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Research Article

Spatio-temporal Distribution of Aedes Mosquitoes (Diptera: Culicidae), in Ed Daein Town, Ed Daein Locality, East Darfur State, Sudan (July 2016 - June 2017)

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Abstract

Aedes aegypti (L.) mosquitoes (Diptera: Culicidae) have a great importance in public health. *Aedes*- borne diseases (ABDs), especially the dengue virus (DENV) has increased and spreaded worldwide. The study is one of a series of studies aimed to map *Aedes* species in the Sudan and determine some aspects of their ecology in Ed Daein town, Ed Daein Locality, East Darfur state (EDS), Sudan. A cross-sectional survey during July 2016 - June 2017 was conducted to determine seasonal and geographical distribution, larval habitats of *Aedes* mosquitoes. Five sentinel sites/neighborhoods/residential areas, namely, Alarab, Almatar, Alnahda, Alguba and Moasker Alneem with different environmental settings were selected as urban and periurban areas. In each neighborhood, water containers in houses and larval habitats were surveyed for larvae and pupae of *Aedes spp*. In each household (HH), all water containers were checked for the presence (positivity) of these immature stages of *Aedes* spp. to describe the larval habitats, their distribution, seasonality and calculate Stegomyia indices, i.e. the house index (HI), the container index (CI), Breteau index (BI) and pupal/demographic index (P/DI). Larvae and pupae from different habitats were also sampled, preserved in 70% ethanol in Eppendorff tubes for species identification. From a total of 382 (89.8%) larvae, were identified as *Ae. aegypti*, while 10.2% were identified as *Ae. albopictus*. From 3,243 containers inspected, 8.2% were found positive for *Aedes* species. The highest percentage of positivity was found in clay pots (zeers) containers (77.9%), followed by barrels (20.2%). High occurrence of larvae and pupae was shown in Alnahda neighborhood with mean of 6.5/5 dips and 2.2/5 dips, respectively. The number of pupae was significantly different in all study sites. The highest mean number of larvae was found to be (10.1/5 dips) in rainy-season in comparison to the dry- season while the high mean umber of pupae was (3.3/5 dips). No significant difference was found between HI during the different months of the study. The highest HI was recorded in July 2016 (98.2%). Significant differences between BI, CI and P/DI, during different months of the study were detected. The high BI was detected in October 2016 (30.0%), High CI was reported in November 2016 (13.9%) and the highest P/DI was recorded in October 2016 (15.7%). *Ae. aegypti* was the prevalent species in the study sites. Zeers and barrels were the key containers for the *Aedes* species breeding. Large-scale studies on spatio-distribution of *Aedes* mosquito in the remain localities of East Darfur State is highly recommended in near future.

Keywords: *Aedes Mosquito; Ed Daein Locality; East Darfur State; Sudan*

Introduction

Aedes mosquitoes (Diptera: Culicidae) are distributed around the world and there are over 950 species [1]. *Ae. aegypti* and *Ae. Albopictus* [2] are vectors of several globally important arboviruses, including dengue virus (DENV) [3], yellow fever virus (YFV; [4]) and Chikungunya virus (CHIKV) [5]. The public health impact of *Aedes* borne-diseases (ABDs), especially the DENV has increased and widely-spreaded. Although, mosquitoes spread several viruses and parasites between people and other animals, however, the viral disease, due to DENV has become growing public health problems in tropical and subtropical regions [5]. An estimated of 2.5 billion people live in areas at risk for epidemic transmission [6] and 50 - 100 million new infections are estimated to occur annually in > 100 endemic countries [8]. In addition, the DF has spreaded widely than before [7], as well as dispersal of its main vectors (i.e. *Ae. aegypti* and *Ae. albopictus*) into new areas [9], resulting in 30-fold increase in incidence.

In Sudan, ABD represents a major health problem. While, YF outbreaks have been reported in disparate regions in Darfur [10], DF and DHF occurred in repetitive outbreak patterns in the coastal area of the Red Sea State [11]. Moreover, DF and DHF have also been reported from Kassala State [12]. However, the disease might probably spread over new areas in the country since *Ae. aegypti* and other *Aedes* species were earlier reported by Lews [13]. The recent studies by the BNNICD group as a series of studies to map the mosquito species in the country, proved the presence of the genus in Port Sudan, Kassala, Sennar state, Blue Nile State, South Kordofan State, West Kordofan State and several localities and towns in the Greater Darfur region, namely Kass, Nyala, Elfashir and Elgenaina. The current study was carried out during July 2016 - June 2017 to determine some aspects on the ecology of *Aedes* mosquitoes in Ed Daein town, Ed Daein Locality in State (EDS), Sudan. The information on spatio-temporal distribution, larval habitats is expected to help in designing proper interventions against these mosquitoes.

Materials and Methods

Study area

East Darfur state (EDS) is located in the southwestern part of the Sudan between 12.48° - 9.24°N and 27: 54 - 25.6°E, about 831 km from Khartoum, the capital of the country. The state occupies an area of 119,500 km^2 and it is bordered from the north by North Darfur State, from the West by south Darfur State and West Kordofan State, from the east and by South Sudan Republic from South (Figure 1). The state is administratively divided into 9 localities; (Figure 2) Ed Daein is the capital of the state. The area is located across three different climatic zones; *viz*. savannah with low and high rainfall and floodplains. The state is mainly flat, but interrupted in some areas with small hills and seasonal streams (khors). Different soil types in different parts occupy the state; however, most areas are dominated by sandy soil. The climate prevailing in the state is tropical continental. The temperatures range in the area between 16 - 40°C. Maximum temperature reaches 47°C in summer (April to June), with a mean monthly temperature of around 35°C and the minimum temperature is 12 - 25°C in winter (December to February). The rainfall varies between 75 - 700 mm in the area according to the different climatic zones prevailing in the state. The population of the EDS is estimated at 1,216,719 people [14]. Most of the inhabitants (75%) live in the rural areas and 25% in towns. The population of the area belongs to different ethnic groups. The people in the state live in different types of houses. In the rural areas, people live in typical African huts built of woods and grasses, whereas in the urban areas they live in houses constructed of bricks and mud. Most of the people are working in farms; they grow different type of crops. Moreover, some people are sheepherders, who raise domestic animals like cows, camels, goats and sheep.

