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Resting behaviors and seasonal variation of *Anopheles arabiensis* in River Nile State, Sudan

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Abstract
Resting behavior and seasonal variation of mosquitoes are of a high degree of importance in the planning of vector control programs and should be well known. A study was carried out to determine the current resting behavior, and the seasonal variation of the malaria vector *Anopheles arabiensis* in selected sentinel sites in River Nile State, Sudan. Mosquitoes were collected in three different seasons; dry- cold, hot- dry, and wet-season. Mosquitoes were collected from the possible resting places at indoor and outdoor sites in four selected sentinel sites. Mosquitoes showed a preference for both indoor and outdoor resting. *Anopheles arabiensis* showed a marked seasonal fluctuation in the occurrence with its peaks of abundance in March, November, and January. The findings of the study might indicate a risk of indoor malaria transmission, therefore, control measures like indoor residual spray should be considered to suppress mosquito population and reduce the possibility of malaria and other vector-borne diseases transmission in the state.

Keywords: *Anopheles arabiensis,* Resting habits, seasonal variation, River Nile State, Sudan

1. Introduction
Vector-borne diseases (VBDs) transmit more than 17% of infectious diseases and annually cause more than 700,000 deaths [1]. Mosquito-borne diseases (MBDs) are transmitted by the bite of infected female mosquitoes belonging to one of the three mosquito genera, *Anopheles*, *Aedes*, and *Culex*. Mosquitoes play a large role in transmitting many pathogens of medical importance, including malaria, rift valley fever, dengue fever, West Nile virus, Zika virus, and chikungunya virus [2].

Most *Anopheles* mosquitoes are active at dusk or dawn (crepuscular) or night (nocturnal). According to the feeding behavior: mosquitoes that feed indoors are called (*endophagic*), while mosquitoes that feed outdoors are called (*exophagic*) [3]. Mosquitoes usually enter houses to feed and rest indoors for a few hours after feeding to digest the blood meal and produce eggs. Indoor resting sites called *endophilic* it happens usually on a wall, on hanging clothes, or under furniture, while outdoors resting sites called *exophilic* usually settle on plants or trees, in pits, caves, on the ground, or in other cool dark places. Due to the scarcity of safe outdoor rest areas, indoor rest is more common in dry or windy areas [4, 5].

Anopheles arabiensis has been classified as a more *exophilic* species in comparison to *An. gambiae* and *culex* species [6,7,8], although the opposite (*endophily*) behavior has also been reported [9]. In Sudan, *An. arabiensis* showed both *endophilic* and *exophilic* behavior [10-13].

Anopheles arabiensis showed different trends of seasonal fluctuation in its relative abundance. The peak of relative density of *An. Arabiensis* was found during February in Zambia [14], March in Zimbabwe [15], and December and January in Madagascar [7]. In Sudan, *An. arabiensis* also showed a marked seasonal variation in its relative density, wherein the northern part, this species showed a drop in its numbers during July and October, and an increase in relative density during November and July [16]. In eastern Sudan, *An. arabiensis* showed a gradual decrease in the relative density after the end of the rainy season in October to February, and an increase in its numbers during the beginning rainy season [17].

This study was conducted in River Nile State to determine the resting behavior and the seasonal variation of *An. arabiensis* populations in the selected sentinel sites in the River Nile State, Sudan.

2. Materials and Methods

2.1 Study Areas

River Nile state is one of the northern states of Sudan and is among 18 Sudanese states (16 – 22´ N and 30 – 32 E) (Figure 1). The area of the state is about 22,123 km^2 (47,152 sq mi), and the estimated population until the year 2017 was 1,472,257. The average rainfall ranges between (150- 25) mm, the relative humidity does not exceed 59, and the average temperatures are between (47 °C - 8 °C) degrees. Water sources include the River Nile, the Atbara River, and the valleys. The state is covered with different types of vegetation such as *Vallechia Flava* (Forssk) (Salam) and *Capparis decidua* (Tondob). The arid semi-desert areas of the state are characterized by sand and silt soils in many parts, with vertisol soil on the riverine ecosystem [18].

2.2 Study Sites

The study was carried out in four sentinel sites; (Albawga, Alzidab, Soola, and Gandato) (Figure 1). The sentinel sites were selected based on; part of the approved national sentinel sites, have intensive agricultural activities, availability of the different breeding places, expected environmental change due to the construction of the Merowe Dam, the impact of the local gold mining activities, and the high densities of mosquitoes in previous entomological surveys conducted during 2014 (unpublished documents).

