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Prevalence, habitat and productivity profiles of *Aedes* mosquitoes (Diptera: Culicidae) in Sennar state, Sudan

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Abstract
The Sudan has suffered through the last few years from several diseases transmitted by Aedes species mosquitoes (Diptera: Culicidae). This study was conducted as cross-sectional surveys during Sept. 2017–May 2018 to determine the existence, characterization, geographical distribution and larval habitats of *Aedes* mosquitoes in Singa, Sennar, Elsuki and Aldaly towns, Sennar State, Sudan, using a cluster sample method. A total of 840 households (HHs) were surveyed every 2wk for *Aedes*, using a specially designed format. All water-holding containers in and around the HHs were inspected for larvae and pupae. Larvae (4th instar) were mounted for species identification and the pupae were reared to the adult stage for species morphologically identification. The data were analyzed using SPSS. A total of 5,951 water containers were inspected. Of these, 2.7% were found to be positive for larvae or pupae. A total of 1,421 larvae and 358 pupae were collected from different containers. The most important habitat containers were clay-pots (Zeers). *Aedes* indices were calculated. Total House Index (HI) was 7.02%, container index (CI) was (2.8%), Breteau index (BI) was 19.6, and pupal/ demographic index (P/D) was 0.44. *Ae. aegypti* was 82.7%, *Ae. africanus* 14.5%, *Ae. vittatus* 1.9%, *Ae. simpsoni* 0.9% and *Ae. scatophagoides* was found as pupae only (9). Clay-pots were the most preferred breeding habitats for *Aedes* mosquitoes. Moreover, *Ae. aegypti*, is the dominant species in the study areas, suggesting a high potential for arbovirus transmission in the study areas. However, effective control strategies aiming to reduce the number of larvae/ pupae will have significant impact in minimizing the risk of arboviral transmission.

Keywords: *Aedes* spp, prevalence, habitat, productivity profile, Sennar, Sudan

1. Introduction
Mosquitoes (Diptera: Culicidae) are of a remarkable importance in the transmission and dissemination of both human and animal diseases [1]. The mosquito constitutes the most medically important group of arthropods [2]. Mosquitoes transmit malaria, dengue virus (DENV), and Zika virus (ZIKV), which cause over one billion infections and one million deaths annually. *Aedes* mosquitoes, lover 950 species, are distributed around the world. Rift Valley fever (RVF) and the equine encephalitis (EE) are important livestock diseases transmitted by mosquitoes. Mosquito species are over 2,500 worldwide, 18 genera and subgenera. Those species of greatest importance as vectors of pathogenic agents *Aedes*, *Culex*, *Anopheles*, and *Mansonia* [4]. *Aedes* borne diseases represent a major health problem. Yellow fever (YF) outbreaks have been reported in disparate regions in Darfur [5]. *Ae. aegypti*, the main vector of DF and YF, was reported in different regions in eastern, central and former southern Sudan [6]. Descriptions of the mosquitoes of the Republic of the Sudan are mostly limited to works published >60 yr ago [7]. Effective VC depends on a good understanding of larval and adult vector ecology.
Therefore, the current study was conducted to characterize larval habitats, determine the larval indices for identifying risk areas of transmission, as well as the species distribution of *Aedes* mosquitoes in four towns, *viz*. Sennar, Singa, Elsuki and Aldaly, Sennar State, the Sudan. The information on distribution and larval habitats is expected to help in designing a proper intervention against these mosquitoes The specific objectives were:
a) To determine the *Aedes* mosquitoes fauna in four towns in Sennar State.
b) To characterize *Aedes* mosquitoes larval habitats in these towns, and

c) To determine the seasonal and geographical distribution of *Aedes* mosquitoes in the study area

2. Materials and Methods

2.1 Study design

This study was conducted as cross-sectional surveys during Sept. 2017 – Feb. 2018, in the four above-mentioned Towns (Map).

