Climatic Factors Affecting Density of *Aedes aegypti* (Diptera: Culicidae) in Kassala City, Sudan 2014/2015

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Abstract

Transmission of Dengue Hemorrhagic fever by *Aedes aegypti* mosquito is influenced by several environmental factors, namely temperature, humidity, and rainfall. This study aims at identifying the relationship between environmental factors and dengue vector population density. A longitudinal entomological study was performed in the city of Kassala, Sudan, in 20 households in each of the 20 clusters during the three seasons of 2014 and 2015. Data were collected using spotlight in any water-holding container where immature stages of *Aedes* spp. were detected. Immature mosquitoes were counted and classified into larvae and pupae. Ambient temperature and relative humidity in each cluster were recorded, and the total rainfall of Kassala City was obtained from the main Meteorological Station in Khartoum. There was a significant positive correlation between rainfall and number of *Ae. aegypti* females at Garb Algash and Khatmia areas. Larval instar 4 and pupae were positively correlated with high humidity at Khatmia and Altora areas. In autumn season, there was no strong correlation of all the stages with all climatic variables. There was also positive significant correlation between ambient low temperature and number of females in autumn season.

Keywords

*Aedes* Aegypti; Climatic Factors; Mosquitoes; Sudan

Introduction

Sudan is characterized by a wide range of climate variation, which differs from desert in the north, through South Savanna belt. Clearly, climatic factors play an important role in the transmission of diseases [1]. Variations in weather and climate can affect *Aedes* mosquitoes and DENV through multiple mechanisms [2]. Climate change can affect the behavior of blood-sucking vectors, and thus may alter the distribution and temporal patterns of diseases transmitted by bite of these vectors [3]. Temperature is important not only in limiting the absolute geographic limits of DENV vector distribution, but also in supporting different levels of endemicity [4]. The immature stages of most species are extremely sensitive to temperatures above 40°C during development [5]. Mosquitoes, like many invertebrates, are directly affected by changes in weather to a greater extent than warm-blooded animals and external temperatures are, therefore, required to be above critical thresholds for adult activity or immature stage development [6]. Many...
species rely on regular rainfall to provide suitable breeding sites, while relative humidity and, to a lesser extent; wind patterns are likely to affect vector population dynamics [7]. Larvae can continue to develop and pupate at temperatures as low as 15°C with duration of approximately 31-32 days, and at temperatures between 8.2-10.6°C development completely ceases [8]. Temperature below 10°C and above 44°C results in the death of larvae. *Ae. aegypti* larvae do not thrive in water temperature much above 34°C and adults begin to die if air temperatures exceed 40°C. Prolonged exposure to a low temperature of 10°C and a high temperature of 40°C resulted in 100% egg mortality [9]. Low mosquito populations are evident in dry and cool seasons and they increase when temperatures increase and wet season commences [10]. Variability of humidity affects the biology and ecology of mosquito vectors and intermediate hosts as well as the risk of disease transmission [11,12]. High relative humidity can give high hatching rates. It is important to allow slow desiccation of eggs as the embryo takes time to develop prior to the drying process [7].

Rainfall is the most important factor that affects *Aedes* spp. breeding [13]. An adequate amount of rain will create natural water bodies and fill artificial habitats, providing opportunities to lay their eggs [14]. Vibrations resulting from the impact of raindrops on the water surface can cause a diving response (alarm reaction) in larvae [15] and may even stimulate hatching of eggs already present in a habitat. Rainfall is positively correlated with larval abundance, especially where rain-filled containers appear to be the primary larval sites especially no body care has been taken for cleaning these containers [16]. High vector abundance was correlated with increased household storage of water during a period of local drought and a simultaneous reduction of vector control activities [17]. Association between rainfall and *Ae. aegypti* abundance almost vary between localities due to both range of container types that are available as larval habitats and differences in the water storage practices of the local population [16].