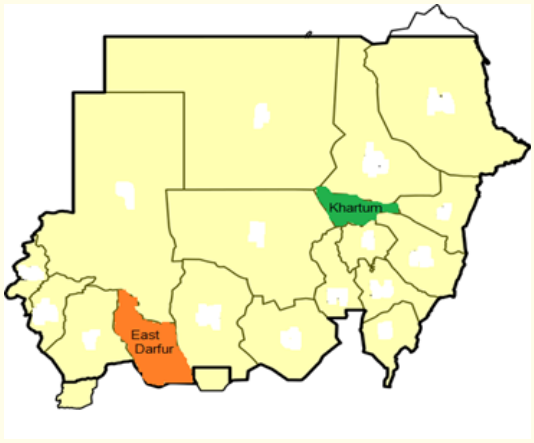

Figure 1: *East Darfur map (Source: Wikipedia, 2016).*

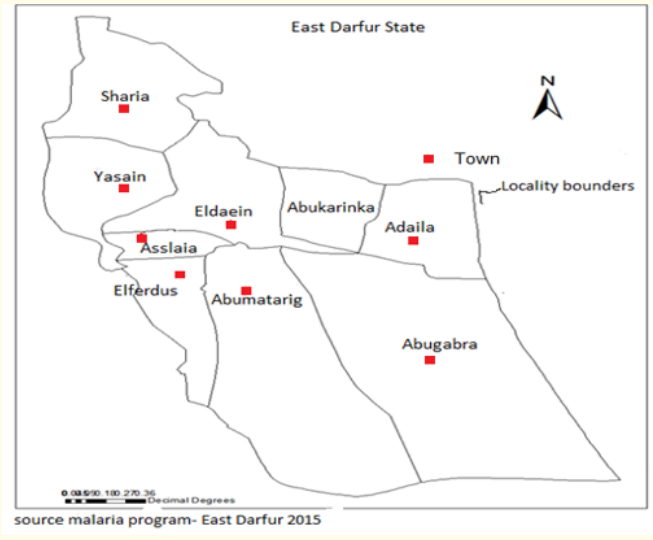

Figure 1: *East Darfur map (Source: Wikipedia, 2016).*

Study sites

The field surveys were conducted in Ed Daein town (El Matar/airport, Elnahda and Elgoba neighborhoods), which are considered as urban areas and the peri-urban (Elneem A), which is classified as internal displaced people camps (IDPs).

Ed Daein town (urban area)

Ed Daein town is the capital of the state. The latitude is 26.12548 E and longitude 11.46468 N. The locality belongs to the savannah area with high rainfall. The population of the locality is 179,723 people, according to expanded program Immunization [14]. Most of activities

practiced in the locality are agriculture and shepherds of animals. The inhabitants obtain water for HH uses from the water network of the Locality (water reservoirs and wells). However, due to the shortage and irregularity of water supply in the locality, inhabitants store water in different types of containers for HH uses. Hence, these containers are suspected to act as the main source of larvae habitats in the area.

IDPs (periurban area)

The locality has four IDP camps, three of them located in the northern side and one in the south. The camps are residence by 141,611 people (EPI, 2014). The majority of inhabitants are farms and Sheepherder.

Study design

This study was conducted as cross-sectional surveys during July 2016 - June 2017 to determine seasonal and geographical distribution, larval habitats. of *Aedes* spp. The survey of *Aedes* mosquitoes was conducted in Ed Daein town, Ed Daein Locality, EDS.

Study population

Aedes mosquitos' larvae, pupae and adults were collected from the suspected larval habitats and resting places in the selected sites. The collection of immature stages from water containers was conducted through inspection of water containers (e.g. barrels, zeers, Jericans,…, etc.) using dipping method, while, wild adult collected from resting places around water containers, using aspirator.

Entomological surveys

Immature stages collection

For this study purpose, at least two areas with different environmental settings were selected; urban and periurban areas (or urban and rural areas). In each area, water containers in houses and larval habitats (i.e. artificial containers, tires, etc.) at outdoor sites close to the human dwellings were surveyed for larvae and pupae of *Aedes* mosquitoes. In each HH, all water containers were checked for presence of larvae and pupae of *Aedes* mosquitoes. The larvae and pupae were recorded and counted in each container in every HH to measure and calculate Stegomyia indices (i.e. HI, CI, BI and P/DI). (Appen. A and B), larvae and pupae from different habitats were also sampled, preserved in 70% ethanol in Eppendorff tubes for species identification. In addition, more samples of larvae were taken to the insectary and reared to adult for species identification.

Entomological surveys were carried out for one year in the two selected areas in Ed Daein Locality, blocks of HHs and the surroundings were randomly selected for inspection of *Aedes* mosquitoes. Entomologic surveillance data was collected monthly during the study period. A well-trained team (3 - 4 health workers) and a supervisor carried out the entomological surveys. The team visited the selected HHs for larval stages of *Aedes* mosquitoes. Information on the number of inspected HHs, types and positive containers (with *Aedes* mosquitoes pupae or larvae) and HHs with positive container were recorded. Moreover, larval surveys were also done at outdoor sites close to the HH. When allocated, the larval habitats were described.

Likewise, wild Adult *Aedes*-mosquitoes was also been collected monthly using aspiration methods. Adult were collected from indoor and outdoor resting places. The collection places of the wild adults *Aedes* mosquitoes were also fully described. Samples of larvae and adults *Aedes* mosquitoes were then preserved into 70% alcohol in 50 ml vials and adults *Aedes* mosquitoes were kept in 1.5 Eppendorff tubes with silica gel for subsequent species identification.

