

Species Composition, Abundance and Population Structure of Malaria Vectors in Two Villages of Sudan

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Abstract

Background

Frequent monitoring of mosquito vector population is a strategy of great importance for reducing risks of disease occurrence. In Sudan, malaria is still a big threat to public health. Insecticide-based control has been undertaken for years, but there is no noticeable decrease of malaria infection nationwide.

Objective

To overcome this situation, a better understanding of the breeding ecology of the vectors is relevant. Here, we investigate the species composition of malaria vectors, seasonal abundance and population structure in two different villages.

Methodology

Monthly samplings were performed in Abu Algoni (Sennar State) and Algerif West (Khartoum State) from June 2010 to May 2011. During each visit, immature stages were collected from potential breeding sites using dipping technique. In addition, adults were collected indoors from houses by aspiration and indoor pyrethrum spray methods. Mosquitoes were identified morphologically, the *Anopheles gambiae* complex diagnosed using PCR and the physiological status of females determined based on appropriate techniques. Environmental parameters namely temperature, rainfall and humidity were measured.

Results

A total of 4,932 mosquitoes comprising of 3047 larvae and 1885 adults (males and females combined) were sampled. Of these, 88.9% were collected from Abu Algoni while 11.1% were from Algerif West. Two species, *An. rufipes* and *An. arabiensis* were encountered. *Anopheles rufipes* was only found in Abu Algoni, while the latter was found in both villages, where it represented more than 99% of the total collection. Mosquitoes were found breeding in many types of places including canals, temporary pools of water, animal hooves, water from broken pipes, and water storage containers. No significant correlation was found between female and temperature ($p > 0.05$). Significant correlation difference was observed between number of females of *An. arabiensis* and rainfall ($p < 0.05$) and humidity ($p < 0.01$).

Conclusions

Anopheles arabiensis is the only member of *An. gambiae* complex detected in the present study. Seasonal abundance of *An. arabiensis* was observed with most during the wet rainy season in both villages. This could be associated with the availability of more breeding sites created by the rainfall. The majority were parous which indicates high survival rates and thus high vectorial capacity in transmitting malaria.

Background

Malaria is a major health problem in Sudan leading to morbidity and mortality. Symptomatic malaria accounts for 7.8% of out-patient clinic visits and approximately 12.2% of hospital admissions [1]. About 87.6% of malaria cases in Sudan are due to *Plasmodium falciparum*, while *P. vivax* accounts for 8.1%. In North Darfur, West Darfur, South Darfur, River Nile and Khartoum states *P. vivax* and mixed infection of *P. falciparum* and *P. vivax* can reach more than

15%. Transmission occurs all year round in the south but is more seasonal in the Northern State, peaking at the end of the rainy season [1].

Thirty one species of *Anopheles* have been identified but only a handful are malaria vectors [2]. *Anopheles gambiae* and *An. funestus*, are important malaria vectors in the southern parts of Sudan and their vectorial capacity may parallel that of *An. arabiensis* [3] which is considered to be the main malaria vector in Sudan [4]. Its distribution extends from the south up to the borders with Egypt [5–6]. Other anopheline mosquitoes present in Sudan such as *An. nili* and *An. rufipes* are of no medical significance due to their predominant zoophilic tendencies and their extremely low densities even during rainy season [2]. In the poor savanna area of Central Sudan, it has been found that *Anopheles* mosquitoes disappear during the dry months of the year and reappear during or soon after the first rainfall [7].

Anopheles arabiensis shows a remarkable tolerance to water shortage and low humidity [8–9]. This vector was documented to even breed during the dry season in areas along the western bank of the White Nile between Khartoum and Jabel Aulia [7]. During the periods of aestivation, often lasting several months, *An. arabiensis* may take several blood meals, but the gonotrophic maturation is arrested or proceeds very slowly. The density of this vector reaches maximum during the rainy season especially in irrigated areas [3]. *Anopheles arabiensis* occurs in desert areas, but is also associated with rivers in Niger, Mali and the River Nile in Sudan [10].

In Sudan the use of insecticides is the most important strategy for controlling malaria vectors through indoor residual house spraying (IRS) and more recently, the use of insecticide-treated bed nets (ITNs) [11]. Current surveys of *An. arabiensis* show high levels of DDT, malathion, Fenitrothion, Bendiocarb, Propoxur, deltamethrin, lambda-cyhalothrin and permethrin resistance in the eastern Sudan, El Rahad, Gezira and Central Sudan [12–15].

This study aims to gather information on the species composition and seasonal abundance of vector *Anopheles* in Algerif West and Abu Algoni in Sudan. Adult females collected were dissected for parity. A survey of the larval population was also carried out.

Material And Methods

Two study areas selected for study were:

Algerif West:

This study was performed in the Algerif West Farm lies on western bank of the Blue River Nile in Khartoum state (latitude 15° 35' 39.4 N and longitude 32° 35' 16.0 E). This farm has citrus fruit trees, cattle and chicken farming. It is irrigated from water supply from the Blue River Nile. The land is composed of fertile flat clay soil.

Abu Algoni village:

Abu Algoni is a village in Sennar State (latitude 13° 31' N and longitude 33° E). The soil, mainly alluvial, is naturally very fertile. The main economic activity is agriculture of sorghum and cotton through the irrigated scheme of Suki, the sugar factory of Sennar and a number of fruit farms (including bananas and mangos) located on the banks of the Blue Nile.