2.2 Study area

Sennar State is located at the central-east Sudan (32.9º - 35º 4' E and 12.5- 14. 7 N). The State total area is 40,680 km^2 and it shares borders. Gezira, White Nile, Gedarif and Blue Nile states, and from the south by the Republic of South Sudan and from the east Ethiopia. Administratively, the State is divided into 7 Localities and 22 Administrative Units; Singa town is the capital. Dindir National Park (DNP) is one of the tourists' attraction sites. The State is flat, but interrupted by small Rocky Mountains, and seasonal streams (khors), in addition to the Blue Nile River. Sennar State is located at the south part of the savannah, with low rainfall biome; the climate prevailing is tropical continental with an annual rainfall of 600 mm and the R.H between 18 and 80%. The year is divided into a hot-dry season (March-May), a warm-wet season (June-October) and moderately cool-dry season (November-February). The temperatures range from 20 to 40 ºC, The area is relatively rich with natural vegetation; forests cover about 34% in addition to small horticultural gardens, the major field crops production areas for sorghum. millet, sesame and groundnuts. Sennar State is wealthy by its wild and domestic animal resources. The dominant domestic animals in the area are sheep, cattle, goats and others, especially in the villages. In contrast, the DNP contains a large variety of wild animals and birds.

2.3 Study Sites

Samples were collected from four towns in the State, *viz*. Sennar, Singa (the capial), Elsuki and Eldaly. Samples were collected (every 2 wk) from certain clusters of each town during the study period (Table 1).

2.4 Study population

Larvae, pupae and adults of *Aedes* mosquitoes were collected from larval habitats and resting places in the four towns.

2.5 Sampling

Samples of *Aedes* larvae and pupae were collected every 2 wk; 20 households (HHs) were surveyed randomly, i.e. 5 HHs/ cluster (40 HHs /month) from Sept. 2017 –Feb. 2018) in each town, except Eldaly town, which was surveyed monthly (20 HHs /month). The total of the HHs surveyed during this period of study was 840.

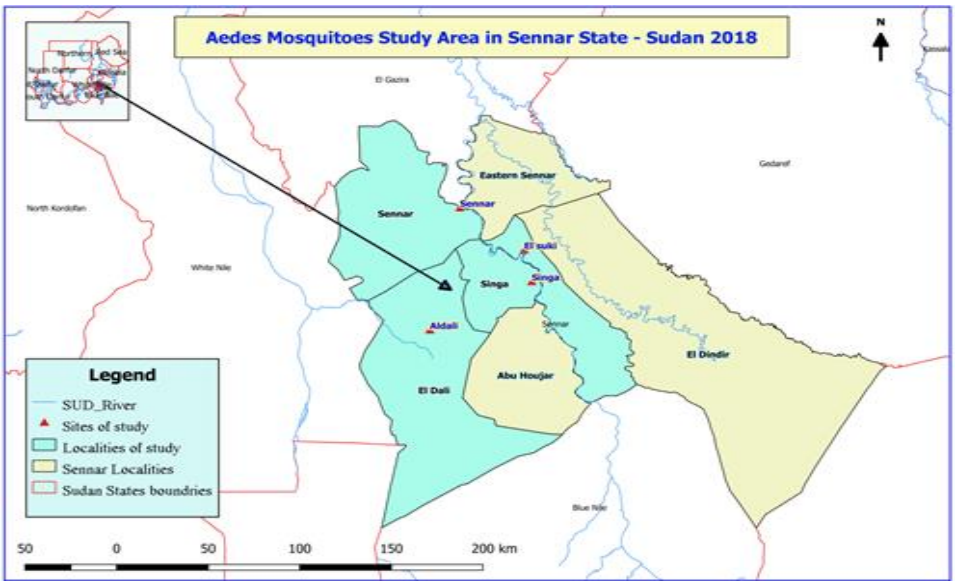

Fig 1: Aedes mosquitoes study area in sennar state – Sudan 2018

Table 1: Selection of clusters choosing in the study site

Site (town)	Cluster (area)
Sennar	Elglaat
	Al-bunian
	Mayierno
	Almazad
Singa	Al-shargi
	14
	Elgla'a
	Alshmali
Elsuki	Almarboo
	Alaftah
	Hasanain
	Berged
Aldaly	Aldaly

2.6 Mosquito collection

Larvae were collected between 07:00-01:00 hr during the study period using the Entomological surveillance kits for collection of *Aedes* mosquito, *viz*. light torch, white enamel pan and fine mesh sieve.