The seasonal abundance of *Aedes* mosquitoes was studied in the study area. The numbers of the larvae and pupae of *Aedes* mosquitoes were recorded and counted every month from indoor and outdoor sites in the area. Samples of 4thinstar (L4) were preserved in 70%

ethanol and identified to species level. Furthermore, wild adults *Aedes* mosquitoes were also collected from the aforementioned sites preserved dry on silica gel in Eppendorff tubes and identified in the laboratory to species level. The numbers of larvae and pupae, as well as the wild adults *Aedes* mosquitoes in different months were counted and recorded.

Dipping method was employed in larval collection. Five dips taken from each water collection. Number of larva and pupae/5 dips was then being counted and recorded to determine larval density. Since, *Aedes* larvae breed on the water margins, dips were taken from the margin of water collection. Moreover, occurrence of *Aedes* mosquitoes in artificial or natural containers with few amount of water (wet -containers) were checked using Pastuer pipettes. Collected specimens were placed in a specimen tube and labeled with location and date of collection. The number of positive containers, as well as the number of water- filled containers inspected was then being recorded. Total larvae in positive (+) containers were counted to give approximate density/larval habitat. Collected larvae and pupae were kept in well-labeled specimen vials and transferred to the laboratory for rearing to adult stage and species identification and verification.

Adult collection

Wild-adult mosquitoes were collected from indoor and outdoor sites by hand catches using mouth aspirator. Wild captured mosquitoes were preserved on silica gel in Eppendorff tubes for species identification.

Wild adult *Aedes* mosquitoes were collected from possible indoor and outdoor sites and resting places by hand catches methods. In each selected area, search for adult mosquitoes was done between 07:00 - 11:00 a.m. for 3 days/month during the study period. Wild adult mosquitoes were captured by a mouth aspirator, put in well-labeled paper cups covered with mesh fixed with rubber band and transported to the laboratory.

Instruments

The study instruments used included; mosquito net, scoops, trays (dishes), sampling tubes, pasture pipettes, bottles (1.5 - 2.0 liters), ethanol (70%), mouth aspirator, torch + 1.5 v batteries, paper cups, fine mesh + rubbers, plastic buckets, ice- box, towels, liquid nitrogen, larval trays (dishes), mosquito cages, larval food (fish food), holding cages, cotton, filter papers, sugar, petri dish, glass slides, slide boxes, cover slip, mountants medium, Eppendorff tubes (1.5 ml), racks, disposable gloves, foil, lens, permanent markers, labels, fine paint brushes, dissecting needles, pens and pencils, digital camera, GPS and notebook.

Sample size of houses

The sample size of HHs to be surveyed for productivity of *Aedes* immature stages was calculated following a simple random sampling of premises or a cluster design (WHO, 2011). Since this study was to provide a baseline data from the area, a survey of all premises within a cluster were done as recommended by WHO (2011). At first, a preliminary survey was carried out to determine the premises infested with immature stages of *Aedes* mosquitoes, because those without larvae and pupae of *Aedes* do not provide any information about key and productive containers and sample size calculations. Therefore, taking in consideration the positive premises, the sample size of HHs was done as described Focks and Alexander, [15].

Mosquito rearing, processing and identification

The field-collected immature stages of *Aedes* mosquitoes kept in well-labeled vials we retransferred separately to paper cups covered with a netting material and fixed with a rubber band. The larvae and pupae were reared in the laboratory to adult stages. When emerged, adult mosquitoes, as well as specimens of larvae (3^{L3} and L4) and pupae were transferred into 70% ethanol in Eppendorff tubes kept for further investigation. Moreover, the wild collected adults also were killed by chloroform and then preserved as described above. The

preserved specimens of mosquitoes were then examined under dissecting microscope to determine the species with the taxonomic Keys of Huang (2004) and Bangs and Focks [16].

In the larval stage, the shape of the comb scales on the 8th segment of the abdomen and the shape of the pecten teeth on the siphon can differentiate the *Aedes* species.

In *Ae. aegypti* larvae, the comb teeth have well -developed lateral denticles, but the pecten teeth have less defined denticles. Whereas, in *Ae. albopictus* larvae, the comb teeth have no lateral denticles, but the pecten teeth have 3 well -defined pointed denticles [17].

Data analysis

The data obtained from this study was analyzed using SPSS v. 20. Data from the entomological surveillance was analyzed. Parametric tests were used for normally distributed data and non-parametric tests were used to analyze non-normalized data. Moreover, *Aedes* mosquito larval indices; HI, CI, BI and P/DI were calculated using the following formulas:

House index (HI; % of houses positive for larvae of *Ae. Aegypti*):

$$HI = \frac{\text{No. of houses positive for } \textit{Aedes} \text{ larvae} \times 100}{\text{No. of houses inspected}}$$

Container index (CI; % of water-holding containers positive for *Aedes* larvae):

$$CI = \frac{\text{No. of positive (+) containers} \times 100}{\text{No. of containers inspected}}$$

Breteau index (BI; Number of positive (+) containers for *Ae. aegypti*/100 HHs):

$$BI = \frac{\text{No. of positive (+) containers} \times 100}{\text{HHs inspected.}}$$

Pupal/demographic (P/DI): $\frac{\text{Total number of collected pupae of } \textit{Aedes} \text{ mosquitoes}}{\text{Total number of inhabitants in the inspected HHs.}}$

Results

Aedes species identified

Table 1 revealed that from a total of 382 larvae, 343 (89.8%) of them were identified as *Ae. aegypti*, while 39 larvae (10.2%) were identified as *Ae. albopictus*. The highest prevalence of *Ae. Aegypti* was recorded during August 2016 (95.3%), followed by October 2016 (92.3%), January 2017 (91.9%) and November 2016 (90.5%). While, *Ae. Albopictus* registered in December 2016 (17.9%), which was followed by September 2016 (15.5%), November 2016 (9.5%) and July 2016 (9.3%).