**Materials and Methods**

**Area and Experimental Design:**

Kassala State is the one of the eastern Sudan states of eleven localities (*mahaliyas*). With a total area of 55,374km², Kassala State lies between longitudes 34° 12’ and 36° 57’ E, and latitudes 15° 12’ and 17° 12’ N. Over 80% of Kassala State consists of flat plains, whereas rocky outcrops and hilly terrain comprise the rest of the area. Alluvial and volcanic deposits cover the state and beneath these, clays lay basement complex formations that are only a poor repository for ground water. Water sources in the state tend to be distributed along the cracks in the geological formations and in the few areas where alluvial deposits accumulate. The largest of the state’s aquifers are the Gash Basin which has an estimated storage capacity of 600 million cubic meters of water and runs north, from the Eritrean highlands through Kassala City [18]. Mean maximum temperature in Kassala State occurs in summer months with an average of 40°C in May and mean minimum temperature is 15°C in January. Kassala State falls within the arid and semi-arid region where rainfall is unreliable for domestic and economic uses. The average total annual rainfall is about 225 mm occurring dominantly between May to October while evaporation amounts to 2- 2.5 mm. The main sources of water supply are surface [19]. Effective use of rainfall is, however, hampered by its short duration, uneven distribution and high rates of evaporation. Overall, a trend of long-term decline in rainfall has been observed in Kassala State since the 1940s [20] and the current rate of depletion is calculated to stand at 2.6 mm per annum [21]. Relative humidity varies from 27% in April to 60% in August in Kassala and from 27% to 48% in Aroma [22].

**Vegetation Cover:**

Kassala State is mainly of desert and semi-desert type with dominating grassland and scattered shrubs. The most important forest trees are Acacias that are represented by one or more species in almost every ecological zone. These trees are abundant, productive, and palatable providing the bulk of the forage for grazing animals within the pasture.

Three important seasonal rivers intersect: Atbara, Gash and Rahad which provide water for the irrigated schemes and the horticulture sector (**Fig-1**).

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Design of entomological study:

A longitudinal entomological study was performed during the three seasons; dry (winter), hot (summer) and wet (autumn) for two consecutive years (2014 and 2015).

Sample size determination of households:

The sample size was calculated using the following formula [23].

\[ n = \frac{z^2pq \times deff}{(ep)^2} \]

Where:
- \( n \) = minimal required sample size (when \( n \) is greater than 10,000)
- \( z \) = \( z \) statistics for the level of confidence of 95% (1.96 for two tailed test)
- \( q = 1 - p = 1 - 0.5 \)
- \( P \) = Prevalence value of Aedes (0.5)
- \( e \) = relative margin of error 12% (e=0.12)
- Deff = the design effect =1.5

\[ n = (1.96)^2(0.5)(1-0.5)/(0.12*0.5)^2*1.5 = 400. \]

Accordingly, the number of houses holds selected is 400.

Entomological survey:

Entomological surveys were carried out at 0600 hrs until 1800 hrs in three seasons; dry (winter), hot (summer) and wet (autumn) in 20 households every season on both sides of Kassala city (East Algash (Alengaz East, Alengazsouth, Biriai, Mukram, Khatmia Blocks 3 and 4, Khatmia Blocks 7 and 8, Alnorab, Altora) and west Algash (Alkormota, BantAlmasna, BantNorth, BantSouth, GarbAlgash 2, GarbAlgash 3, Mastora a, Mastora b, Mastora d, Alsawagi South, Alshahid, Alsog Alshabi) in each of the 20 clusters in 2014 and 2015. All the water holding containers inside the households and around the households were inspected for immature stages of Ae. aegypti. All larvae and pupae were collected and numbers per container were recorded on
entomological survey forms (in/outdoor). Aedes spp. breeding sites were identified using spotlight in any water-holding container where immature stages of Aedes spp. were detected. A container was considered ‘positive’ for Aedes spp. when one or more larvae or pupae were present. Different types of containers were examined depending on size. For large water storage containers (>100 l) such as large clay pots, water tanks, and barrels, water was emptied gradually, using a pipette and a sieve as a filter for collection of immature stages and were then counted and classified to larvae and pupae. For small water containers (less than 20 l) e.g. vase and small clay-pots, water was directly filtered using fine mesh in white-enamel trays [24].