Entomological survey

Detection of Anopheles breeding sites: Detection of mosquito breeding sites in and around Algerif West and Abu Algoni villages took place during the study period. All possible sites were visited including the surrounding farms, and the presence of larval anopheline mosquitoes were checked by dipping or netting collection methods.

Mosquito sampling techniques: Anopheline populations at Algerif West and Abu Algoni villages were sampled over 12 months beginning in June 2010 to May 2011. Larval surveys and spray captures of resting adults inside houses were done once a month throughout the period following the standard procedures [16]. Three mosquito collectors assisted in the field work throughout the study.

Larval collection: Dipping collection method

Larval samples of various life stages were routinely collected every month from a fixed productive site near the main waterworks of the village from water pools created by the running water, water draining into the vegetation from canals as well as from animal hooves.

Standard dipping collection using a ladle of 8 cm diameter and 3 cm deep with a metal handle of 50 cm length was used. Ten random dips were taken from the fixed breeding site for every sample. The ladle was lowered gently at an angle of about 45° until one side was just below the surface. Then the samples were collected in large plastic containers using a pipette and aquatic natural predators (dragonflies, water beetles, tadpoles, etc.), when found were removed. The water samples were then transferred to the laboratory.

Adult collection

Two methods were used for the collection of adult *Anopheles* mosquitoes resting inside houses. These were indoor spray collection and aspiration collection methods.

Indoor spray collection method:

Indoor spray collection (pyrethrum spray collection or knock down collection) method was carried out early in the morning, usually between 6-10am. For this collection 10 houses were randomly selected as fixed capture stations, taking into consideration the following criteria; each house is occupied by a number of people and is near to one or more of mosquito breeding sites. People were first requested to leave the house and then the whole floor surfaces as well as beds and other areas were completely covered from wall to wall with white cloth sheets. All windows, door, eaves and other openings through which mosquitoes could otherwise escape were firmly closed. The house was filled with mist of 0.2% solution of pyrethroid diluted in kerosene using hand atomizer (spray pump) at minimum capacity. After spraying, the house was kept closed for 10 minutes and then opened to collect mosquitoes found on the sheets. Dead mosquitoes were placed into a plastic cup lined with moist cotton wool and covered with a damp filter paper. Samples were transported to the laboratory for examination.

Aspiration method

Resting adult females of morphologically identified *An. arabiensis* from unsprayed houses were aspirated using sucking tubes (aspirator). The aspirator consisted of a glass tube 24 cm in length and one cm internal diameter. A fine mosquito net was fixed on the one end of the tube. This end of the tube was inserted into a piece of rubber 60 cm long. The mosquitoes collected were emptied in a plastic cup covered with mosquito net with a central hole plugged with a wet piece of cotton wool. Samples were transported to the insectary, well protected to prevent mortality.

Laboratory techniques:

Adults and larval mosquitoes were morphologically identified to species level using the morphological keys of Gillies and De-Meillon [17] The numbers were counted for *An. arabiensis*.

Specimens were classified based on the abdominal appearance according to the Sella scale. The ovaries were dissected following the procedure described by WHO [16] to determine the parity rate.

PCR assay for species identification

PCR analysis was conducted for species identification using the rDNA-PCR method because individual species within the *Anopheles gambiae* species complex cannot be precisely identified by morphology alone[18].

Data collection and analysis

All *An. arabiensis* females were eye-checked for physiological status and the resulting numbers of fed, unfed, half-gravid and gravid counted, taking into account location and season of collection. These numbers were used to calculate overall and seasonal mean feeding rates for both villages as follows: (number of fed females + half-gravid / the sum of the numbers of fed + unfed + gravid females) x 100.

Fed and unfed females were also dissected under a binocular microscope for their ovaries and their parity status, determined based on the Detinova method as described by Gillies[19], which relies on the presence or absence of coiled tracheolar skeins in fresh ovaries. The parity rate was calculated as the (number of parous females /number of parous + nulliparous females) x 100, scored population age. All data collected during the study was analyzed using the computer program SPSS (Statistical Package for Social Science) for windows version 18.

Results

PCR results in figure1 showed that all anopheles were identified as *An. arabiensis*. Table 1 shows the *Anopheles* collections and their seasonal variations in Abu Algoni and Algerif West. A total of 4,587 mosquitoes were collected during this survey, 73.5% of which originating from Abu Algoni. In this latter village, 59.4 (2,007/3,377) and 40.6% (1,370/3,377) of the mosquitoes collected were at the larval and adult stages, respectively. In Algerif West, the larval population collected (85.9% = 1,040/1,210) was much higher than that of adults (14.1% = 170/1,210). The population sizes of the two developmental stages exhibited different seasonal variation patterns according to village. In both villages, the larval population size increased sharply from cool dry season (CDS), reaching peaks during rainy season (RS); however, there was far more larvae in Abu Algoni during hot dry season. (HDS). In both villages, more than 80% of the total adult collections resulted from pyrethrum spray method. In Abu Algoni, the adult population size gradually increased when progressing from HDS to CDS to RS, which recorded 75.8% (1,039/1,370) of the collections. Similar variation pattern of abundance was observed in Algerif West, but there were far more adult mosquitoes in Abu Algoni.

Table 2 depicts the *Anopheles* faunal composition in Abu Algoni and Algerif West. *Anopheles gambiae* and *An. rufipes* were the two species found. *Anopheles arabiensis*, the only member of the *An. gambiae* complex, as revealed by PCR, was encountered throughout the survey period in both villages, but in greater numbers in the Abu Algoni. In both Abu Algoni and Algerif West, this species was more abundant during CDS and RS.