Month	No. of *Aedes* spp. identified	No. of *Ae. aegypti*	% *Ae. aegypti*	No. of *Ae. albopictus*	% *Ae. albopictus*
July 2016	54	49	90.7	5	9.3
August	43	41	95.3	2	4.7
September	71	60	84.5	11	15.5
October	65	60	92.3	5	7.7
November	84	76	90.5	8	9.5
December	28	23	82.1	5	17.9
January 2017	37	34	91.9	3	8.1
February	ND	ND	ND	ND	ND
March	ND	ND	ND	ND	ND
April	ND	ND	ND	ND	ND
May	ND	ND	ND	ND	ND
June	ND	ND	ND	ND	ND
Overall mean	382	343	89.8	39	10.2

Table 1: *Monthly distribution of Aedes species in Ed Daein town, East Darfur State, Sudan (July 2018 - June 2017).*

ND: Not Detected.

Breeding habitats of *Aedes* species

Table 2 shows the distribution of positivity of different types of containers for *Aedes* species. From 3,243 containers inspected, 267 (8.2%) containers were found positive for *Aedes* species. High breeding was found in clay- pots (zeers, where their number out of the total containers was 2,313, of which 208 proved positive: i.e. 77.9%, followed by barrels (700), 54 of the barrels were positive; i.e. 20.2%.

Item	Total containers inspected	Jars (zeers)	Barrels	Tanks/other
No. (%)	3,243	2,313 (71.3%)	700 (21.6%)	230 (7.1%)
No. (+) (%)	267 (8.2%)	208 (77.9.%)	54 (20.2%)	5 (1.9%)

Table 2: *Distribution of Aedes species in the water-storage containers in Ed Daen town, East Darfur State, Sudan.*

Larval and pupal occurrence

By neighborhood

The overall mean of the number of the larvae for the locality was 4.6 larva/5 dips, with a C.V. 17.5%, while the overall mean of number of pupae was 1.2 pupa/5 dips, with a C.V. of 25% (Table 3).

Sentinel site	No. larvae (Mean ± SD)	No. pupae (Mean ± SD)
Alarab	3.1 ± 1.2	0.6 ± 0.3
Almatar	6.1 ± 2.2	2.2 ± 0.8
Algoba	6.1 ± 1.8	0.8 ± 0.3
Alnahda	6.5 ± 2.3	2.2 ± 0.9
MoaskarAlneem	1.1 ± 0.8	0.2 ± 0.02
Overall mean	4.6	1.2
Overall SD ±	0.8	0.3
C.V.%	17.4	25
P-value	0.135	0.040*

Table 3: *Number ofAedes larvae and pupae/5 dips in different neighborhoods within Ed Daein town, East Darfur State, Sudan.*

P-value significant at less than 0.05 levels.

High occurrence of larvae and pupae was detected in Alnahda neighborhood (6.5 larvae/5 dips and 2.2 pupa/5 dips, respectively). The second highest number of larvae (6.1 larva/5 dips) was found in both Almatar and Algoba neighborhoods. Alarab neighborhood registered 3.1 larvae/5 dips and the lowest was detected in MoaskarAlneem (1.1 larvae/5 dips).

The number of pupae was significantly different between the neighborhoods. Almatar and Alnahda registered 2.2 pupa/5 dips, whereas Algoba and Alarab revealed the presence of 0.8 and 0.6 pupa/5 dips, respectively. For pupae also, MoaskarAlneem registered the lowest number, i.e. 0.2 pupa/5 dips.

By months

It is worth mentioning that from February to June no larvae or pupae were detected. Table 4 show the overall mean numbers of larvae, for all study months in all neighborhoods, was found to be 4.5 larvae/5 dips (C.V. = 33.3%) and that of the pupae was 1.1 pupae/5 dips (C.V. = 36.4%). The highest number of larvae was recorded in September 2016 (13.6 larva/5 dips) and that of the pupae was detected in July and September 2016 (3.6 pupae/5 dips). The range of the larval numbers from July to January was from 4.2 (September) to 13.6 larvae/5 dips (November). August registered the 2nd highest count (11.6 larvae/5 dips). The counts started with 5.2 larvae/5 dips in July and ended by 6.6 larvae/5 dips during January. With regard to the pupae, the data showed that the range was from 1.2 (January) to 3.6 pupae/5 dips (July and September).

Month	No. larvae (Mean ± SD)	No. pupae (Mean ± SD)
July 2016	5.2 ± 1.5	3.6 ± 1.2
August	11.6 ± 3.5	2.6 ± 1.1
September	13.6 ± 4.6	3.6 ± 1.9
October	7.3 ± 1.9	1.3 ± 0.7
November	4.2 ± 1.6	ND
December	5.8 ± 1.7	1.4 ± 0.6
January 2017	6.6 ± 2.8	1.2 ± 0.0
February	ND	ND
March	ND	ND
April	ND	ND
May	ND	ND
June	ND	ND
Overall mean	4.5	1.1
Overall SD ±	1.5	0.4
C.V %	33.3	36.4
P-value	0.000*	0.004*

Table 4: *Mean monthly number of Aedes larvae and pupae/5 dips in different months in Ed Daein town, East Darfur State, Sudan.*

P-value significant at less than 0.05 levels; ND: Not Detected.

By season (dry and rainy)

The differences in counts between the two seasons were drastic. The dry- season average was 2.7 larvae/5 dips and 0.5 pupae/5 dips (Table 5). On the other hand, in the rainy- season, from July to October, the situation changed drastically. The larval average was 10.1 larvae/5 dips and the pupal count was 3.3 pupae/5 dips.