If the container is large (>100 l) and has high number of larvae/pupae, they were collected by sweeping the surface using a hose pipe with a net. Larvae and pupae were kept in plastic cups with 250 ml of water and labeled indicating location, date, time, house number, type of container, number of sample, indoor or outdoor collection, then, transported to the laboratory for identification. The 3rd and 4th larval instars of Ae. aegypti were separated from the samples for identification before they pupated. Samples of larvae and pupae were placed in hot water (60°C) for a few minutes to quickly kill them. They were, then, placed in 70% denatured ethyl alcohol. These were identified according to the methods described by [25-27]. Throughout the entomological survey, ambient temperature and relative humidity in each cluster were recorded, and the total rainfall of Kassala city was obtained from the main Meteorological Station in Khartoum.

Statistical analysis:
Data collected on immature stages were subjected to an appropriate general linear model procedure of statistical analyses using SAS package and mean separations were performed using Ryan-Einot-Gabriel-Welsch (REGWQ) multiple range test [28]. Immature stages counts were transformed to log_{10} (\bar{x} ± 1). Correlation analysis was carried out to relate immature stages counts to meteorological data.

Results
Correlation analysis between climatic variables and mean number of female Ae. aegypti:
The mean number of the female Ae. aegypti in Garb Algash was positively significantly correlated with rainfall (r^2 = 0.37935, n = 52, P ≤ 0.01) (Table 1). The mean number of the 3rd and 4th larval instars and pupae were negatively insignificantly (P > 0.05) correlated with low and high temperatures (r^2 =0.05630, 0.03355, 0.08363, 0.06971, 0.04145, 0.03687, n=52), respectively. The mean number of the 3rd larval instar was negatively insignificantly correlated with low and high humidity (r^2 = -0.16097, -0.02202, n=52, P>0.05), while for the rainfall, it was positively insignificantly correlated (r^2=0.06782, n=52, P>0.05). In Alnorab area, the mean number of adults were positively significantly correlated with low humidity (r^2=0.48048, n=20, P≤0.05). The mean number of the 3rd and 4th larvae and pupae and adults were positively insignificantly correlated with rainfall and low and high temperatures (n=20, P>0.05). The mean number of the 3rd larval instar at Altora was negatively insignificantly (P>0.05) correlated with low temperature and low and high humidity (r^2=0.14648, 0.17198, 0.34987, n=12), respectively.

At high temperature and rainfall, they were negatively insignificantly (P > 0.05) correlated. The mean number of the 4th instar larvae at Altora was positively significantly correlated with low and high humidity (r^2=0.68328, n=12, P≤0.05) and (r^2=0.57868, n=12, P≤0.05), respectively, while at amount of rainfall and high temperature they were negatively insignificantly (P>0.05) correlated. The mean number of pupae were positively insignificantly (P>0.05) correlated with low temperature and low and high humidity (r^2=0.11421, 0.44573, 0.51846, n=12), respectively. The number of females were negatively insignificantly correlated (P>0.05) with all climatic variables (r^2=-0.43248, -0.40261, -0.02347, -0.267272, n=12), respectively, except for low humidity which was positively insignificantly correlated (r^2=0.23006, n=12, P>0.05). The mean number of pupae in Birai was significantly positively correlated with low temperature (r^2=0.55928, n=12, P≤0.05), and positively insignificantly correlated with high temperature (r^2=0.24222, n=12, P>0.05), but at low and high humidity and rainfall, they were negatively insignificantly correlated (r^2= -0.19098, -0.26899, -0.28174, n=12, P>0.05), respectively.
**Table 1: Correlation analysis between climatic variables and developmental stages of *Ae. aegypti* in residential areas during 2014/2015 in Kassala City**