Table 3 summarizes the different physiological status of *An. arabiensis* and their seasonal variations. A total of 1,532 *An. arabiensis* exhibiting four feeding status were collected, comprising of 1,362 from Abu Algoni and 170 from Algerif West. Overall, the mean feeding rate of *An. arabiensis* recorded in Abu Algoni (768/1,362 = 56.4%) was similar to that obtained in Algerif West (99/170 = 58.2%), but the seasonal feeding patterns differed between the two villages.

In Abu Algoni, the feeding rate of *An. arabiensis* population was 53.6% (21/41) during HDS. This rate slightly decreased during CDS (135/282 = 47.9%) and peaked during RS (612/1039 = 59.0%). Similar variation patterns was observed in Algerif West, but the feeding rates obtained during HDS (3/6 = 50.0%) and RS (76/122 = 62.3%) were slightly higher than those recorded during the same periods in Abu Algoni. The mean parity rates of *An. arabiensis* in Abu Algoni tended to be slightly greater than that obtained in Algerif West [79.4% (312/393) vs. 72.1% (49/68)]. In Algerif West, the parity rate during CDS was 72.7% (8/11) and similar to RS's rate (41/57 = 71.9%). The values of this parameter during the same seasons (CDS and RS) tended to be high in Abu Algoni [75.0% (51/68) and 81.2% (247/304)] when compared to those from Algerif West. Note that parity was nil during HDS in this village because no fed or unfed female was collected; during the same period. Approximately 66% (14/21) of dissected *An. arabiensis* females from Abu Algoni was parous. It is interesting to note that in both villages, the number of *An. arabiensis* gravid females increased as season progressed, but the seasonal numbers of such females were far higher in Abu Algoni.

Figure 2 shows that adult abundance was relatively high during the period June-September 2010, and peaked in October in Abu Algoni. Their abundance sharply decreased thereafter and mosquito adults were nearly imperceptible from January to May 2011. In Algerif West as shows in Figure 2, mosquito abundance was very low from June to December 2010 and adults were unnoticeable from January to May 2011.

In Abu Algoni, larval abundance in 2010 gradually increased from June, peaked in August and progressively decreased up to 3 individuals in December of the same year. The larval populations rebounded during early 2011, with major peak (100 individuals) recorded in April (Figure 2). In Algerif West, a similar variation of larval abundance was observed, but the peak was attained in September and was minor compared to that of Abu Algoni. In Algerif West, larval abundance was very low in January 2011 and larvae were not found thereafter (Figure 3).

The parity of *An. arabiensis* exhibited different variation patterns between Algerif West and Abu Algoni. In the first village, this parameter increased from June to attain a first peak in August. A similar trend was observed in Algerif West, but the number of parous *An. arabiensis* was lower compared to Abu Algoni. In this latter village, the parity rate recorded a second peak in August thereafter until October, a time when the number of parous females peaked in Algerif West. In Algerif West as well as Abu Algoni, the number of parous females increased from November 2010, attaining major peaks in January 2011. The parity rate in both villages sharply decreased in the next month. No *An. arabiensis* was found for the rest of 2011 in Algerif West, whereas parous females were still present in considerable numbers from March to May of the same year (Figure 4).

Correlations between *An. arabiensis* abundance (larval and female) with-climatic factors

In Abu Algoni, *An. arabiensis* female populations varied considerably from one month to another, as did measured climatic factors. From June to July 2010, the number of females increased with increasing rainfall, but decreased in August, the month that recorded the highest rainfall. After August 2010 the female population rebounded and peaked in October. During this period (August-October 2010), there was much less rainfall and the environment was drier. At the end of the rainy period (November), the number of females was low and remained almost constant when rains were scarce and humidity low (December-May 2011) (Figure 5). Larval populations in Abu Algoni (AA), moisture conditions were low and almost constant during the survey period, except the slight increase observed from July to September 2010. Furthermore, it often rained during the early months of the survey (especially in July and August 2010); however, there were no rainy days thereafter. Concomitant with increased rainfall events and relative humidity, mosquitoes were present in large numbers, in particular larvae. Larval populations sharply decreased from August towards September 2010, a period that corresponded with decreasing rainfall events. This, in combination with the

reduced mosquito populations (larvae and adults) from November 2010 to May 2011, is likely to suggest rain as a driving force of mosquito breeding in this village (Figure 5).

In contrast to Abu Algoni, there was not marked variations of climatic factors in Agerif Wesy. Here, *An. arabiensis* females maintained a low and nearly invariable population size throughout the study (Figure 6). A similar trend of larval population (Figure 6) variations were observed in Algerif West (AW) but with several differences; (i) a lower rainfall throughout 2010, (ii) the peak of larval abundance was recorded later (September), (iii) larvae were less numerous and (iv) absence of larvae when no rain event occurred (2011).

Discussion

Our study revealed that *An. arabiensis* is the predominant species found and is also the only member of the *An. gambiae* complex observed at the two study sites. In Abu Algoni, all larvae collected were identified as *An. arabiensis*. Except for several *An. rufipes* individuals encountered, all trapped adults were also from the same species. Similarly, at Agerif West, only *An. arabiensis* was found. Overall, the prevalence and abundance of both larval and adult populations exhibited a seasonal pattern and were influenced by changes in the monitored climatic parameters. Finally, there were increased parity rates, thus indicating aged *An. arabiensis* populations in both villages.