Season	Larvae (Mean ± SD)	Pupae (Mean ± SD)
Dry	2.7 ± 0.6	0.5 ± 0.2
Rainy	10.1 ± 2.1	3.3 ± 0.8
Overall mean	6.4	1.9
Overall SD ±	1.3	0.5
C.V. (%)	20.3	26.3
P-value	0.000*	0.000*

Table 5: *Means of Aedes larvae and pupae/5 dips during dry- and rainy- season in Ed Daein town, East Darfur State, Sudan. P-value significant at less than 0.05 levels.*

By container

Table 6 shows comparison of positivity of HHs, zeers, barrels and tanks/others. High positive of HHs was significantly shown in Algoba area (5.8 larvae/5 dips) and high positive of zeers was significantly recorded in Algoba also (5.6 larva/5 dips). The overall mean of positive HHs was found to be 4.6 larvae/5 dips (C.V. of 23.9%), overall mean of positive zeers was 3.4 (C.V. of 29.4%), the overall mean of positive barrels was 0.9 (C.V. of 33.3%) and the positive tanks/others was 0.1 (C.V. of 10%). High number of positive barrels was not significantly recorded in Almatar (1.3/5 dips) and high positivity of tanks/other was shown in Almatar (0.2/5 dips).

Site	(+) HHs	(+) jars (zeers)	(+) barrels	(+) tanks/others
Alarab	3.0 ± 1.1	2.9 ± 1.1	0.8 ± 0.3	ND
Almatar	4.9 ± 1.5	4.4 ± 1.3	1.2 ± 0.4	0.2 ± 0.01
Algoba	5.8 ± 0.4	5.6 ± 1.4	1.1 ± 0.4	0.1 ± 0.01
Alnahda	4.8 ± 1.4	4.2 ± 1.2	1.3 ± 0.4	0.1 ± 0.01
MoaskarAlneem	ND	ND	ND	ND
Overall mean	4.6	3.4	0.9	0.1
Overall SD ±	1.1	1.0	0.3	0.01
C.V.%	23.9	29.4	33.3	10
P-value	0.012*	0.010*	0.105	0.566

Table 6: *Means of positivity of houses, barrels and tanks/others in different sentinel sites within Ed Daein town, East Darfur State, Sudan. P-value significant at less than 0.05 levels; ND: Not Detected.*

Table 7 compares the mean number of positive HHs, barrels and tanks/others in different months. October (2016) showed significantly high positivity of HHs (7.5), while the high positivity of zeers was recorded in August and September 2016 (7.6). However, high

positivity of barrels was detected in October 2016 (2.2). The overall mean of positive HHs was (3.7; CV of 16.2%), positive zeers (3.2; CV of 25%), positive barrels (0.9; CV = 44.4%) and positive tanks/others (0.1; CV = 20%).

Month	(+) HHs	(+) jars (zeers)	(+) barrels	(+) tanks/others
July 2016	6.0 ± 1.4	5.4 ± 2.1	0.4 ± 0.1	0.4 ± 0.08
August	6.8 ± 1.2	7.6 ± 1.5	1.8 ± 0.8	0.2 ± 0.01
September	7.4 ± 1.3	7.6 ± 1.6	1.4 ± .3	0.2 ± 0.04
October	7.5 ± 1.1	6.5 ± 1.5	2.2 ± 1.2	ND
November	7.2 ± 1.4	6.7 ± 1.4	1.0 ± 0.8	0.2 ± 0.1
December	5.0 ± 0.8	4.2 ± 1.6	1.8 ± 0.8	ND
January 2017	4.6 ± 0.5	3.6 ± 1.3	2.0 ± 0.9	ND
February	ND	ND	ND	ND
March	ND	ND	ND	ND
April	ND	ND	ND	ND
May	ND	ND	ND	ND
June	ND	ND	ND	ND
Overall mean	3.7	3.2	0.9	0.1
Overall SD ±	0.6	0.8	0.4	0.02
C.V.%	16.2	25.0	44.4	20
P-value	0.000*	0.000*	0.002*	0.628

Table 7: *Monthly means of positive houses (HHs), barrels and tanks/others in Ed Daein Town, East Darfur State, Sudan. P-value significant at less than 0.05 levels; ND: Not Detected.*

Distribution of *Aedes* mosquitoes

By season

Table 5 indicates that the highest number of larvae and pupae occurred in the rainy- season (10.1 larvae/5 dips and 3.3 pupae/5 dips, respectively), compared to the dry-season. The overall mean of larvae was found to be 6.4 larvae/5dips (C.V. = 20.3%) and the overall mean of pupae was 1.9 pupa/5 dips (C.V. = 26.3%).

By container and neighborhood

Table 8 shows that, the high mean number of positive zeers was reported during rainy- and dry- season in Algoba sentinel site were 9.7 and 4.4, respectively. However, the proportion of positive zeers during rainy season in Algoba area was found to be 30.8% versus 20.8% in dry season. Regarding high mean number of positive barrels reported in Almatar sentinel site was (2.3) during rainy- season with proportion of 9.2% and (1.4) in Algoba sentinel site during dry season with percentage of 10.8%. In adiition, the high mean number of positive tanks/others was reported in Almatar sentinel site during rainy- season (0.7) with proportion of 17.5% and (0.1) Algoba sentinel site during dry- season with proportion of 5.3%. The overall mean of houses inspected was (25), positive houses 3.7 (14.8%), zeers inspected (45.2), positive zeers 3.5 (8.4%), barrels inspected (13.7), positive barrels 0.9 (7.7%), tanks/others inspected (1.3) and positive tanks/others was 0.08 (61%).

