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Breeding Biology and Ecology of *Anopheles Stephensi* in Kota Region, Rajasthan

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***Abstract*: Mosquito borne disease are transmitted typically by the bite of an infected mosquito. Different vectors carry different diseases such as malaria, dengue, encephalitis, yellow fever, etc. which frequently occur in India and cause heavy morbidity and mortality annually. The dynamics of disease transmission by these vectors is governed by an array of factors such as climate change, environmental disturbances, and several other man-made factors which include change in land use, population density, community hygiene and human behaviour. The major mosquito vectors of India belong to the genera Culex, Aedes, and Anopheles. Malaria is one of the important factors responsible for the slow growth of nation's agricultural, industrial and economic progress. Rajasthan provides favourable environment for mosquito survival. Kota lies on latitude 25°10` N and longitude 75°50` with the intervention of Chambal river coming from Madhya Pradesh and running throughout Kota city, irrigating various rural areas via man made channels. Kota city has been a favourable place for mosquito breeding reporting a record number of malaria and dengue cases. Maximum cases of malaria are reported during August to October. The mosquitoes are more active in humid atmosphere. The rainy season falls between June to September. Rainfall provides water as well as increases humidity of the atmosphere for breeding and survival of the mosquitoes. Community hygiene and housing also play an important role in the spread of malaria. Poorly ventilated and dim lit houses provide resting place for mosquitoes.**

***Keywords*: Breeding Biology, Anopheles Stephensi**

1. Introduction

Mosquitoes are ubiquitous in distribution and are found in all regions of world, except the regions near the two poles and altitudes beyond 2000 marks. There are 3500 species of mosquitoes, out of which most of them exist in tropical climate. In India mosquitoes have been known from very ancient times and are considered as a cause of both nuisance and deadly human diseases. Mosquito borne disease are transmitted typically by the bite of an infected mosquito. Different vectors carry different diseases such as malaria, dengue, encephalitis, yellow fever, etc. which frequently occur in India and cause heavy morbidity and mortality annually. The dynamics of disease transmission by these vectors is governed by an array of factors such as climate change, environmental disturbances, and several other man made factors which include change in land use, population density, community hygiene and human behaviour. The civil developmental activities in the urban areas prove favourable for mosquito breeding as a result of which Malaria is freely disseminated from urban areas to rural areas by the free movement of the people to the big cities and towns in search of employment for various developmental activities like industries, constructions etc. While moving out of urban areas the infection borne individuals carry the infection even to the distant rural areas thus establishing a new source of transmission to the distant areas. Rural migration leads to the establishment of "urban slums" with poor housing and sanitary conditions. These areas have a heavy breeding potential of *Anopheles stephensi, A. culifacies, Culex quinquefasciatus* and *Aedes aegypti*. The major mosquito vectors of India belong to the genera *Culex*, *Aedes*, and *Anopheles*. While *Culex* is the major vector of filiariasis and Japanese encephalitis, *Aedes* carries dengue, and *Anopheles* transmits malaria (Hemingway *et al.*, 2006).

In India over 80% of country population is exposed to the risk of malaria due to the wide distribution of mosquitoes. Malaria is one of the important factors responsible for the slow growth of nation's agricultural, industrial and economic progress.

Rajasthan also provides favourable environment to mosquitoes. Kota lies on latitude 25°10` N and longitude 75°50` with the intervention of Chambal river coming from Madhya Pradesh and running throughout Kota city, irrigating various rural areas via man made channels. Kota has also been a favourite place for mosquito breeding and hence a record of malaria and dengue cases reported are more from neighbouring Bundi (Roteda) and other districts of Rajasthan. The Kota barrage made for irrigation purpose serves as a reservoir for mosquito breeding. The right canal passes around Kota fort and falls in "Kishore Sagar Talab" which is almost in the centre of the city. The left canal runs near Thermal power plant towards Bundi, irrigating the area of Talera, Keshorai Patan and Kapren. Kishore Sagar is situated in the heart of Kota city from which right canal passes towards Madhya Pradesh irrigating Kaithun, Digod, Sultanpur, Itawa and Anta. Certain seepage areas are formed en route this canal. Kotari, Ramchandrapura and Kansua areas lie on the right side of the canal and Bajrang Nagar, Thengra Village on left side of right canal. Small seepage pockets of water enroute the canal invites mosquitoes for breeding.