2.6.1 Larvae and pupae

2.6.1.1 From small containers

Larvae and pupae were collected from clay- pots (locally called zeers), discarded tires, flowers vases, tins, small containers, air coolers, tree- holes, rocky hollows, etc. These were emptied directly into a tray or filtered through a sieve. The larvae and pupae were removed with a pipette and kept in water jugs, then transported to the laboratory.

2.6.1.2 From large containers

Collection by comprehensive netting was performed in large, round containers with low densities of larvae and pupae. The net was immersed carefully to about 7.5 cm beneath the water surface of the container and moving around the perimeter in a downwards spirally, creating a funnel-like in which the larvae and pupae were concentrated at the bottom of the container. All collected larvae and pupae were kept in water jugs (for each breeding-site) and transported to the laboratory. Each sample was labeled with the pertinent information [8].

2.7 Mosquito rearing, processing and identification

The field-collected immature stages of *Aedes* were kept in well-labeled water jugs and were transferred to Elgadal Center (Sennar) for rearing and maintenance, using standard procedure. The early stages of larvae were transferred to the dishes, provided with rice powder, and after pupation, pupa were transferred into paper cups and placed in mosquito cages. The emerged adults were supplied with sugar. The adults were used for species identification.

Fourth-instars (L4) larvae were transferred directly from preservative into slide and were mounted dorsal side up. Slides were left to dry at room temperature for 3 days, labeled with the location and date of collection, type of habitat and species name. Specimens were identified using Hopkins [9] for larvae and Edwards [10] for adults.

2.8 Entomological survey

Entomological surveys were carried out by a well-trained team (4 health workers) and a supervisor every 2wk for 6 months in the selected clusters of each town**.** The team visited the selected HHs for larval stages of *Aedes*. Information on the number of inspected HHs, types and positive containers (with pupae and/ or larvae), and HHs with positive container were recorded. Moreover, larval surveys were conducted at outdoor sites close to the HHs and when located, the larval habitats were described. Locations positive to *Aedes* larvae and adult collection sites were marked using GPS. Samples of larvae were preserved in 70% alcohol in 50 ml vials and adult *Aedes* mosquitoes were kept in 1.5 Eppendorff tubes with silica gel for subsequent species identification.

2.9 Data analysis

The data were analyzed using SPSS. *Aedes* mosquitolarval indices, i.e. House index (HI), container index (CI), Breteau index(BI) and pupal/demographic index (P/DI) were calculated using the following formulae:

HI = No. of houses positive for *Aedes* larvae / No. of HHs inspected x 100

CI = No. of positive containers /No. of containers inspected x 100

BI = No. of positive containers/100 HHs inspected.

P/D = Total no. of collected pupae of *Aedes* mosquitoes / Total number of inhabitants in the inspected HHs.

2.10 Ethical clearance

Ethical clearance to conduct the study was obtained from the state Ministry of Health after approval by Ethical Committee

3. Results

Over the period of 6 months (Sept. 2017 – Feb. 2018), 42 field surveys were carried out in the four towns. A total of 1,421 *Aedes* larvae were collected and all were identified using morphological key up to the species level. The results showed that 1,175 (82.7%) were *Ae. aegypti*, 206 (14.5%) were *Ae. africanus*, 27 (1.9%) were *Ae. Vittatus*, 13 (0.9%) were *Ae. simpsoni* and 7 pupae identified as *Ae. scatophagoides* (Table 2).

A total of 840 HHs were inspected during the study period of which 59 (7%) were found to be positive for larvae (Table 3). HHs positive percentages recorded in Singa (9.5%), followed by Sennar (7.9%), Elsuki (7%) and Aldaly (only 7 pupae).

Table 2: Collected and identified *Aedes* larvae collected from the four study sites.