2.2 Entomological sampling method

By using Pyrethrum Spray Catches (PSCs), mosquitoes were collected from each sentinel site in three different seasons; hot-dry season (March -June), wet or rainy season (July – October), and cold- dry season (November –February). In each sentinel site, mosquitoes were knocked down in (7-9) rooms of different types (bricks and mud-built rooms) every month from March 2015 to February 2016. In each sentinel site, mosquitoes were knocked down between 06:00-8:00 a.m. The knocked down mosquitoes in each room were then placed in Petri dishes (and/or paper cups) lined with moist cotton and filter paper and transported to the insectary. The persevered mosquitoes were identified to species level.

The field-collected mosquito species were categorized based on abdominal status following WHO Protocol; the unfed (U), freshly-fed (FF), semi-gravid (SG), and gravid (G) female mosquitoes [4], and measured according to the ratio of the full-fed: semi-gravid and Gravid (FF:SG and G) female mosquito by PSC [19-21].

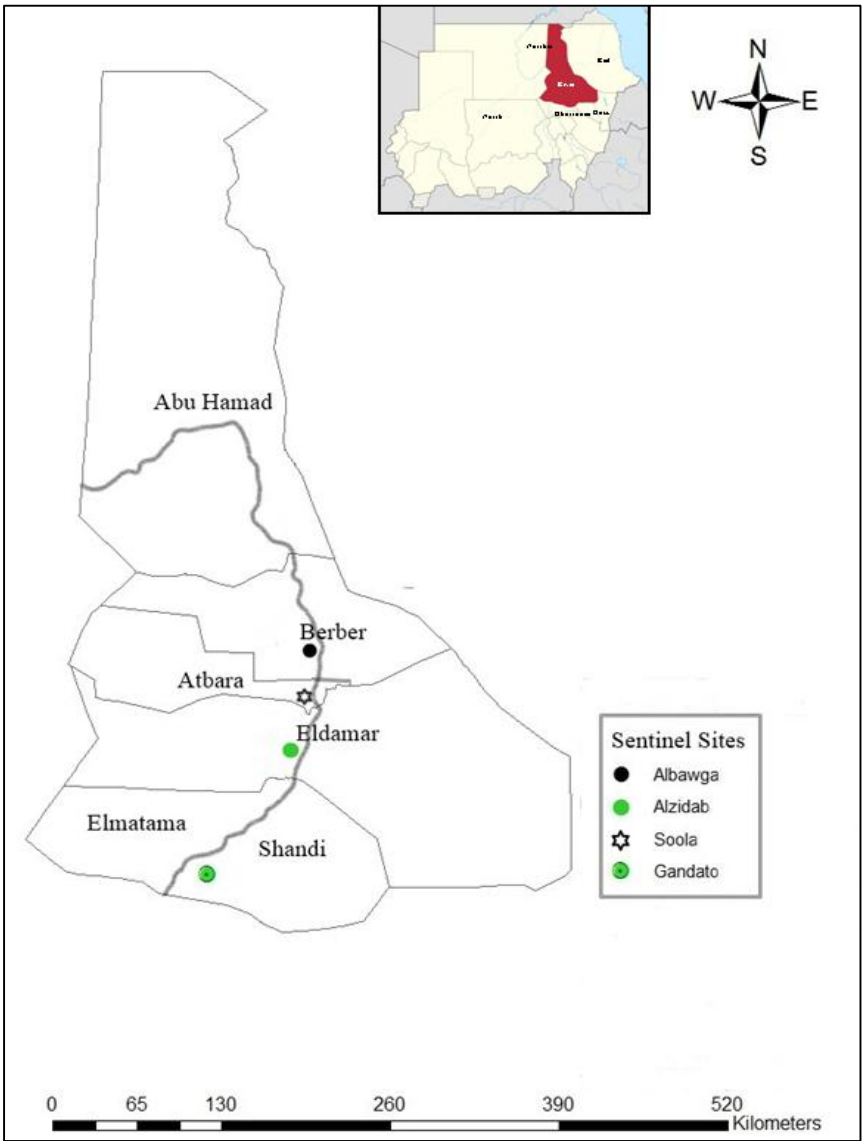

Fig 1: A map of the River Nile State, Sudan showing the study sites

2.4 Statistical analysis
Entomological data were analyzed using the computer software SPSS ver. 22. Several statistical tests were used; descriptive analysis was used to determine *endophilic* behavior of mosquitoes. Kruskal-Wallis test was used to compare the relative abundance of *Anopheles arabiensis* mosquito during 12 months of collection periods.