<table>
<thead>
<tr>
<th>Stages of <em>Ae. aegypti</em></th>
<th>GarbAlgash (52)</th>
<th>Alnorab (20)</th>
<th>Altora (12)</th>
<th>Biriai (12)</th>
<th>Mastora (48)</th>
<th>Khatmia (28)</th>
<th>Alsawagi (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larva3</td>
<td>0.0563</td>
<td>-0.24943</td>
<td>0.14648</td>
<td>0.16971</td>
<td>0.02761</td>
<td>-0.11666</td>
<td>0.30351</td>
</tr>
<tr>
<td>Larva4</td>
<td>0.08363</td>
<td>-0.24491</td>
<td>0.08731</td>
<td>0.28256</td>
<td>-0.06128</td>
<td>-0.09167</td>
<td>0.31136</td>
</tr>
<tr>
<td>Pupae</td>
<td>0.04145</td>
<td>-0.26911</td>
<td>0.11421</td>
<td>0.55928*</td>
<td>0.55928*</td>
<td>0.14822</td>
<td>0.30351</td>
</tr>
<tr>
<td>Adult</td>
<td>0.10049</td>
<td>-0.09382</td>
<td>-0.43248</td>
<td>0.14585</td>
<td>0.14585</td>
<td>0.07686</td>
<td>0.31136</td>
</tr>
<tr>
<td>Total</td>
<td>0.08965</td>
<td>-0.25495</td>
<td>-0.23969</td>
<td>0.28794</td>
<td>0.28794</td>
<td>0.28794</td>
<td>0.28794</td>
</tr>
</tbody>
</table>

**Climatic variables**

- Low Temperature
- High Temperature
- Low Humidity
- High Humidity
- Rainfall

**Correlation coefficients**

- * indicates significance at p < 0.05
- ** indicates significance at p < 0.01

For example, for GarbAlgash (52), the correlation coefficient between Larva3 and Low Temperature is 0.0563, and between Larva3 and High Temperature is -0.24943.
The mean number of the 3rd larval instar in Biriai was negatively insignificantly correlated (P≤0.05) with high temperature and rainfall ($r^2= -0.24274$, $n=43$, P>0.05), and positively insignificantly correlated with low temperature, low and high humidity ($r^2=0.02761$, $0.11899$, $0.21309$, $n=12$, P>0.05) (Table 1). The mean number of the 4th larval instar in Biriai was positively insignificantly correlated with low and high temperature and high humidity ($r^2=0.28256$, $0.00106$, $0.05003$, $n=12$, P>0.05), respectively, and the females at Biriai were positively insignificantly correlated with low temperature and low and high humidity ($r^2=0.14585$, $0.12335$, $0.12705$, $n=12$, P>0.05), respectively, and negatively insignificantly correlated with high temperature and rainfall ($r^2=-0.31735$, $-0.39076$, $n=12$, P>0.05), respectively. The total mean number of all stages was positively insignificantly correlated with low temperature and high humidity ($r^2=0.28794$, $0.03979$, $0.07622$, $n=12$, P>0.05), respectively, and negatively insignificantly correlated with high temperature and rainfall ($r^2=-0.13592$, $-0.16984$, $n=12$, P>0.05), respectively.