Species composition

Throughout the survey, we observed increased prevalence of *An. arabiensis* at both larval and adult stages in AL and AW. This is in line with a report from Osman [20] who surveyed the *Anopheles* populations in Sennar State. Observed that 92% of collected *Anopheles* individuals were *An. arabiensis* and considered it as the main malaria vector in the area. This species also accounted for more than 99% of total anophelines collected in AL, which is also located in Sennar. Similarly, Himeidan *et al.*, (2004) showed that *An. arabiensis* is the main malaria vector in Kassaala State. Seidahmed *et al.*, [21], Yagoop *et al.*, [15] and [22] confirmed that *An. arabiensis* is the main malaria vector in Khartoum, El Rahad Central Sudan and in Ed Dueim, White Nile State respectively.

Variations in population abundance relative to climatic factors

Algerif West area is characterized by a low density of *An. arabiensis* and restricted only to the rainy season. This reduction has been accomplished by the efficient control activity in Khartoum State through the Malaria-Free Khartoum programs.

The population density of *An. arabiensis* was monthly recorded during the study period except in June. The major peak was recorded in October and two minor ones in February and April 2011. Our current study revealed that there is a direct correlation between the amount of rainfall and density of *An. arabiensis*. This study shows that the survival and development of large range due to the climatic and environmental factors. The high abundance of *An. arabiensis* during the rainy season could be associated to the availability of more breeding sites created by the rainfall. Interestingly, there was a reduction in female mosquitoes during August although this is the wettest month. This observation could be attributed to the cleansing of the breeding sites which effectively washes down the larvae and the eggs, consequently reducing the abundance of adult *An. Arabiensis*. This is in agree with the findings of Adeleke *et al*, [23].

Population structure

Mosquito age composition plays an important role in malaria transmission. Parous females that have previously blood fed, have a higher possibility of being infected with malaria parasites. Lemasson *et al.* [24] conducted a study comparing the behavior and vector competence of *An. gambiae* and *An. arabiensis* in Senegal during 1994-1995. They observed that *An. gambiae* had a higher parity rate than *An. arabiensis*, but data collected during 1995-1996 showed that the parity rate of *An. gambiae* was significantly lower than *An. arabiensis* [24]. These findings are similar to the current study where the parity rate was high in *An. arabiensis* in the two study sites in Sudan. Ndiath *et al.* [25] studied the dynamics of transmission of *Plasmodium falciparum* by *An. arabiensis* and the molecular forms M and S of *An. gambiae* in Dielmo, Senegal. They found that the mean parity rate was 70.9% for *An. arabiensis*, 68.7% and 80.1% for *An. gambiae* M and S forms, respectively. These findings are in line with the present study where the mean parity rate was 77.19% in Abu Algoni village and 63.85% in Algerif West village. The low parity rate in Algerif West village maybe due to the effective vector control programme in this area. In contrast Himeidan *et al.* [13] who studied the biology and behaviour of *An. arabiensis* in New Halfa eastern Sudan recorded a parous rate of 32.23%. He explained that the low level of parity rate might be due to the application of insecticide during the study period.

Dukeen and Omer[26] studied the ecology of the *An. arabiensis* by the Nile in northern Sudan. They observed that the 1398 mosquitoes collected could be categorised as; 2.2% (30) unfed, 54.6% (763) freshly fed and late fed, and 43.3% (60) gravid. Abdelwhab *et al.*[27] found 644 anopheline were blood fed, 393 were unfed, while 117 were gravid in Central and Eastern Sudan, In the current study, out of a 1358 females collected, 6.7% (91 females) were unfed, 21.94% (298) were fed, 34.61% (470) were half gravid and 37.04% (503) were gravid. High proportion of gravid, half gravid and fed females in the present study concluded that continuous breeding and blood-feeding mean high persistent transmission during the year.

Conclusions

In both villages, the larval population size increased sharply from CDS, reaching peaks during RS; however, there was far more larvae in Abu Algoni during HDS. In Abu Algoni, the adult population size gradually increased when progressing from HDS to CDS to RS. Similar variation pattern of abundance was observed in Algerif West, but there were far more adult mosquitoes in Abu Algoni. This study has established that *An. arabiensis* is the most important vector of malaria in Sudan. *Anopheles* populations are naturally subject to environmental factors in which they inhabit. These factors play a major role in controlling their population dynamics, population genetic structure and their effectiveness as vectors. The climatic changes in Sudan would predictably cause fluctuations in the *An. arabiensis* populations. This would ultimately affect the population dynamics and population genetics of these vectors. Mosquito age composition plays an essential position in malaria transmission. Parous females that have previously blood fed; have a higher opportunity of being infected with malaria parasites and then transmitting the disease.

Abbreviations

IRS: Indoor residual house spraying

ITNs: Insecticide-treated bed nets

PCR: Polymerase chain reaction

WHO: World Health Organization

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The data supporting the results reported in this article are included within the article.

Competing interests

The authors declare that they have no competing interests

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Authors contributions

MSM: did the field and practical laboratory work, data analyses and prepared the manuscript. ZJ and SAMN supervised the study and edited the manuscript. SA supervised the field work.

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Tables

Table 1. Numbers of larvae and adults collected in Abu Algoni (Sennar State) and Algerif West (Khartoum State) from June 2010 to May 2011 in relation to season and collection techniques. “HDS” stands for Hot Dry Season; “CDS” stands for Cool Dry Season; “RS” indicates Rainy Season. ASP stand for Aspiration collection methods and IS indoor spray collection.

