. caspius* in only 3 sites; Albawga, Abusleem, and Alfadlab (Figure 6).

Table 2: Mosquito species recorded in different surveyed sentinel sites in the River Nile State, Sudan.

Species	Constant sites				Cross-check					
	Albawga	Alzidab	Soola	Gandato	Almikharif	New Manaseer	Abusleem	Alfadlab	Alsyalla	Alsheriq
<i>Anopheles arabiensis</i>	+	+	+	+	+	+	+	+	+	+
<i>An. pharoensis</i>	-	+	+	+	-	-	-	-	-	-
<i>Culex quinquefasciatus</i>	+	+	+	+	+	+	+	+	+	+
<i>Cx. univittatus</i>	+	+	+	+	+	-	-	+	+	-
<i>Cx. poicilipes</i>	+	+	+	+	-	+	-	-	+	-
<i>Cx. bitaeniorhynchus</i>	-	+	-	-	-	-	-	-	-	-
<i>Aedes vexans</i>	+	+	+	-	-	-	-	+	-	-
<i>Lutzia tigripes</i>	-	-	+	-	+	-	-	-	-	-
<i>Ochlerotatus caspius</i>	+	-	-	-	-	-	+	+	-	-

(+) = presence and (-) = absence, * New Manaseer.

Table 3: Numbers and percentages of mosquito species recorded in selected sentinel sites in the River Nile State, Sudan

Species	Constant sites								Cross-check sites										Total		
	Albawga		Alzidab		Soola		Gandato		New Mnasir		Alfadlab		Abosleem		Askerik		Alsyalla			Almikharif	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%		No	%
<i>Anopheles arabiensis</i>	581	29.8	397	43.6	223	39.3	224	25.3	1	25	15	18.5	5	19.2	4	100	8	32	15	4.2	1473
<i>An. pharoensis</i>	0	0	5	0.6	2	0.4	2	0.2	0	0	0	0	0	0	0	0	0	0	0	0	9
<i>Culex quinquefasciatus</i>	619	31.8	153	16.8	152	26.8	200	22.6	1	25	48	59.3	0	0	0	0	11	44	328	92	1512
<i>Cx. univittatus</i>	708	36.3	235	25.8	154	27.1	359	40.6	0	0	10	12.3	0	0	0	0	5	20	6	1.7	1477
<i>Cx. poicilipes</i>	19	1	112	12.3	18	3.2	100	11.3	2	50	0	0	0	0	0	0	1	4	0	0	252
<i>Cx. bitaeniorhynchus</i>	0	0	1	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>Aedes vexans</i>	20	1	7	0.8	5	0.9	0	0	0	0	6	7.4	0	0	0	0	0	0	0	0	38
<i>Lutzia tigripes</i>	0	0	0	0	13	2.3	0	0	0	0	0	0	0	0	0	0	0	0	4	1.1	17
<i>Ochlerotatus caspius</i>	1	0.1	0	0	0	0	0	0	0	2	2.5	21	80.8	0	0	0	0	0	0	0	24
Overall total	1948		910		567		885		4		81		26		4		25		353		4803

Percentages of mosquitoes were calculated from the total in each sentinel site (i.e. from the overall total in the column).