At Mastora, the mean number of the 3rd larval instar was positively insignificantly correlated with low temperature and low and high humidity ($r^2=0.02761$, $0.40795$, $0.11313$, $n=43$, P>0.05), respectively, but negatively insignificantly correlated with high temperature and rainfall ($r^2=-0.00392$, $-0.06040$, $n=43$, P>0.05), respectively. The mean number of the 4th larval instar was negatively insignificantly correlated with low and high temperature and rainfall ($r^2=-0.06128$, $-0.04104$, $-0.05085$, $n=43$, P>0.05) respectively, and positively insignificantly correlated with low and high humidity, respectively ($r^2=0.45211$, $0.14011$, $n=43$, P>0.05). In Mastora the mean number of pupae was negatively insignificantly correlated with low and high temperature and rainfall respectively ($r^2=-0.05437$, $-0.03818$, $-0.05168$, $n=43$, P>0.05), and positively insignificantly correlated with low and high humidity and rainfall ($r^2=0.43004$, $0.13820$, $n=43$, P>0.05), respectively while no significance was recorded for females mean numbers with all climatic variables (Table 1).

At Khatmia, the mean number of the 3rd larval instar was negatively insignificantly correlated with low and high temperature and low humidity ($r^2=0.11666$, $-0.02635$, $-0.06378$, $n=28$, P>0.05), respectively, and positively insignificantly correlated with high humidity and rainfall ($r^2=0.11588$, $0.55228$, $n=28$, P>0.05), respectively. The mean number of the 4th larval instar were negatively insignificantly correlated with low and high temperature ($r^2=-0.09167$, $-0.33454$, $n=28$, P>0.05), respectively, but at low and high humidity and rainfall it was positively insignificantly correlated ($r^2=0.26105$, $0.28215$, $0.15469$, $n=28$, P>0.05), respectively. The mean number of pupae at Khatmia, was positively significantly correlated with low and high humidity and rainfall ($r^2=0.39200$, $0.36079$, $n=28$, P≤0.05) and ($r^2=0.61725$, $n=28$, P≤0.01), respectively. The mean number of female were positively significantly correlated with rainfall ($r^2=0.42277$, $n=28$, P≤0.05). Mean number of the female at Khatmia was positively correlated with low temperature and rainfall ($r^2= -0.06128$, $-0.04104$, $-0.05085$, $n=43$, P>0.05) respectively, and positively insignificantly correlated with low and high humidity, respectively ($r^2=0.43121$, $0.14011$, $n=43$, P>0.05). In Mastora the mean number of pupae was negatively insignificantly correlated with low and high temperature and rainfall respectively ($r^2=-0.05437$, $-0.03818$, $-0.05168$, $n=43$, P>0.05), and positively insignificantly correlated with low and high humidity and rainfall ($r^2=0.43004$, $0.13820$, $n=43$, P>0.05), respectively while no significance was recorded for females mean numbers with all climatic variables (Table 1).
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In Alsawagi, it was positively significantly correlated with low and high temperature (r\textsuperscript{2}=0.70888, n=8, P≤0.05), respectively. In 2014, mean total numbers of stages of Ae. aegypti and 4

Table-2: Correlation analysis between climatic variables and developmental stages of Ae. aegypti during 2014 / 2015 in Kassala City

<table>
<thead>
<tr>
<th>Climatic variables</th>
<th>Stages of Ae. Aegypti</th>
<th>2014 (90)</th>
<th>2015 (90)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Larvae3</td>
<td>Larvae4</td>
<td>Pupae</td>
</tr>
<tr>
<td>Low temperature</td>
<td>0.11422</td>
<td>0.05669</td>
<td>-0.0276</td>
</tr>
<tr>
<td>High temperature</td>
<td>0.01242</td>
<td>0.07397</td>
<td>-0.0526</td>
</tr>
<tr>
<td>Low humidity</td>
<td>-0.1061</td>
<td>0.12332</td>
<td>-0.0498</td>
</tr>
<tr>
<td>High humidity</td>
<td>-0.0967</td>
<td>0.06994</td>
<td>-0.008</td>
</tr>
<tr>
<td>Rainfall</td>
<td>0.11128</td>
<td>0.02929</td>
<td>0.06162</td>
</tr>
</tbody>
</table>

*P ≤ 0.05.