	Abu Algoni				Algerif West				Total
	HDS	CDS	RS	Sub-total	HDS	CDS	RS	Sub-total	
Larvae	241	132	1,634	2,007	6	79	955	1,040	3,047
Adult (ASP)	12	54	160	226	2	14	17	33	259
Adult (IS)	29	236	879	1,144	4	28	105	137	1,281
Total	282	422	2,673	3,377	12	121	1,077	1,210	4,587

Table 2. *Anopheles* faunal composition in Abu Algoni (Sennar State) and Algerif West (Khartoum State) sampled from June 2010 to May 2011.

	Abu Algoni				Algerif West			
	HDS	CDS	RS	Sub-total	HDS	CDS	RS	Sub-total
<i>An. arabiensis</i>	41	278	1039	1,358	6	42	122	170
<i>An. rufipes</i>	0	12	0	12	0	0	0	0
Total	41	290	1,039	1,370	6	42	122	170

Table 3. Population structure of *An. arabiensis*

	Abu Algoni				Algerif West			
	<i>HDS</i>	<i>CDS</i>	<i>RS</i>	<i>Sub-total</i>	<i>HDS</i>	<i>CDS</i>	<i>RS</i>	<i>Sub-total</i>
Unfed	1	24	66	91	1	2	18	21
Fed	19	41	238	298	0	9	48	57
Half-gravid	2	94	374	470	3	11	28	42
Gravid	19	123	361	503	2	20	28	50
Nulliparous	7	17	57	81	0	3	16	19
Parous	14	51	247	312	0	8	41	49

Figures



Figure 1

Ethidium bromide stained 2% agarose gel electrophoregram of PCR products obtained from the amplification of *Anopheles arabiensis* DNA for species identification. of *Anopheles gambiae* complex. Lane 1 100bp markers Lane 9 negative control; 2, 8 and 10-12 wild- caught *An. arabiensis* 315bp.

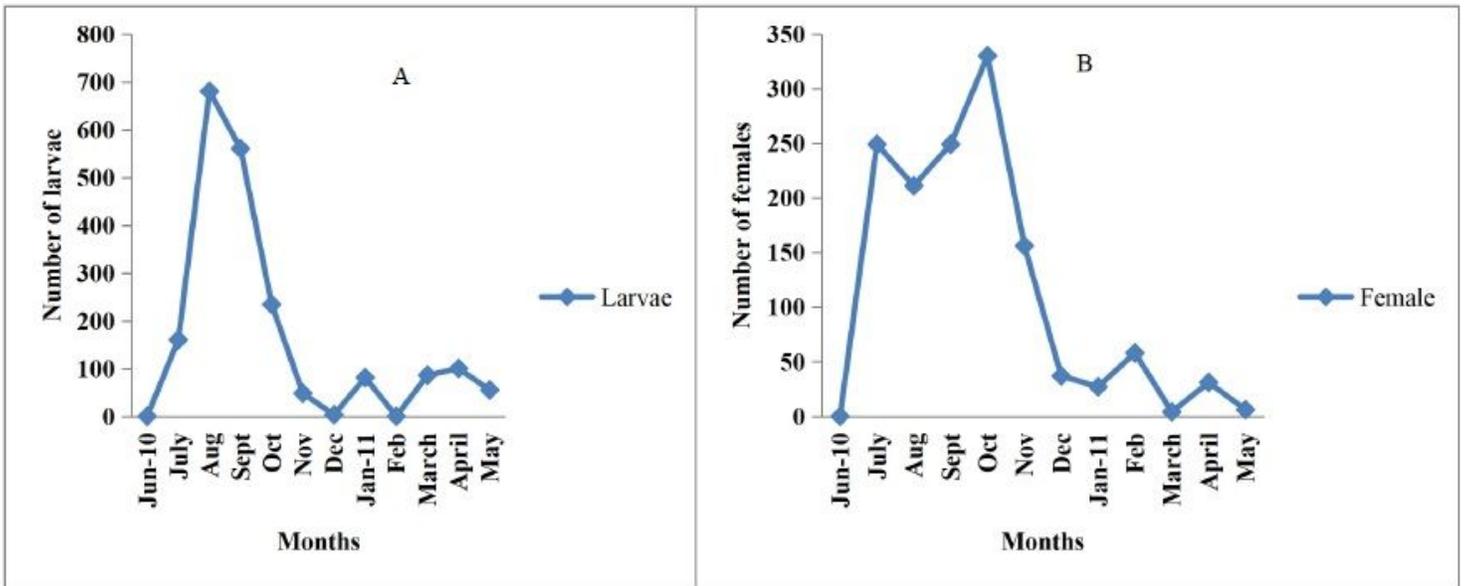


Figure 2

Monthly variations of numbers of A= larval and B= adult mosquitoes in Abu Algoni Village Sennar State during study period from June 2010 to May 2011.