3. Results
3.1 Resting habitats of *Anopheles arabiensis* species in the selected sentinel sites
Using the Pyrethrum Spray Catches (PSCs) method, A total of (417) specimens were examined for their abdominal status. The proportion of the freshly-fed (FF) to semi-gravid (SG), and Gravid (G) females varied for different mosquitoes (Table.1). Overall, the ratio for *Anopheles arabiensis* showed a preference for *endophilic* behavior (FF: SG and G = 1:1.1) (Table 1).
In the four sentinel sites, the female *Anopheles arabiensis* had different abdominal status. Of the total females examined (417); 179 (42,9%), 153 (36,7%), 65 (, and 20 were from Albawga, Alzidab, Soola, and Gandato, respectively (Table 2). *Anopheles arabiensis* showed a preference for *endophilic* behavior in Albawga (FF: SGG=1: 1.5) and Soola (1: 1.4), and a relative preference for *exophilic* behavior in Alzidab (1.14:1) and Gandato (4:1) sites, respectively (Table 2).
Of the total females examined (417); 145, 58, and 214 were in hot-dry, wet and cold-dry seasons, respectively (Table 3). *Endophilic* and *exophilic* behavior varied among the three different seasons in the study area. *Anopheles arabiensis* had a preference to rest indoors during the hot-dry season and wet-season (FF: SGG = 1:1.2, and 1.3 respectively) and a tendency to rest outdoor during the cold-dry season (1.6:1 (Table. 3).

3.2 Seasonal variation of *Anopheles arabiensis* in the sentinel sites
The seasonal oscillations of overall *An. arabiensis* population collected by PSCs is shown in (Figure 2). *Anopheles arabiensis* showed distinct seasonal fluctuation in their abundance during 12 months of collection periods (Figure 2). Using the Kruskal-Wallis test, a significant difference was observed in the mean density of *An. arabiensis* between the different months of the study (Chi-sq. = 36.78; *P = 0.00*). In the study area, *An. arabiensis* occurred throughout the months of the surveys and showed three high peaks of abundance in March (5.5±1.9), November (4.0±1.5), and January (2.2±0.9).

Table 1: Abdominal status of female *Anopheles arabiensis* mosquitoes captured indoors**.**

Total females examined	Abdominal Status				Status Ratio FF: SG and G
	UF	FF	SG	G	
417	75 (18%)	162 (38.8%)	156 (37.4%	24 (5.7%)	1:1.1

Table 2: The abdominal status of females *Anopheles arabiensis* mosquito recorded indoors.

Sentinel sites	Total females examined	Abdominal Status				Status Ratio FF: SG and G
		UF	FF	SG	G	
Albawga	179	30 (16.7%)	60 (33.6%)	77 (43%)	12 (6.7%)	1: 1.5
Alzidab	153	33 (21.6%)	64 (41.8%)	53 (34.6%)	3 (2%)	1.14:1
Soola	65	12 (18.5%)	22 (33.8%)	22 (33.8%)	9 (13.8%)	1: 1.4
Gandato	20	0 (0%)	16 (80%)	4 (20%)	0 (0%)	4:1

Table 3: Ratio of females *Anopheles arabiensis* mosquito with different abdominal status recorded indoors during three seasons.

Seasons	Total females examined	Abdominal Status				Status Ratio FF: SG and G
		UF	FF	SG	G	
Hot- dry	145	47 (32.4%)	34 (23.4)	42 (29%)	22 (15.2%)	1:1.2
Wet	58	0 (0%)	14 (24.1%)	44 (75.9%)	0 (0%)	1:3
Cold- dry	214	28 (13.1%)	114 (53.3%)	70 (32.7%)	2 (0.9%)	1.6:1

UF = unfed females., FF = freshly-fed females., SG = semi -gravid females., G = gravid females.