Sentinel site	Season	No. HHs	(+) HHs (%)	No. Jars (zeer)	(+) Jars (zeer) (%)	No. barrels	(+) barrels (%)	No. tanks/ others	(+) tanks/others (%)
Alarab	Dry	25.0	2.0 ± 0.6 (8%)	43.0	1.7 ± 1.1 (3.9%)	11.9	0.5 ± 0.1 (4.2%)	0.7	0.0 ± 0.0 (0.0%)
	Rainy	25.0	6.0 ± 1.1 (24%)	53.0	6.7 ± 1.5 (12.6%)	18.0	1.7 ± .2 (9.4%)	2.3	0.0 ± 0.0 (0.0%)
Almatar	Dry	25.0	3.1 ± 0.8 (12.4%)	38.7	2.9 ± 1.0 (7.5%)	10.7	.7 ± 0.2 (6.5%)	1.1	0.0 ± 0.0 (0.0%)
	Rainy	25.0	9.7 ± 1.2 (38.8%)	66.0	8.7 ± 1.9 (13.2%)	25.0	2.3 ± 0.1 (9.2%)	4.0	0.7 ± 0.1 (17.5%)
Algoba	Dry	25.0	5.2 ± 1.6 (20.8%)	36.8	4.4 ± 1.3 (11.9%)	13.0	1.4 ± 0.3 (10.8%)	1.9	0.1 ± 0.03 (5.3%)
	Rainy	25.0	7.7 ± 0.7 (30.8%)	46.7	9.7 ± 1.1 (20.8%)	21.7	0.3 ± 0.01 (1.4%)	1.7	0.3 ± 0.01 (17.6%)
Alnahda	Dry	25.0	3.0 ± 0.4 (12%)	38.5	2.5 ± 0.4 (6.5%)	9.4	1.2 ± 0.1 (12.8%)	1.1	0.0 ± 0.0 (0.0%)
	Rainy	25.0	10.3 ± 1.24 (41.2%)	56.3	9.3 ± 0.3	21.7	1.7 ± 0.4 (7.8%)	3.3	0.3 ± 0.01 (9.1%)
Moas-karAl-neem	Dry	25.0	0.0 ± 0.0 (0.0%)	34.2	0.0 ± 0.0 (0.0%)	2.4	0.0 ± 0.0 (0.0%)	0.0	0.0 ± 0.0 (0.0%)
	Rainy	25.0	0.0 ± 0.0 (0%)	38.7	0.0 ± 0.0 (0.0%)	3.7	0.0 ± 0.0 (0%)	0.0	0.0 ± 0.0 (0.0%)
Overall	Dry	25.0	2.7 ± 0.7 (10.8%)	38.2	2.3 ± 1.7 (6.0%)	9.5	0.8 ± .3 (8.4%)	0.9	0.02 ± 0.01 (2.2%)
	Rainy	25.0	6.7 ± 0.8 (6.8%)	52.1	6.9 ± 0.8 (13.2%)	18.0	1.2 ± 0.3 (6.7%)	2.3	0.3 ± 0.02 (13.0%)
	Overall mean	25.0	3.7 ± 1.5 (14.8%)	45.2	3.5 ± .9 (8.4%)	13.7	0.9 ± 0.1 (7.7%)	1.3	0.08 ± 0.01 (6.1%)
	Overall SD ±		0.8	0.9	0.8	0.6	0.1	0.2	0.01
	C.V. %		21.6	2.0	22.8	4.4	11.1	15.4	12.5
	P-value		0.012**	0.024**	0.01**	0.000**	0.105	0.019	0.566

Table 8: *Numbers and positivity of houses inspected in each neighborhood and the different water -storage containers and their positivities during the wet- and dry seasons in Ed Daein town, East Darfue State, Sudan.*

P-value significant at less than 0.05 levels.

There are significant differences between positive households (+HHs) inspected, zeers inspected, positive zeers, barrels, tanks/others inspected during dry- and rainy- season in the different study areas.

Aedes indices

By neighborhood

Table 9 presents the mean percent of HI, BI, CI and P/DI. BI was significantly higher in Algoba area (23.1%) and HI was not significantly high in Alarab area (93.6%) while CI was significantly high in Algoba area (10.9%), in addition P/DI was significantly increased in Algoba

area (12.8%). The overall mean HI was (91.7%) with C.V. of 4.9%, BI (14.8%) with C.V. (29.7%), CI (6.1%) with C.V. of (29.5%), while overall mean of P/DI was found (7.3%) with C.V. of (28.8%).

Sentinel site	%HI	%CI	%BI	%P/DI
Alarab	93.6 ± 4.6	4.5 ± 1.8	12.0 ± 4.6	5.6 ± 2.2
Almatar	91.1 ± 5.2	7.4 ± 2.3	19.6 ± 5.9	8.7 ± 2.7
Algoba	91.7 ± 3.7	10.9 ± 2.9	23.1 ± 5.8	12.8 ± 3.2
Alnahda	92.1 ± 5.8	7.8 ± 2.1	19.3 ± 5.5	9.4 ± 2.6
MoaskarAlneem	89.8 ± 3.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Overall mean	91.7	6.1	14.8	7.3
Overall SD ±	4.5	1.8	4.4	2.1
C.V.%	4.9	29.5	29.7	28.8
P-value	0.985	0.008*	0.012*	0.006*

Table 9: *Aedes indices in Ed Daein town, East Darfur State, Sudan.*

P-value significant at less than 0.05 levels.

By month

Table 10 indicates monthly mean % of HI, BI, CI and P/DI. High HI was not significantly recorded in July 2016 (98.2%), while high BI was significantly shown in October 2016 (30.0%), High CI was significantly shown in November 2016 (13.9%) and the high P/DI was significantly recorded in October 2016 (15.7%).