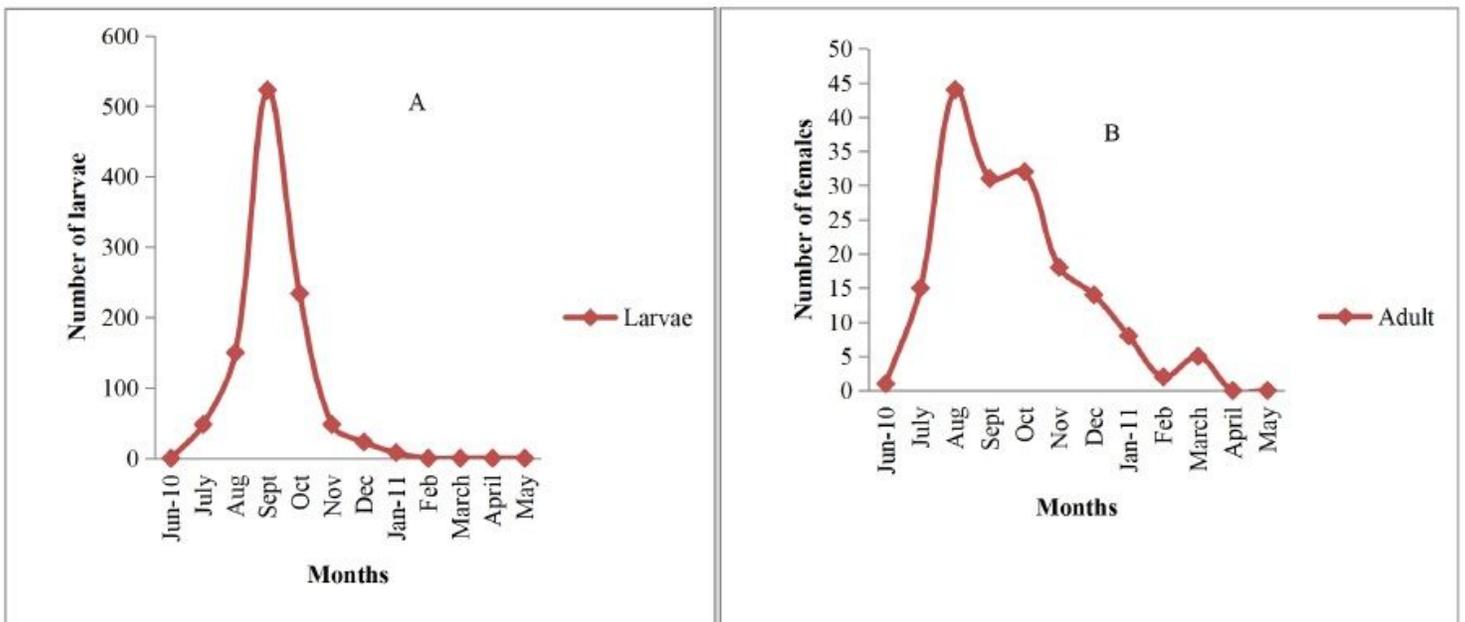


Figure 3

Monthly variations of the numbers of A= larvae and B= adult mosquitoes in Al gerif West village, Khartoum State during the study period from June 2010 to May 2011.

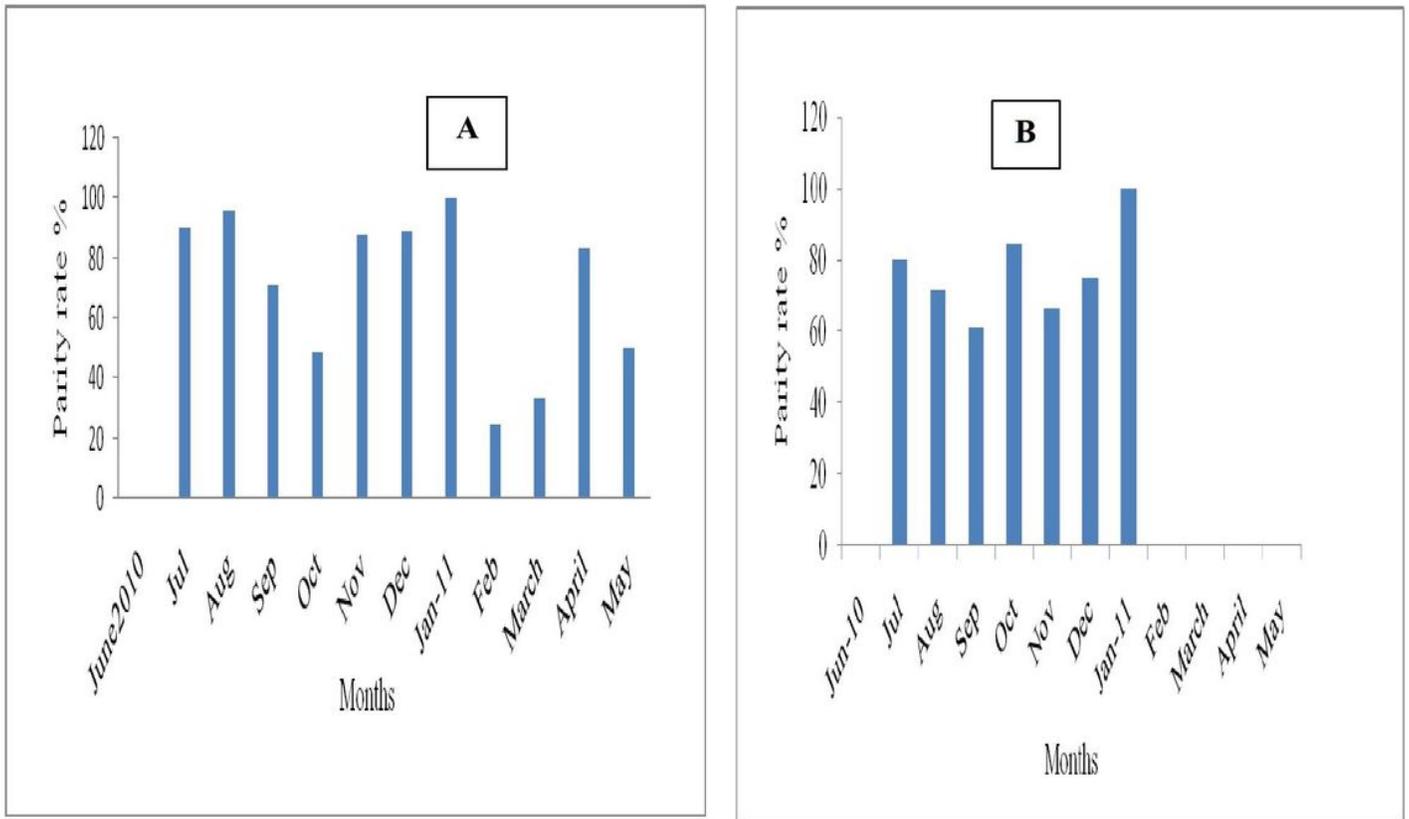


Figure 4

Parity rates of *An. arabiensis* in A=AA, and B=AW during the period from June 2010 to May 2011. AA= Abu Algoni, AW=Algerif West.

