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THE ECOLOGY OF ANOPHELES ARABIENSIS AND INSECTICIDE RESISTANCE/ SUSCEPTIBILITY STATUS IN KASSALA AREA, EASTERN SUDAN.

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Abstract:

Objectives: The study was carried out to investigate the ecology of *A. arabiensis* and their susceptibility to insecticides in Kassala area, eastern Sudan.

Methods: Both adult anopheline mosquitoes and immature stages were sampled monthly following the standard WHO procedures during the period November 2000 through January 2002. The susceptibility of *A. arabiensis* to insecticides was conducted using the WHO standard technique.

Results: *A. arabiensis* was the main malaria vector; its density fluctuated throughout the year with bimodal peaks during the rainy (6.1 females/room and 56.1 larvae/dip) and the cool dry seasons (4.8 females/room and 47.5 larvae/dip). The difference was not statistically significant (P>0.05). An indication of partial aestivation of *A. arabiensis* to adapt the harsh environmental conditions of the dry season was confirmed in the area. Adult populations like to cluster around breeding sites in an area of less than 200 m. The susceptibility to DDT and fenitrothion was 88.3% and 100% respectively.

Conclusion: *A. arabiensis* is the sole malaria vector in Kassala area and it was highly susceptible to the insecticides tested and these should be considered for malaria vector control in the area.

Keywords: malaria, vector, Anopheles arabiensis, insecticides, Sudan.

Introduction:

Malaria is a major health problem in the tropical Sub-Saharan Africa, where about 90% of the clinical cases occur.(1) The disease constitutes around 40% of all infectious diseases in Sudan and over 90% of the infections are caused by *P.*

falciparum.[2] *Anopheles arabiensis* is the main malaria vector being reported from all parts of the country.[3] Due to the worldwide spread of resistance of *P. falciparum* to antimalarials [4], the efforts to reduce vector abundance are urgently needed to supplement the drug-based curative approach. The vector control remains as the best approach for protecting the community against malaria .[5] Investigating and understanding the ecology of the malaria vector in a particular area is of paramount importance of planning and deciding the best approach for vector control.

The use of the chlorinated hydrocarbon insecticides (DDT) in mosquito control was started in Sudan in 1965 to replace BHC, but by the early 1970s, resistance to DDT was reported. Thereafter, the organophosphates malathion and fenitrothion insecticides replaced DDT. [6-7] The susceptibility of *A. arabiensis* to these insecticides in New Halfa agricultural area was recently reported and in the eastern Sudan [8] This study was conducted to investigate the dynamic of the vector *A. arabiensis* population and their susceptibility to insecticides in Kassala area, where no data exist.

Methods:

The study area:

The study was carried out at New Shukria village, which lies 10 km south of Kassala town (altitude 450m, lat.14.45-17.15 N & long.34.40-37.00 E) and about 3 km of the western bank of the seasonal Gash River. The climate in the region is typical of the semi-arid belt of the Sudan (dry savannah) characterized by three seasons, the short rainy season (July-October), cool dry season (November-February) and hot dry season (March-June). May is the hottest month of the year (34.7^{o} C). The average annual rainfall is about 250 mm. The horticulture fields of vegetables and fruits are distributed along the riverbanks of Gash River. The village is populated by 1560 individuals most of them live together in compounds as families in huts as reported in details somewhere. [9]

Mosquito collections*:*

Both adult and immature stages of anopheline mosquitoes were sampled monthly following the standard WHO procedures [10] during the period of November 2000

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through January 2002. Immature stages of *A. arabiensis* were encountered in water pools in the Gash River, bed irrigation canals, water containers, rainfall, rock holes, neglected wells and ditches used by inhabitants. The surveys were carried out using standard dipper. Netting method was used only in neglected wells. Pyrethrum spray method knock-down was used to collect indoor resting mosquitoes from a 20 inhabited huts selected randomly as fixed capture stations. These huts were divided into two groups according to the distance from the sampling-breeding site (more and less than 200m). 0.2% pyrethrum in kerosene was sprayed and knocked-down mosquitoes were collected from 06.00 to 9.00 am. Females collected were classified according to their blood meal stages as unfed, fed, half-gravid and gravid. The ovaries of females were dissected for the identification of the ovarian development and then classified according to Christopher's stages. Mosquito species were identified by external morphology following a standard key.[11] *Anopheles gambiae* specimens were recorded as *A. arabiensis*, which is the only member of the complex found in eastern Sudan.[3]

Human blood index (HBI):

Blood spots from freshly fed females of *A. arabiensis* knocked down by pyrethrum spray were harvested on filter paper, the source of blood was determined as the human biting index using Ouchterlony radial diffusion technique.[9]

Susceptibility test:

The susceptibility of *A. arabiensis* (heterogeneous population) to two insecticides was tested according to the WHO procedure.[12] Fed females were exposed to paper impregnated with 4% DDT and 1% fenitrothion for one hour to determine their susceptibility to these insecticides.