Month	%HI	%CI	%BI	%CI	%PI
July 2016	98.2 ± 3.8	7.1 ± 2.3	24.0 ± 6.8	7.1 ± 2.3	7.2 ± 2.4
August	86.4 ± 7.5	9.1 ± 2.7	27.2 ± 8.1	9.1 ± 2.7	12.7 ± 3.4
September	91.4 ± 10.3	9.8 ± 2.8	29.6 ± 9.4	9.8 ± 2.8	12.5 ± 3.3
October	96.8 ± 8.1	13.6 ± 2.8	30.0 ± 6.4	13.6 ± 2.8	15.7 ± 3.3
November	94.5 ± 4.3	13.9 ± 5.9	29.0 ± 12.4	13.9 ± 5.9	15.3 ± 5.6
December	92.6 ± 7.1	12.1 ± 5.3	20.0 ± 8.3	12.1 ± 5.3	14.4 ± 3.2
January 2017	90.6 ± 9.1	8.7 ± 3.8	18.4 ± 8.1	8.7 ± 3.8	10.7 ± 1.2
February	77.7 ± 10.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
March	90.7 ± 6.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
April	95.2 ± 2.9	.00 ± 0.0	0.0 ± 0.0	.00 ± 0.0	0.0 ± 0.0
May	86.4 ± 3.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
June	91.4 ± 4.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Overall mean	91.7	6.1	14.8	6.1	7.3
Overall SD ±	4.5	1.8	4.4	1.8	1.7
C.V.%	4.9	29.5	29.7	29.5	23.3
P-value	0.700	0.000*	0.000*	0.000*	0.000*

Table 10: *Monthly Aedes indices in Ed daien town, East Darfur State, Sudan.*

P-value significant at less than 0.05 levels.

By season

Table 11 shows the comparison of mean % of HI, BI, CI and P/DI in dry- and rainy- season. The highest mean of HI was 93.7% in the rainy- season and the highest BI was 26.9% in the same season, while the CI of 8.6% was also in the rainy- season (8.6%). However, in the same season, the highest mean of P/DI was 10.8%. The overall mean of HI was 92.3% (C.V. =1.4%), BI 18.8% (C.V. = 18.1%), CI 7.0% (C.V. =18.6%), whereas the overall mean of P/DI was 8.5% (C.V. = 18.8%).

Season	%HI	%BI	%CI	%PI
Dry	91.0 ± 2.2	10.8 ± 2.5	5.4 ± 1.3	6.2 ± 1.4
Rainy	93. 7 ± 4.5	26.9 ± 4.4	8.6 ± 1.4	10.8 ± 1.8
Overall mean	92.3	18.8	7.0	8.5
Overall SD ±	1.3	3.4	1.3	1.6
C.V.%	1.4	18.1	18.6	18.8
P-value	0.563	0.003*	0.172	0.101

Table 11: *Aedes indices in rainy and dry seasons in Ed Daein town, East Darfur State, Sudan.*

P-value significant at less than 0.05 levels.

Discussion

VBDs account for significant morbidity and mortality worldwide. This is especially true for resource-poor countries and many VBDs are neglected tropical diseases (NTDs), which disproportionately affect poor and marginalized populations [18]. A vector is an animal or object which carries a pathogen from one host to another; typically an invertebrate arthropod [19]. These authors also stated that for VBDs to complete a cycle of transmission; a vector must feed on an infected and infectious host, undergo a pathogen incubation period and transmit the pathogen to a new host. Seven of the 10 neglected tropical diseases targeted for control programs by WHO are VBDs.

The present study was carried out during July 2016 - June 2017 to determine some aspects on the ecology of *Aedes* mosquitoes in Ed Daein town, Ed Daein Locality in State (EDS), Sudan which aimed to determine the *Aedes* mosquitoes fauna in the study areas; seasonal and geographical distribution of *Aedes* mosquitoes and to determine the Stegomyia indices (HI, CI, BI andP/DI). The present study showed that *Ae. aegypti* was more prevalent in the study sites, followed by *Ae. albopictus*. This might be attributed to the availability of suitable environmental and climatic conditions in the study sites, which match with previous literature that showed the *Ae. aegypti* and *Ae. albopictus* share similar biological and behavioral characteristics; they are both container-breeding mosquitos and often compete with each other for resources [20,21]. However, several earlier studies proved that these mosquito species coexist in man-made containers in urban, sub-urban and rural settlements in tropical and subtropical regions [22,23]. This situation is not varied from the classification which was reported in the present study.

The present study agreed with majority of the studies conducted in other Sudanese states regarding mosquito fauna identified, *viz*. previous studies conducted by BNNICD teams on the distribution of *Ae. aegypti* reported on the eastern States of the Sudan, i.e. Red Sea State [24] and Kassala (Abdalmajed and Shaeldine, 2008), in addition to Elfasher Town, North Darfur State [25], Sharif [26] and Fadol (2019) in ELgenina Town, West Darfur State; and Alfahal [27], Abu Jebaiha Locality, South Kordofan State,

The study revealed that higher numbers of larvae and pupae appeared in the rainy- season, compared to the dry-season. Therefore, it can be concluded that the climate plays a major role in the distribution of these species similar to other insect species. The current results support previous findings that the number of *Ae. aegypti* larvae were higher than that of *Ae. Albopictus* larvae in the winter and the rainy-

seasons [28,29] in Samui Island and Thailand. Similar results were reported by Fadol [27] in Elgenina Town, West Darfur State, Abdelbage [30] in Kass Town, SDS and Altoum [31] in Abu Jebaiha Locality, South Kordofan State, Sudan.

The key containers for *Aedes* breeding were jars (zeers), followed by barrels in the study area. This is due to lack of water in the study sites; people may use these containers to store water. Also, containers that retained water for long periods of time make good or suitable breeding habitats for mosquitoes, such as the artificial containers, e.g. underground cement tanks and basins tanks. The finding in a study conducted in Port-Sudan, Red Sea State, revealed that the main containers for indoor breeding of dengue vector in clay pots (zeers) and barrels [32]. Moreover, the findings of several other studies among the series of previous studies conducted in the Sudan showed common containers between all the states, localities, towns and villages. These containers are three types, viz. zeers, barrels and water-storage tanks [24-27,30,33-35]. WHO (2012b) reported that clay-pots (zeers) were the main and preferred receptacles infested by *Ae. Aegypti* followed by barrels and water -storage tanks in the Sudan [10].

The *Aedes* indices remain central to the monitoring of dengue vector populations. The most commonly used indices, as mentioned earlier, are the HI (or 'premise') % HHs infested with larvae and/or pupae) the CI)%, water-holding containers infested with larvae and/or pupae and the BI, number of positive containers/100 HHs inspected) [36].