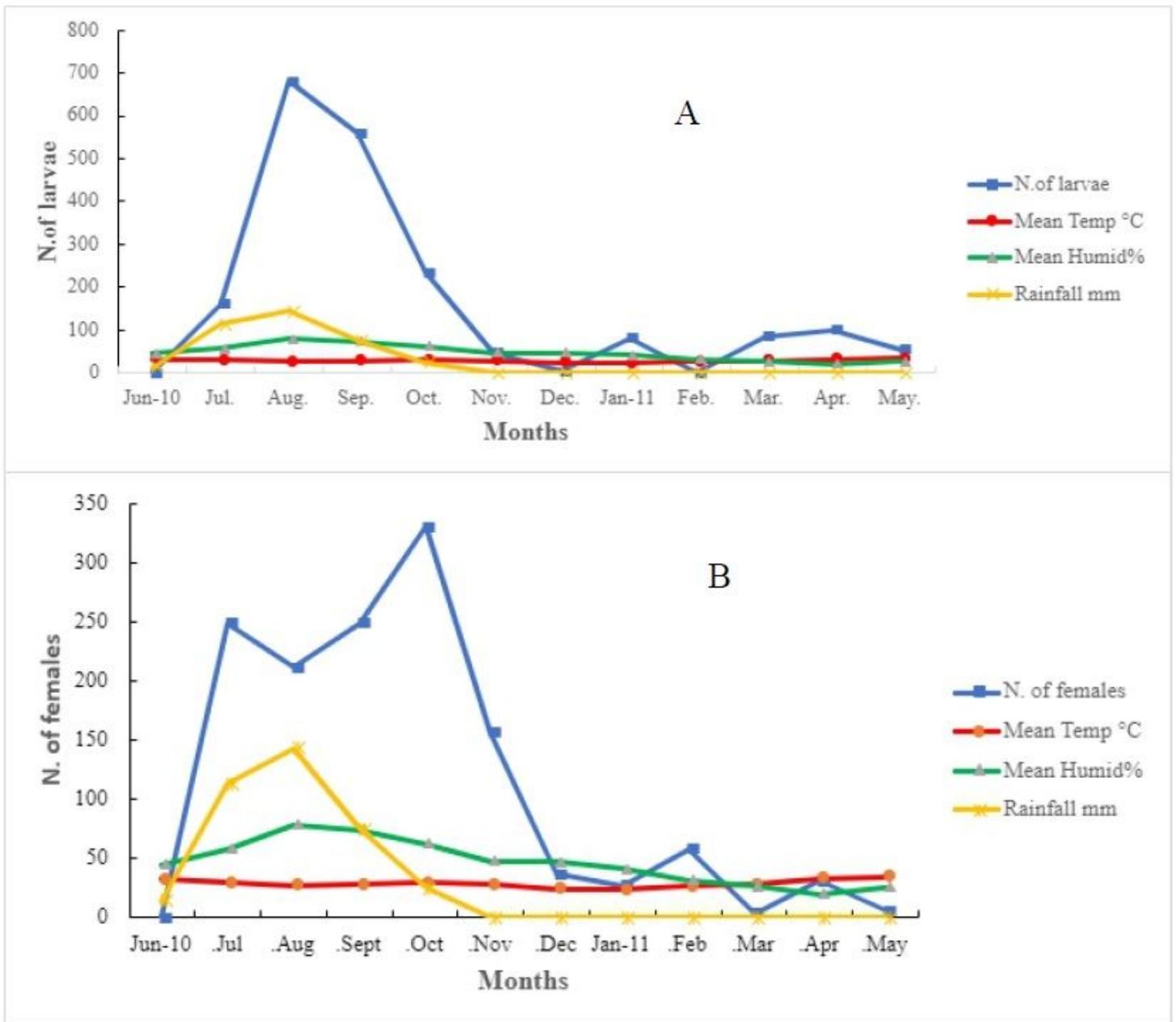


Figure 5

Seasonal abundance of the larvae (A) and adult (B) of *Anopheles arabiensis* and their relationships with rainfall, temperature and humidity in Abu Algoni during the period from June 2010 to May