Statistical analysis

Statistical analysis was carried out using *SPSS* (version 7.5) for WINDOWS. Variations in vector densities between different seasons were examined using ANOVA. Qualitative variables concerned with distribution of adult populations of *A. arabiensis* were compared using the Binomial test. Spearman's correlation analysis was conducted to test the relationship between monthly climatic variables and the density of immature stages. *P* value of less than 0.05 was considered significant.

Results:

Anopheline species:

A. arabiensis was the predominant species in the area, which accounted for 99.99 of all those caught (725/1), while 0.01% was *A. pharoensis* and some Culex specimens were occasionally captured.

Seasonal densities:

The densities of both adult and immature stages of *A. arabiensis* fluctuated throughout the year with bimodal peaks during the rainy and cool dry seasons (Fig. 1). The numbers of adult females and larvae detected rose rapidly after the onset of the rains in June to reach the peak in September-October (56.1 larvae per dip, 6.8 females per room). The males disappear during the period of April to July and began to increase and reach the peak in August-September (1.2 males per room). During the cool dry season the numbers of both adult and immature stages remain relatively high with a minor peak in January (47.5 larvae per dip, 4.8 females/room) and decreased to a low level in the hot dry season (6.7 larvae per dip, 0.75 females/room). Complete absence of both females and immature stages was reported in May. The difference in densities between seasons was not statistically significant ($P>0.05$) (Table 1). The highest number of immature stages (24.28 larvae per dip) was detected during the cool dry season in the pools resulting from the buddle of irrigation canals, which were the only type of breeding habitats existing throughout the year. Spearman's correlation revealed that both minimum temperature and rainfall have a significant impact on breeding activity rather than maximum temperature and relative humidity (Table 2). Generally the mean density of males throughout the study period was very low in comparison to mean density of females, the ratio of female to male was 4.7:1.

Distribution of adult population:

About 97% of the females and 83% of the males were found in the houses close to breeding sites (distance

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less than 200m), while the others were distributed in an area less than one km away. Adult populations tended to cluster around breeding sites and to be distributed according to distance from it. No relationship in the distribution was found between the two sexes (P<0.05)

Resting behaviour:

The females of *A. arabiensis* showed regular blood feeding, which was accompanied by the normal gonotrophic cycle development during the study period. Out of 725 females collected at indoor sites, 4% (27), 36% (259), 20% (145), 27% (200) and 13% (94) were unfed, freshly fed, late fed, half gravid and gravid respectively. The data gave a fed (freshly and late fed) to gravid (half gravid and gravid) ratio of 1.4:1 indicating an endophilic vector behaviour in the area. The outdoor resting was confirmed only in humid and shaded water container used for household purposes.

Reproductive conditions:

Dissection of the ovaries revealed various Christopher's stages of ovarian development invariably represented in the catches of successive months of the dry season. Of a total 281 females dissected, 15% (43) were found to be in stage I, 1% (3) in early stage II, 10% (29) in late stage II, 30% (84) in stage III, 25% (70) in stage IV, 13% (36) in stage IV-V and 6% (16) in stage V. Abnormal pattern of ovarian development was observed during April 2001 (maximum temperature 42.2° C). Two gravid and half gravid females of *A. arabiensis* (stage V and IV-V) were fed again after ovaries development instead of ovipositing inspit of the presence of breeding sites. These females had fresh blood meal in their stomach and their abdominal appearance was not accompanied the ovary development.

Human blood index (HBI):

The HBI has a seasonal variation in the area with 71.9%, 93.8% and 100% in the rainy, cool dry and hot dry seasons respectively. The average HBI was found to be 83.8% of the total mosquitoes examined. The source of non-human blood observed in the females was not identified.

Susceptibility test:

The mortality rates of *A. arabiensis* after exposure to impregnated papers of 4% DDT and 1% fenitrothion for one hour was 88.2% and 100% respectively.

Table 1: Seasonal density of *A. arabiensis* in the study area during the period November 2000-January 2002

Seasons	Larvae/dip	Males/room	Females/room
Cool dry (Nov-Feb)	17.55	0.36	2.14
Hot dry (Mar-Jun)	6.70	0.13	0.75
Rainy (Jul-Oct)	17.05	0.50	2.50
Mean	14.50	0.33	1.87
S.D	17.84	0.39	1.80
P value	0.63	0.41	0.36

Table 2: Spearman correlation between monthly climatic variables and monthly larvae densities detected during November 2000-January 2002.

limatic variables	R^2 value (calculated)	S. E	P value
Max T	-0.510	0.218	0.090
Min	-0.790	0.137	0.002
RH	0.493	0.296	0.103
Rain	-0.595	0.127	0.041

Max T = monthly mean maximum temperature, Min T = monthly mean minimum temperature, RH = monthly mean relative humidity, Rain = total monthly rainfall.