The present study showed that the highest HI was recorded in July 2016, while highest BI was in October 2016; the highest CI was recorded in November 2016 and the highest P/DI was recorded in October 2016. This was attributed to the suitable R.H. and temperature. Furthermore, the study showed that the HI was highest in Alarab neighborhood, while CI was highest in Algoba neighborhood. The highest P/DI was that of in Algoba neighborhood. These neighborhoods are depending mainly on storing water in different containers, due to the shortage of water. Several studies proved that R.H. influences longevity, mating, dispersal, feeding behavior, egg production, oviposition of mosquitoes and dengue virus transmission in other countries [37,38]. Adult survival and hatching rate are affected by the rise in temperature and lower humidity as reported by Costa., *et al* [39].

The present study showed that the *Aedes* indices are very high during rainy season. The overall mean of HI was 92.3%, BI 18.8%, CI 7.0%, whereas the overall mean of P/DI was 8.5%. These figures of indices are high enough to maintain risk of transmission of arbo-viral diseases such as yellow fever, dengue fever and dengue haemorrogic fever and Chikungunya. However, HI values for *Ae*. *aegypti* were used to estimate risk of YFV transmission for the individual species with values of HI > 35%, BI > 50 and CI > 20% considered as high risk of urban transmission of YFV; HI < 4% BI < 5 and CI < 3% considered as unlikely or low risk of the disease transmission [40]. Similarly, the Pan American Health Organization (PAHO) has established threshold levels for dengue transmission based on HI for *Ae*. *aegypti* with low HI < 0.1%, medium an HI 0.1%-5% and high an HI > 5% [41]. Similar findings were obtained by Bakr., *et al*. [33] in Elfasher town, North Darfur State. She found that the wet-season showed higher percentage of HI (17.8 ± 4.0%), compared to dry -season (11.4 ± 2.0%). The BI was also higher in the wet- season (29.5 ± 8.8percentage) than the dry-season (14.1 ± 2.7%). Moreover, the CI was (9.2 ± 2.7%) in the wet-season and (3.6 ± 0.6%) in the dry-season. However, the P/DI was found to be (7.8 ± 5.2%) in the wet-season and (0.1 ± 0.1%) in the dry-season.

The study showed that there are significant differences in all stegmoyia indices between the two seasons. The finding of the study clearly showed that prevalence of *Aedes* mosquito was found to be in rainy- season which matched with studies conducted in South Kordofan State [31]. This might be due to increased rain with environmentally deteriorated conditions around human settlement being the main reason for increased vector abundance of both immature and mature forms of *Aedes* mosquitoes.

Moreover, the present study showed high positivity of HHs during rainy- season and the positivity of jars/zeers were higher in the rainy- season and the same was reported for the storage tanks/others. These results are similar to those observed in some Brazilian cities mainly in Sao Paulo city by Favier., *et al*. [42], who reported that there was a record of the highest proliferation of eggs and larvae of *Ae.*

aegypti during the rainy-season. A similar situation was reported in Brasalia city [43]. In Roraima city, the dynamics of mosquito population was influenced by rainfall [42]. In Sao Luas, State of Maranhao, Bezerra., *et al*. [44] showed a trend for the development of mosquitoes during the temperature reduction periods, increase in R.H. and precipitation. *Aedes mosquitoes* proved to have a strong seasonal pattern associated with temperature and rainfall. The occurrence of heavy and continuous rain, interspersed with short periods without rainfall, is associated with the outbreak of mass eggs and an increase in the number of mosquitoes (Estallo., *et al*. 2015). Likewise, the study found that the high mean of HI was shown in rainy- season (93.7%), high BI was reported in the rainy- season (26.9%), while the highest CI occurred in the rainy- season (8.6%). Similar trend was found for P/DI (10.8%). Therefore, it can be concluded that these findings are in line with the various studies, which emphasized that the abundance of *Aedes* mosquitoes is affected by air temperature and rainfall, e.g. Mellor and Leake [45]. However, air temperature affects the breeding activity of the adult female *Aedes* mosquito, as it has to take blood for the nutrients required for egg production and the biting activity of the females' increases with increasing air temperature as reported by Martens [46]. On the other hand, the presence, absence, size [47] and persistence of breeding sites for *Aedes* mosquitoes [48] are affected by rainfall as the immature stages of the mosquitoes are aquatic. Rain can help build breeding sites, but very heavy or prolonged rain may disrupt the sites and wash the eggs and larvae away or kill the mosquitoes directly [45].

The current study shed light on the seasonal occurrence and abundance of *Ae. aegypti* and *Ae. albopictus* throughout different months of the year. The results of the study confirmed the presence of the two species in 7 months and disappear during 5 months, i.e. from February to June 2017, which indicated that the two species are seasonal species. *Aedes* mosquito showed marked seasonal variations in terms of distribution and abundance, where most of their presence was from July to January in the study area. The finding of the study to some extent not varied from a study of South Kordofan which revealed that a marked seasonal fluctuations in its occurrence and abundance. *Aedes* larvae occurred during five months of the year, i.e. July, August, September, October and November and completely disappeared during the remaining months, the peak of the relative abundance was in September. The seasonal fluctuation in its occurrence and abundance in both study sites might be due to rains, suitability of temperature and R.H. common factors that affect the abundance of *Aedes* mosquito [31].

Conclusion

The study concluded that *Ae. aegypti* was the main species in all the study sites (89.8%), followed by *Ae. albopictus* (10.2%) in Ed Daein town, Ed Daein Locality in State (EDS), Sudan; the key container for *Ae. aegypti* and *Ae. alobopictus* were jars/zeers (77.9%), followed by barrels (20.2%); c) Larvae and pupae increased during the rainy- season, *viz*. from July to January, compared to dry -season (from February to June); all *Aedes* indices were also higher during rainy- season. However, the study area is at risk for arbo-viral diseases. Therefore, more efforts are needed from the health authorities and the community.

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