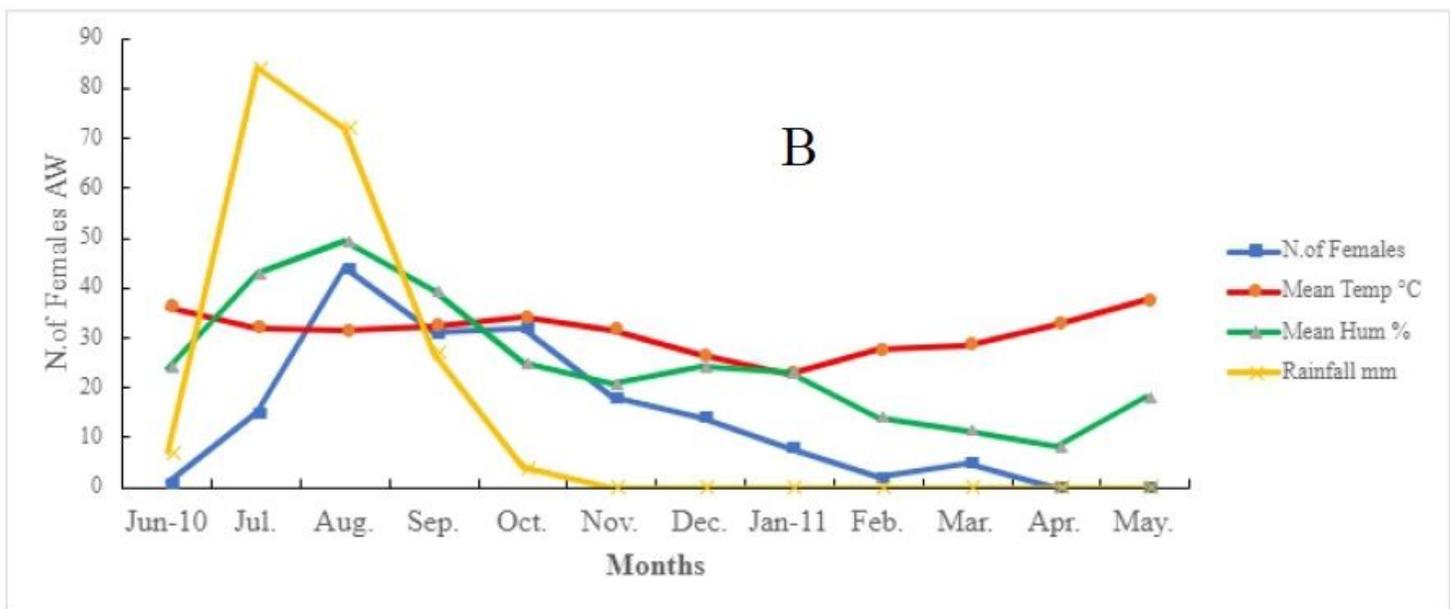
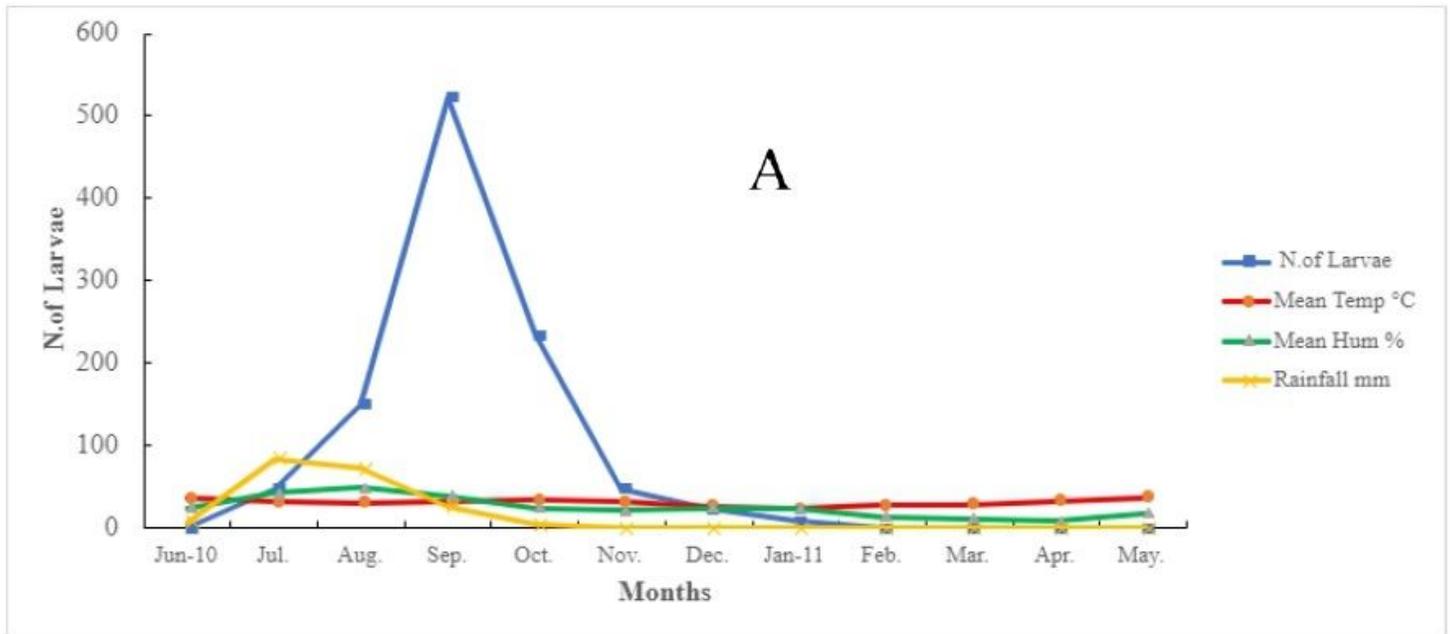


Figure 6

Seasonal abundance of the larvae (A) and adult (B) of *Anopheles arabiensis* and their relationships with rainfall, temperature and humidity in Algerif West during the period from June 2010 to May

Supplementary Files

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- [Graphicalabstract.jpg](#)