No collected and Identified	***Aedes* species**								
	Ae. aegypti		***Ae. africanus***		***Ae. vittatus***		***Ae. simpsoni***		***Ae. scatophagoides***
	No.	***%***	***No.***	***%***	***No.***	***%***	***No.***	***%***	***No***
1,421	1,175	82.7	206	14.5	27	1.9	13	0.9	7(pupae)

Table 3: Results of positivity of inspected households (HHs) for *Aedes* spp. larvae and pupae in the studied towns

Town	Total of inspected HHs	Total of (+) HHs	(%)	Total of larvae collected	Total of pupae collected
Sennar	240	19	7.9%	297	64
Singa	240	23	9.5%	709	185
Elsuky	240	17	7%	388	102
Aldaly	120	0	0%	27	7
Total	840	59	7%	1,421	358

3.2 Availability and positivity of container

The most available container habitats (table 4) encountered were clay-pots, which constituted 49% of the total container sampled, followed by barrels (18.6%), flower vases (16.4%), used tires (6.9%) and other container (8.5%). In Sennar town, clay-pots (zeers) and other containers registered the highest positivity- rate (4.5% and 3.3%, respectively). Whereas, used tires in Singa area had 6.7% positivity rate, followed by clay-pots (5.5%) and other containers with (37).For Elsuki town, clay-pots registered 5.8% positivity rate, followed by flower vases (0.3%), and for Aldaly town all habitat types were negative, except the other containers (0.5%).

Table 4: Container type, availability and positivity rate of *Ae. aegypti* larval habitats (Sept. 2017– Feb. 2018)

Type	Sennar			Singa			Elsuki			Aldaly			Combined		
	Total	+	%	Total	+	%	Total	+	%	Total	+	%	total	+	%
Clay-pots	719	30	4.1	884	49	5.5	974	57	5.8	362	0	0	2,939 (49%)	136	4.6
Barrels	269	4	1.4	254	2	0.7	244	0	0	342	0	0	1,109 (18,6%	6	0.5
Used Tires	120	2	1.6	89	6	6.7	85	0	0	119	0	0	413 (6.9%)	8	1.9
Flower Vase	357	3	0.8	318	1	0.3	304	1	0.3	0	0	0	979 (16.4%)	5	0.5
Others	151	5	3.3	117	4	3.4	65	0	0	178	1	0.5	511 (8.5%)	10	1.9
Overall Total	1,616	44	2.7	1,662	62	3.7	1672	58	3.4	1001	1	0.09	5,951 (100%)	165	2.7

3.3 Aedes density indices

Table (5) shows that a total of 5,951 water containers were inspected in the four studied towns taken from 840 HHs. Different indices used to assess the levels of *Ae. aegypti* infestation. HI, CI, BI and P/DI. In this study the overall HI was found to be 7.02%, highest in Singa town (9.6%), followed by Sennar town (7.9%), Elsuki town (7.08%) and none was found in Aldaly town. The CI was highest in Singa town (3.7%), followed by Elsuki town (3.4%), Sennar town (2.7%) and lowest in Aldaly town (0.09%).BI was highest in Singa town (25.8), followed by Elsuki town (24.16), Sennar town (18.3) and lowest in Aldaly town (0.12).

3.4 Aedes density seasonality

Noticeable reduction in *Aedes* mosquito density was recorded in January and February (zero) (Fig. 1). Then the density started to increase during the rainy month September, when the maximum mean of larvae/pupae was recorded (73/20.2). In October larvae/pupae means were 63.25/10.75. The *Aedes* mosquito density started to decrease gradually with reduction in BI, and sharp reduction continually occurred during Nov., Dec. (28.5/3, and 17.75/6.25, respectively).

3.5 Indoor and outdoor collection

There was a significant difference between CI mean and positivity mean of *Ae. aegypti* immature stages in outdoor (17.8%) and indoor (15.8%) in the different container types. This with noticeable reduction in total container mean in outdoor (351.8) with (843.8) in total container mean in indoor. Table (6) illustrated different container types and positivity in two collection areas. Clay-pots were common *Aedes* container habitat in two collection areas, followed by other containers and the last one had a higher positively rate.