Values in parenthesis = number of observations

Total= Larvae3+Larvae4 + Pupae + Females

In 2014, the mean number of pupae was negatively insignificantly correlated with low and high temperature and low and high humidity (r\textsuperscript{2}=-0.0276, -0.0526, -0.04982, -0.00797, n=90, P>0.05), respectively, and positively insignificantly correlated with rainfall (r\textsuperscript{2}=0.06162, n=90, P>0.05). In 2015, the mean number of pupae was negatively insignificantly correlated with low and high temperature and low and high humidity and rainfall (r\textsuperscript{2}=-0.02350, -0.08125, -0.10026, -0.09539, n=90, P>0.05).
The number of pupae in winter 2014 and 2015 was significantly negatively correlated with high temperature ($r^2 = -0.04302$, $n=56$, $P \leq 0.05$) but was positively insignificantly correlated with rainfall ($r^2 = 0.06162$, $n=56$, $P > 0.05$) (Table 3). The mean number of females in winter was significantly negatively correlated with low humidity ($r^2 = -0.02060$, $n=56$, $P \leq 0.05$), significantly negatively correlated with high humidity ($r^2 = -0.42053$, $n=56$, $P \leq 0.001$), and significantly positively correlated with rainfall ($r^2 = 0.25087$, $n=56$, $P \leq 0.05$). However, the total mean number of stages was significantly negatively correlated with high temperature ($r^2 =$-}
Discussion

Environmental factors like temperature, relative humidity and rainfall have been described as the most important physical factor affecting Aedes biology. The result revealed that there was a positive correlation between rainfall and number of females. *Ae. aegypti* at Garb Alhash and Khatmia areas. This finding is in line with that of Jansen and Beebe (2010) [16] who found that rainfall is positively correlated with larval abundance, especially where rain-filled containers appear to be the primary larval sites and no care has been taken for cleaning them. Some stages of *Ae. aegypti* like larval instar 4 and pupae were positively correlated with high humidity at Khatmia and Altora areas, a finding that agrees with that of Hayden et al. (2010) [29] who concluded that *Ae. aegypti* are positively associated with areas of high relative humidity and high vegetation cover. In autumn season, there was no strong correlation of all the stages of *Ae. aegypti* with all climatic variables. This finding disagrees with Jansen and Beebe (2010) [16] who recorded an association between rainfall and *Ae. aegypti* abundance varying among localities due to both range of container types and differences in water storage practices of the local people.

Correlation between ambient low temperature and number of females was positive in autumn season. This agrees with Ibarra et al. (2013) [30] who found the effect of climate on *Ae. aegypti* abundance varying within a region due, in part, to a suite of social factors that interact with climate to influence vector dynamics. Studies from drier and wetter regions of Puerto Rico found that *Ae. aegypti* densities were positively correlated with rainfall only. According to Vezzani (2004) [31,32], ambient temperatures over 20.8 °C and rainfall higher than 150 mm are suitable for *Ae. aegypti* population increase. With reference to these reports and to the climatic conditions obtained in the present study, positive effects of rain, temperature and relative humidity are more likely to have occurred during the rainy season. It is also likely that the high temperatures that prevailed during the dry period (36 to 46 °C) had adverse effects on the larval life and host as well as oviposition sites-seeking activities.

Conclusions

In conclusion, the results show that human population density positively affects the number of *Ae. aegypti* females within the residence. Climatic variables also affected mosquito population’s particular in some areas. Similarly, rainfall, high humidity, and temperature were positively correlated with the number of females *Ae. aegypti* collected in the residences. *Ae.aegypti* in some areas and also between ambient low temperatures in autumn season.

Recommendations

Entomological indicators and climatic factors of females *Ae. aegypti* must be part of an interconnected data set when evaluating and controlling this mosquito.

Conflict of Interest

The authors declare no conflict of interest in relation to this work.

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