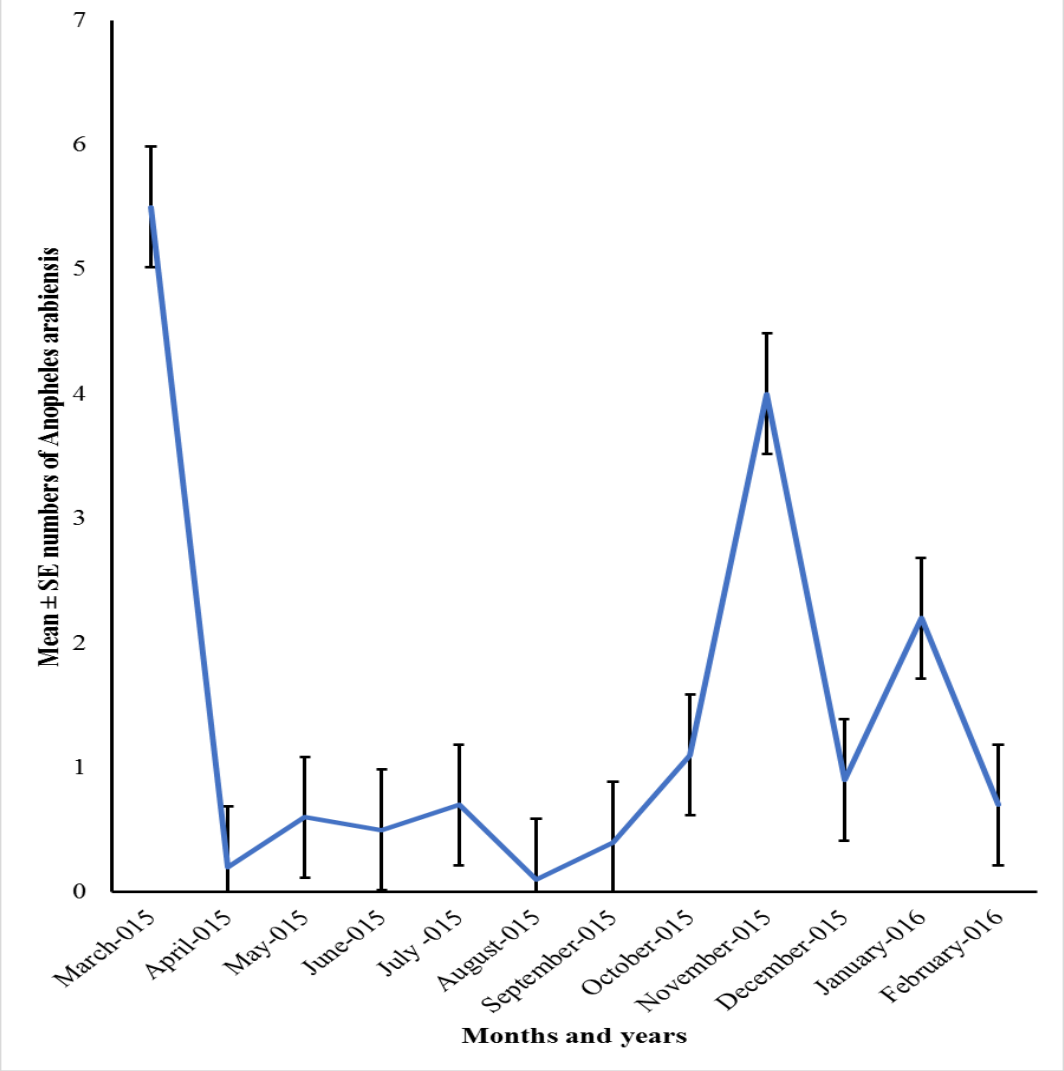

Fig 2: The seasonality of *Anopheles arabiensis* collected indoors using the Pyrethrum Spray Catches method in the River Nile State, Sudan (March 2015- February 2016)

Discussion

Information on the indoor resting and seasonal dynamics of female mosquito species is important to understand the epidemiology as well for designing better control strategies against mosquito-borne diseases. This study was conducted to determine the *endophilic* behavior and seasonality of the malaria vector *Anopheles arabiensis* in River Nile state, Sudan. Such information will be of great value for the control program in the area to focus on effective intervention measures to suppress the mosquito vectors in the area.

Three hundred and twenty-three bedrooms were sprayed in the four constant sentinel sites in the study area. Moreover, the occurrence of the *Anopheles arabiensis* at indoor sites in the area is of epidemiological interest. This finding might indicate a risk possibility of indoor transmission of malaria, lymphatics filariasis, and other mosquito-borne diseases [22-25]. During the study period, indoor residual spray (IRS) is not carried out at the correct time due to the scarcity of budgets, and covering with bed nets is not one of the control strategies adopted in the state. IRS has limited impact if it is carried out too late in relation to peak transmission [26]. The use of insecticides on bed nets (ITNs), eaves curtains, durable linings, or IRS, all have the potential to keep drive malaria vectors outdoors [27-29].