Table 5: *Aedes aegypti* mosquito indices (HI, CI, P/DI and BI) n the studied towns (Sept. 2017– Feb. 2018), Sudan

Town	HI			CI			P/DI			BI	BI/ Month
	Inspected HHs	(+)	%	Inspected container	(+) container	%	Collected pupae	inhabitation HHs	%		Sept / 19%
											Oct/ 19%
Sennar	240	19	7.9	1.616	44	2.7	64	1,355	0.04	18.3	Nov /14%
Singa	240	23	9.6	1.662	62	3.7	185	1,328	0.13	25.8	Dec /11%
Elsuki	240	17	7.08	1,672	58	3.4	102	1,448	0.07	24.16	Jan/ 0%
Aldaly	120	0	0.0	1,001	1	0.09	7	807	0.01	0.12	Feb/ 0%
Total	840	59	7.02	5951	165	2.8	358	4938		19.6	

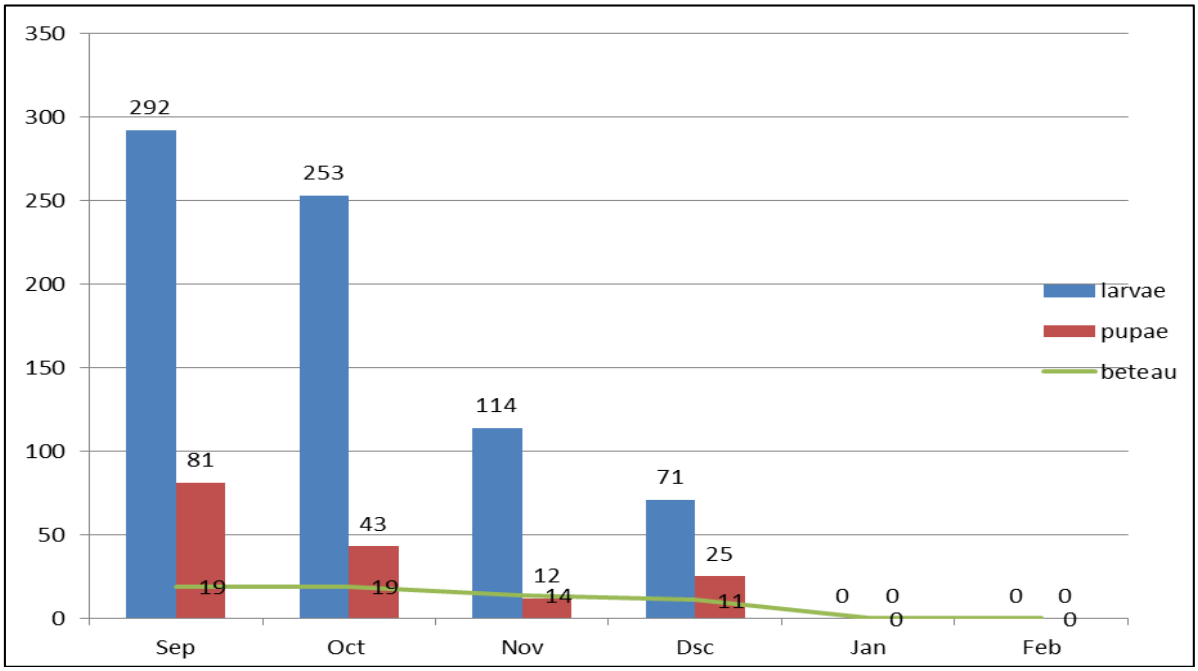

Fig 1: Relationships between Breteau Index, larvae, pupae collected at (Sept. 2017– Feb. 2018), Sinner State- Sudan (Y axis = BI, X axis = month)

Table 6: The different container types and their positivity indoors and outdoors

Container Type	Collection area					
	Indoor			Out door		
	Total	(+)	%	Total	(+)	%
Clay-pots	1,587	58	3.6	1,352	78	5.7
Barrels	1,039	5	0.4	70	1	1.4
Used Tire	166	3	1.8	274	5	1.8
Flower Vase	979	5	0.5	0	0	0.0
Other Containers	448	8	1.7	63	5	7.9
Total	4,219	79	1.8	1759	89	5
Mean	843.8	15.8		351.8	17.8	
CI mean			1.8	5%		
BI mean			7	Not implemented		
P/DI mean			3.2	Not implemented		
HI mean			86.9	Not implemented		