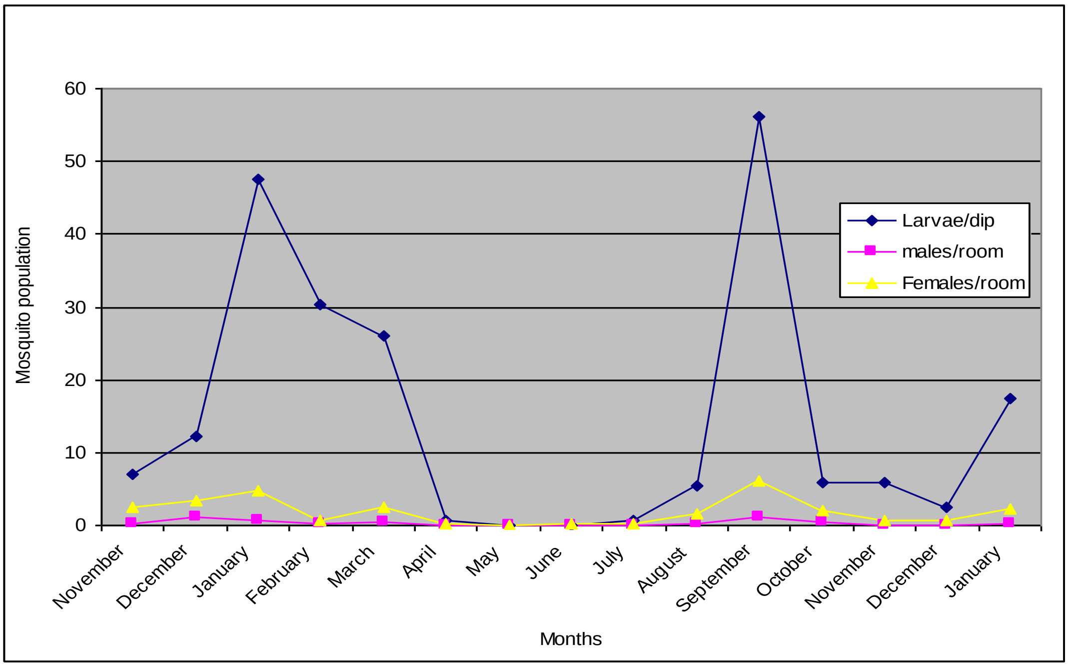

Figure 1: Seasonal density of *A. arabiensis* collected from the study areas from November 2000-January 2002

Discussion:

The study showed that *A. arabiensis* was the predominant species found in the area. This species was the sole malaria vector, recently identified cytogenetically and morphologically from El Girba, New halfa and El Gedaref areas of eastern Sudan. [3, 8, 9]

The vector *A. arabiensis* showed bimodal annual peaks in the area. The high density during the cool dry season might be explained by the irrigation of horticultural crops. Similar findings were reported from irrigated areas in the Gezira and New Halfa schemes in the central and eastern Sudan. [8,13] Previously, it has been reported that the irrigation had led to extension of the breeding season of the malaria vectors [8,14] Fortunately, during this season, the density is limited by long larval duration due to the decrease in minimum temperature[15] (Table 2). As it well known that temperature and humidity was adversely affecting adult and immature stages,[16] unlike the one peak of the vector density observed at the end of the short rainy season and dropped gradually to disappear in the long, hot dry season in El Gadaref area.[9] In the present study *A. arabiensis* passed the unfavourable conditions of dry season in a state of lowered physiological activity (in rested ovarian development) described as incomplete aestivation as an adaptation to the harsh summer conditions similar to the situation in the desert valley of the White Nile more than 20 km south east of Omdurman.[17] This was confirmed by the phenomenon of re-feeding of the gravid females instead of ovipositing despite the presence of breeding sites. Hence, the abundance of the vector *A. arabiensis* in this area is governed by both rainfall and irrigation of horticultural crops, and at no time during the year did the temperature go above or fall below the survival range of the mosquito. [18]

The preference of host feeding by mosquitoes varies according to the species, [19] but many studies confirmed that *A. arabiensis* was more anthropophilic than zoophilic.[20] This supported our observation of the high human biting index (HBI) record (83.3%), similar to that in Gedaref state and New Halfa area.[8, 9]

The susceptibility to DDT and fenitrothion was found to be 88.2%, and100% respectively, indicating that the mosquito population in this area had low level of resistance to DDT. Similar finding was reported by Himeidan *et al*.[8] in New Halfa area, eastern Sudan, and they stressed that the come-back susceptibility of

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A.arabiensis to DDT may be due to the stoppage of chlorinated hydrocarbon insecticides for more than 20 years. Nevertheless, in the Gezira Scheme the vector developed resistance to several insecticides in 1970s that had been influenced by the overusing in spraying crops.(6)

Conclusion and recommendation:
The bimodal annual density and the susceptibility of field population to insecticides should be considered, particularly to inform the implementations of malaria vector control in the study area.

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