Knocked-down mosquitoes from indoors were graded according to their abdominal conditions, the freshly-fed: semi-gravid and gravid (FF: SGG) [19-21]. *Anopheles arabiensis*, showed a varied preference of resting behavior in the surveyed sentinel sites. The ratio of freshly-fed to semi-gravid and gravid (FF: SGG) for *An. arabiensis* indicated that this species is *endophilic* and it had a tendency to rest indoors (FF: SGG= 1: 1.5 and 1: 1.4 in Albawga, and Soola sites, respectively). In contrast, *An. arabiensis* showed a preference for outdoor resting in Alzidab (FF: SGG= 1.14:1) and a greater tendency to rest outdoor in Gandato site (FF: SGG= 4:1). However, the difference in resting behavior is common for *An. arabiensis*, which is known to be *exophagic*, *zoophilic*, and *exophilic* [13, 30-33].

The results of abdominal status also showed that *An. arabiensis* had a different resting behavior during three seasons (*i.e*. cold-dry, hot-dry, and wet seasons). The ratio of (FF: SGG) for *An. arabiensis* showed that this species had a moderate tendency to rest indoors during hot-dry and wet seasons (FF:SGG= 1:1.2 and 1:1.3), respectively. In addition, this species showed a high preference for outdoor resting during the cold dry season (FF: SGG= 1.6:1). A similar observation was reported in India recorded monthly variation in the densities of several mosquito species resting indoors [34].

Moreover, the high proportion of freshly-fed mosquitoes

caught from indoors in different sentinel sites in the area might indicate that these mosquitoes are more *endophagic* and *anthroponotic* species. These mosquitoes might also be *exophagic* species but have *endophilic* trendy and hence after taking a blood meal they rest at indoor sites. These differences in feeding and resting behavior are common in mosquito species. In the Blue Nile and Khartoum states, *An.arabiensis* was found infected with (RVF) virus, so it becomes important to connect between human and entomological studies [35, 36]. Otherwise, *An. arabiensis* might have a high tendency for indoor resting than the other mosquito species recorded in the PSCs. A previous study conducted in the Blue Nile State revealed that *An. arabiensis* was collected in a higher proportion at indoor sites [10]. *Anopheles arabiensis* was found more *exophilic* and *zoophilic* in Northern State [11], in contrast, in eastern Sudan, showed both *endophilic* and *exophilic* behavior [12], while in Khartoum state it showed *endophilic* and *anthropophilic* behavior [30], also, in Ethiopia [31] and Tanzania [32, 33].

The monthly fluctuation of *An. arabiensis* was investigated in the surveyed sentinel sites using PSCs. The overall data showed that this species occurred throughout the year with three peaks of abundance during March (5.5 ± 1.9), Nov. (4.0 ± 1.5), and Jan. (2.2 ± 0.9). More precisely, the major peaks of abundance for this species were in March and November. This finding indicates that *An. arabiensis* is non-seasonal species and it is consistent with the data obtained above on variation of the mean density of *An. arabiensis* during the three seasons (*i.e*. cold-dry, hot-dry, and wet), since the months of the three peaks of abundance, were during cold-dry (November and January) and hot-dry (March) seasons. This finding is consistent within data on the malaria cases and their peaks of transmission in the State [37].

In Gedarif State, the density of *Anopheles* mosquitoes including *An. arabiensis* peaked in October, November, and December [38]. Although *An. arabiensis* also showed distinct monthly fluctuation in the mean density in each of the four surveyed constant sentinel sites, this species was completely absent from during Aug. in Albawga, May – Aug. in Alzidab, April – May, and July in Soola sites. In addition, *An. arabiensis* were collected only during four months (March, December, January, and February) in the Gandato site. However, the seasonal dynamics of *An. arabiensis* in Albawga and Alzidab sites had a similar pattern to that of the overall data whereas, the peaks of abundance for this species in Soola and Gandato showed a different trend. This variation could be due to the difference in availability of larval habitats between the surveyed sentinel sites which contributes to the distribution and abundance of adult mosquitoes. It has been suggested that larval habitats have a direct influence on the relative density of adult mosquitoes in a certain area [39].

Conclusions

Anopheles arabiensis has a preference for both indoor and outdoor resting. This finding might indicate the risk of indoor and outdoor MBDs transmission. On the other hand, *An. arabiensis* showed a significant pattern of seasonality, with three peaks in March, November, and January, these findings are of importance in the planning of suitable intervention for mosquito control in the River Nile State.

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