5. Discussion

Aedes mosquitoes are vectors for transmitting many arboviruses. Knowledge of the prevalence, fauna, breeding habitat and productivity profiles of this vector is vital for implementing appropriate interventions. Thus, this study was conducted to determine the breeding habitats and presence of *Aedes* species in the study area**.** This study is the first of its kind in Sennar state since the reports published by Lewis [11, 12] surveyed the entire Anglo-Egyptian Sudan and he recorded several species of *Aedes* mosquito. The present study identified 5 species of *Aedes* in the study area: *Ae. aegypti*, which constituted 82.7% of total *Aedes* larvae identified, followed by *Ae. africanus* (14.5%), *Ae. vittatus* (1.9%), *Ae. simpsoni* (0.9%) and *Ae. scatophagoid*es (7 pupae). This result agreed with previous studies conducted by Lewis [6, 11, 12]. A recent study conducted in Khartoum State, the capital of Sudan, identified only *Ae. caballus*, *Ae. vexansarabiensis* and *Ae. caspius* [7]. Generally, similar studies in the country have reported occurrence of *Ae. aegypti* only [13, 14, 15].

Ae. aegypti is native to Africa and is now globally distributed in tropical, subtropical and mild temperate regions. Globalization of trade has influenced the distribution of this invasive species [16]. In this study, the dominant *Aedes* species collected and identified in all study areas in Sennar State and in different types of containers inspected during the study period was *Ae. aegypti*. Studies in urban forest in Rio de Janeiro [17], Central Africa [18] Laos [19] and East Ethiopia [20] showed *Ae. aegypti* was strongly associated with urban environments. The presence of *Ae. aegypti* in this study is likely attributable to the abundance of suitable water-holding containers that are favorable for its breeding, in addition to the availability of adequate organic matter as larval food [21]. This species is usually found in close proximity to human residences and feeds preferentially on human blood [22]. In Sudan, *Ae*. *aegypti* is responsible of transmission of DENV in eastern Sudan [14]. A study conducted in Darfur States sated that Sudan is subject to repeated outbreaks, of Viral Hemorrhagic Fever (VHF). And YF, which have been reported from the 1940s through 2005. In 2012, a new outbreak of YF occurred in the Darfur region [23]. The presence of *Ae. aegypti* as a dominant species in the study area poses risks of disease outbreaks.

Ae. africanus is abundant in east and central Africa. It has a role in the jungle transmission of YF [24]. The low infection rates found in both species could be due to the origin of the chikungunya virus (CHIKV) strain in the sylvan mosquito *Ae. Africanus* [25]. It is very aggressive to humans [26]. In the present study *Ae*. *africanus* was found in two areas, *i.e*. Singa and Sennar, in different types of containers, *viz*. barrels, clay-pots and old tires indoor and also outdoor around houses.

Ae. vittatus acts as a vector of pathogens causing animal and human diseases *e.g*. CHIK, Zika (ZIKV) and dengue. *Ae. vittatus* is also able to breed in artificial containers [27]. This species is known as sylvan YFV vector in several African countries [24]. In addition, ZIKV was isolated from *Ae. vittatus* in a Senegalese village [28]. *Ae. vittatus* in this study was found only in Aldaly mountainous area and it was collected from rocky pits filled with rainfall water during September. This might indicate that this species occurs as sylvan in Sennar State, This is in agreement with Diallo *et al*. [29], and Roberts [30], who found that this species is sylvatic mosquito that mainly breeds in rock pits filled with rainfall water.

Ae. simpsoni consists of a complex of mosquito species including vectors of important arbovirus diseases, such as YF [31]. The species was also recorded in this study in Singa area in few numbers and it was found during September in discarded tires around HHs together with *Ae*. *aegypti* in the same breeding habitat, This could be attributed to the presence of *Ae. aegypti* in most outdoor containers as dominant species in the study area. A study conducted in Tanzania revealed that, presence of *Ae. simpsoni* and other *Aedes* species in the same breeding area with *Ae. Aegypti* [32].

Ae. scatophagoides widely distributed in the southern and central Sudan, Lewis [11, 12] recorded 33 species and one variety of *Aedes* in the Anglo-Egyptian Sudan including *Aedes* (*Mucidus*) *scatophagoides* In this study *Ae. scatophagoides* was recorded in El-suki area in few numbers of pupae (7) in a pool filled with rain water during October, also recorded in Sennar as adults by accident during night collection by using aspirator, and from Singa by light trap during entomological survey conducted by vector surveillance unit in the State.