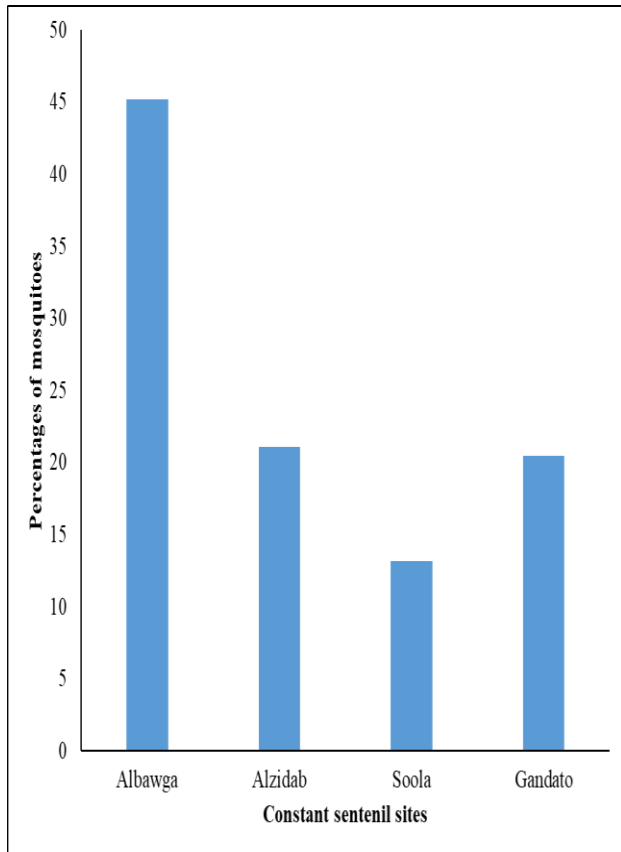


Fig 3: Percentages of mosquito specimens collected from the constant sentinel sites in the River Nile State, Sudan.

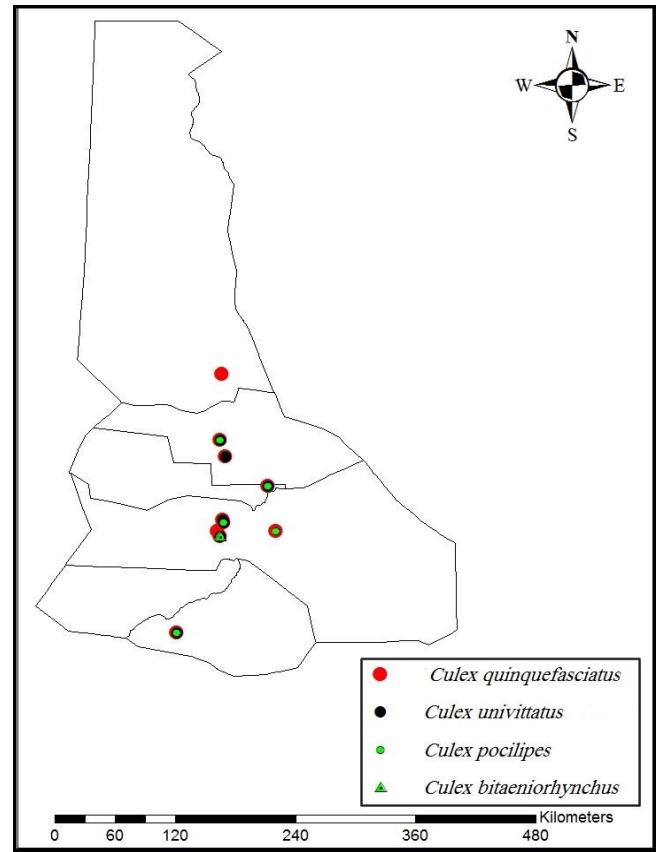


Fig 5: The distribution map of *Culex* spp. mosquitoes in the River Nile State, Sudan.