From Singa area *Ae. aegypti*, *Ae. africanus* and *Ae. simpsoni* were collected indicating that the area is at risk of outbreaks. Although, most parts in Sudan are at risk of *Aedes* borne-diseases, only a few studies were conducted on its distribution, ecology and biology (Kassla and Port Sudan). But information on the *Aedes* mosquito of other states is lacking.

The study indicated that *Aedes* mosquitoes species prevalent in Sennar state preferred to breed primarily on water storage containers, especially clay-pots, discarded tires and barrels. The most key- containers were found most positive and formed the major breeding sites were clay-pots that constituted (49%) of the total container sampled, followed by barrels (18.6%), flower vases (16.4), used tires (6.9%) and other containers (8.5).These containers are used store the water for long time that creates suitable habitat for *Aedes* mosquitoes to complete their life- cycle. Same was reported by other authors [33, 34]. Moreover, the micro-habitat inside tires, such as cool temperature, humidity, and reduced light, create a suitable environment for *Aedes* mosquito breeding. Significant deference between CI mean and positivity mean of *Aedes* mosquitoes immature stages in outdoor (17.8) and indoor (15.8%) in deferent container type could be attributed to availability of different suitable kinds of containers around HHs. Moreover, many containers found outdoors are not covered and filled with rainwater. This is s in agreement with Chareonviriyaphap *et al*. [35]. Clay-pots, the main containers

found positive indoors and outdoors, many of them are usually filled with dirking water out of HHs for street people (Sabeel), most of them are uncovered. This finding is in agreement with previous studies conducted in Port Sudan and Kassala towns [13, 14]. Discarded tires had an especially high positivity rate for larvae, especially during the rainy -season. This is consistent with other studies done in India and northwest Ethiopia [36, 37].

The study monitored different Indices used to assess the levels of *Ae. aegypti* infestations. The total HI was found to be 7.02%. However high HI was observed in Singa 9.6%. The CI was 2.8%. CI increased in Singa and Elsuki towns (3.7, and 3.4% respectively). BI was 19.6%. Singa town showed high BI (25.8), followed by Elsuki town (24.16%).These indices indicated the risk of YF in the study area according to the WHO; there is an epidemic risk when these indices are more than the thresholds of 5% for the BI, 3% for CI and 4% for HI [38]. Season, specially rainfall, can increase the mosquito density [39], by increasing the availability of potential breeding sites [40]. Many studies have shown that rain plays an important role in DF epidemiology [41]. In this study the density started to increase during the rainy month September, when the maximum mean of larvae/pupae was recorded (73/20.2). In October, larvae/pupae means were (63.25/10.75). *Aedes* Mosquito density started to decrease gradually with reduction in BI, and sharp reduction occurred during November, and December (28.5/3, and 17.75/6.25, respectively). Noticeable reduction in *Aedes* density was recorded in January and February (zero).

6. Conclusions

This study concluded the following

- The presence of *Ae. aegypti* is the dominant species in all selected area of study in Sennar State, in addition to *Ae. africanus*, *Ae. vittatus*, *Ae. simpsoni* and *Ae. scatophagoides* which have role in transmission.
- The most key- containers, which were found most positive and formed the major breeding sites were clay-pots (49%) of the total container sampled, followed by barrels (18.6%) and (4.6%) positivity rate, followed with used tires (1.9%).
- *Aedes* mosquitos' density started to increase during the rainy months, and then decreased gradually, especially in outdoor containers.
- Most clay-pots found around the houses were uncovered and created suitable breeding place for *Aedes* mosquitoes.
- Discarded tires on streets, inside houses, establishments and tire services stations might also create a suitable environment for *Aedes* mosquito breeding.

6.2 Recommendations

- Conduct source reduction by suitable method to eliminate mosquitoes, *i.e.* by covering all water containers around living and working areas, and proper disposal of tires should be implemented.
- Conduct active surveillance system to determine abundance, distribution, and type of containers in the area.
- Conduct public education campaigns focusing on reducing or eliminating larval habitats for *Aedes* spp and personal protection measures.
- Further studies regarding distribution and other bionomics factors are required for better conduct.
- Space-spraying is to be applied during out- breaks or high densities of the mosquito (*i.e.* when justified).

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