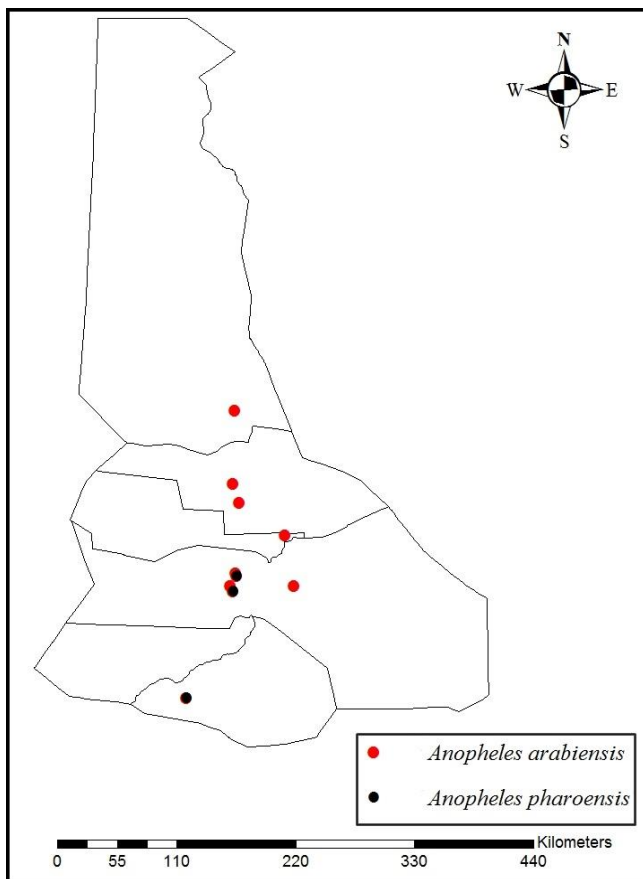


Fig 4: The distribution of *Anopheles* mosquitoes in the River Nile State, Sudan.

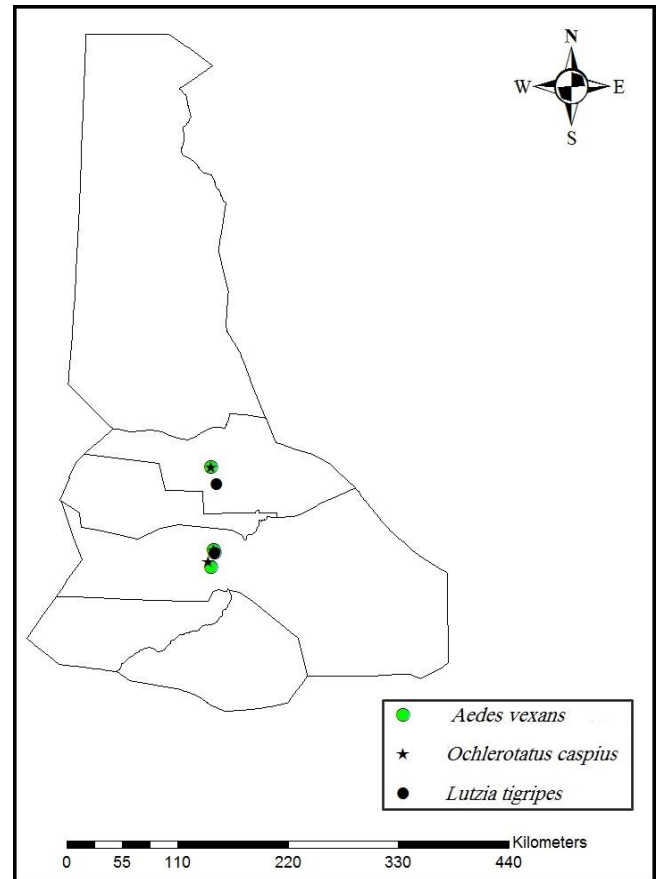


Fig 6: The distribution of *Aedes vexans*, *Ochlerotatus caspius*, and *Lutzia tigripes* mosquitoes in the River Nile State, Sudan.

4. Discussion

Nine mosquito species were recorded in surveyed sentinel sites. These species namely were; *An. gambiae* s.l., *An. pharoensis*, *Cx. quinquefasciatus*, *Cx. univittatus*, *Cx. poicilipes*, *Cx. bitaeniorhynchus*, *Ae. vexans*, *L. tigripes* and *O. caspius*. The results of mosquito fauna of River Nile State are consistent with general distribution maps of mosquitoes of Sudan [6, 13, 17, 18]. Besides, these species have been reported in different regions of Sudan [7, 9, 10, 18, 19].

The anopheline mosquitoes in the State have comprised 2 species; viz. *An. gambiae* s.l. and *An. pharoensis*. Based on molecular analysis, *An. gambiae* complex was identified as *An. arabiensis*. *Anopheles arabiensis* was recorded in all studied sites; whereas; *An. pharoensis* was recorded in very low densities in only 3 sites; viz. Alzidab, Soola, and Gandato. *An. arabiensis* is a common and one of the major malaria vectors throughout sub-Saharan Africa [20, 21]. Besides, it is the sole malaria vector in a different region in Sudan [22, 23]. *Anopheles pharoensis* is widely distributed in Ethiopia, Somalia, and Sudan and also extends into Egypt [24]. The species is as a potential vector in Egypt [25].

Culex species recorded in this study were *Cx. quinquefasciatus*, *Cx. univittatus*, *Cx. poicilipes* and *Cx. bitaeniorhynchus*. These species were reported from different areas in Sudan [7]. *Culex quinquefasciatus* is known to be a domestic annoying mosquito and it has been found to transmit lymphatic filariasis (LF) in the Blue Nile area and former southern Sudan [26]. *Culex quinquefasciatus* was recorded in all study sites. *Culex univittatus* is the commonest and the most widely distributed Culicine in the Sudan [10, 18]. Berge [27] found that *Cx. univittatus* has a major role in the transmission of West Nile Virus (WNV) in Sudan and the Republic of South Sudan [28]. *Culex univittatus* was recorded in all constant sites and 3 cross-check sites. *Culex poicilipes* is widely distributed worldwide and is considered as a potential vector of many serious human and animal diseases, such as the Rift Valley Fever (RVF) infection [29, 30]. This species was recorded in all the surveyed constant sites in the present study, as well as in 2 of the cross-check sites. In a previous study in the Sudan, adults, and larvae of *Cx. poicilipes* collected from Khartoum and White Nile States were found infected with the Rift Valley virus [31]. *Culex bitaeniorhynchus* is an extremely common and widespread mosquito species [32]. However, this species was not found to have a role in the transmission of the disease in Sudan. The species was collected as a single specimen from the Alzidab site, which agrees with the study done by Lewis [6].

Aedes vexans, are capable of transmitting WNV, St. Louis encephalitis virus, Western and Eastern Equine Encephalitis viruses, and RVF virus [33, 34]. Moreover, *Ae. vexans* was found infected with RVF viruses in Khartoum State, the Sudan [35]. This species was collected in 3 constant and 1 cross-check sentinel sites.

Lutzia tigripes occurs in Africa and Asia [36, 37]. Although it is widely distributed in southern and central regions of Sudan [7], this was the first record for this species in River Nile State. However, the larvae act as predators that eat other mosquito species and immature stages of aquatic insects, and more specifically, they prefer larvae of *Ae. Aegypti* [7]. A similar observation was recorded during the present study, where one larva of *L. tigripes* fed on several mosquito larvae during mosquito rearing and maintenance in the laboratory. This species was only recorded in one constant and one cross-check sentinel site.

Ochlerotatus caspius is widely distributed worldwide and

mainly occurs in the coastal areas [38, 39]. This species has been recorded in the Sudan; however, its role in the transmission of human disease has been discussed [19]. The species was recorded in 1 constant and 2 cross-check sentinel sites.

The spatial distribution of the mosquito species recorded in all surveyed sentinel sites was mapped. The distribution maps showed that only *An. arabiensis* and *Cx. quinquefasciatus* were recorded in all surveyed sentinel sites. The distribution of mosquito vectors is influenced by climatic and environmental conditions, such as availability of aquatic habitats, hosts, and sugar meal sources, a shelter for resting, as well as the ambient temperature, RH%, and rainfall [3, 39]. The findings on the distribution pattern of adult mosquitoes surveyed in sentinel sites in the state might be ascribed to the behavior and performance of mosquitoes. The distribution of both *An. arabiensis* and *Cx. quinquefasciatus* in the surveyed area may be explained to a large extent by the availability of preferable larval habitats and the presence of suitable hosts. On the other hand, the wide distribution of these 2 species might represent a risk of malaria transmission, LF, and related arboviruses to the later species as well. In contrast, the occurrence of other species in limited sites in the study area might be due to the unavailability of suitable larval habitats rather than hosts. Hence, these species could be of medical importance in only these sites unless they can spread to other areas in the future.

5. Conclusions

The study has revealed nine mosquito species belonging to five genera which indicates the presence of diverse species. This finding might suggest a risk of transmission of several MBDs in River Nile State. Also, the findings are of importance in the planning and implementation of malaria vector control strategy in the State.

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