In Kota city maximum cases of malaria occur during August to October. The mosquitoes are more active in humid

atmosphere. The rainy season falls between June to September. Rainfall provides water for mosquito breeding as well as increases humidity of the atmosphere for survival of the mosquitoes. Though the annual incidence of malaria has stabilized around 2 million during the last one decade, the country witnessed sudden upsurge of malaria epidemic during 1994 in Rajasthan, Manipur, Nagaland and a few other states with four fold increase in deaths due to malaria. In most cases man is responsible for creation of malaria. Pits, garden pools, unplanned housing, improper drainage, irrigation channels and engineering projects have led to the breeding of mosquitoes and increase in malaria cases.

Community hygiene and housing also play an important role in the spread of malaria. Poorly ventilated and dim lit houses provide resting place for mosquitoes. The slum environment is conducive to spread of several ailments. In the old areas of cities, lack of planning/layout/bye- laws of consideration to improve environment is missing. Dwelling units continue to be built wherever any space is available irrespective of ventilation, lighting and sanitation. Due to ease in transportation people readily travel from one place to another thus importing malaria parasite in their blood and spreading it to the malaria free places where they come to work and study. One such example of this is Thar Desert situated in north-west Rajasthan. Cerebral malaria here is supposed to be brought by the workers coming from Bihar and U.P. to dig the Indira Gandhi Canal (Singh and Prakash, 2004).

2. Work Plan

The present study is based on surveys in Kota city and extensive laboratory studies. Field trips were conducted in the slum areas of Kota, which were prone to incidence of mosquito borne diseases. The effect of temperature, rainfall and community hygiene was studied by visiting the specific sites. The number of cases reported suffering from Malaria vivax, Malaria falciparum and Dengue were collected from the CMHO office, Primary Health Centres and Government hospitals of Kota. The experiments in the laboratory were conducted on Anopheles stephensi in five cages for colour preference, preference of female mosquito for oviposition, mating time, preference of food material and attraction towards male or female sweat. Fixed number of Anopheles stephensi were left in cage and observations were recorded. During all the experiments water mixed with glucose was provided ad libitum. The mean of all the readings were taken to reach any conclusion.

3. Observations and Results

Various parameters for the ecology and breeding biology of *A. stephensi* were studied in Kota region in both field and laboratory conditions.

A. Preference of mosquitoes to different colours

Method of Treatment : Contact
Number of mosquitoes : 50 (male + female)
Temperature and Humidity : 28 ±1° C, 70 -80 % RH
Dark: Light Period : 14: 10 hours
Factor: varying colors (Red, Green, Yellow, Blue, White and Black)

50 adult *A. stephensi* collected at random were released in a cage containing different colored cloth strips of same size and texture hung at equal distance from the center of the cage. The numbers of mosquitoes resting on each cloth strip were counted after a definite interval of time. The experiment was performed 5 times and the mean readings were taken for interpretation. The percent adults attracted to the different colors are tabulated below:

Color	% adults attracted
Red	7.2
Black	4.0
Blue	2.8
Yellow	0.8
White	0.4
Green	0.0

The adult *A. stephensi* mosquitoes have a preference for certain colours. Red colour appeared more attractive to the adults. Black colour was the next in order of preference. Very few adults were attracted to the blue color. Yellow and white colors were also not much preferred whereas green color was not at all liked by the mosquitoes.

B. Preference of female mosquitoes for oviposition

Studies on the preference for oviposition of *A. stephensi* were conducted on the following parameters:

- Different pH
- Different Surfaces
- Vessel with coloured bottoms
- Different kinds of Water

1) At different pH

Method of Treatment : Contact
Number of pairs : 10
Temperature and Humidity : 28 ±1° C, 70 -80 % RH
Dark: Light Period : 14: 10 hours
Factor : Different pH (4, 7, 9)

Three Petri dishes of equal size and depth containing varying pH solution were kept in the cage. 10 pairs of *A. stephensi* mosquitoes were released in the cage. Mosquitoes were provided glucose solution as food. The numbers of eggs laid in each of the Petri dish were counted. The experiments were performed 5 times and mean readings were taken for interpretation. The number of eggs laid on different pH is given below:

pH	No. of eggs laid
4	62.8
7	Nil
9	44.0
Tap Water	102.0

The female *A. stephensi* are also selective in laying eggs at particular water sites. The females prefer only certain media to lay their eggs on. They use their tarsal sense organs to detect the acidic and alkaline media. The females are found to lay eggs on both the media.

2) At different Surfaces

Method of Treatment : Contact
Number of pairs : 10
Temperature and Humidity : 28 ±1° C, 70 -80 % RH
Dark: Light Period : 14: 10 hours

Factor: Different surfaces – wood, Aluminium, glass, steel, tyre, cement, earthen plates.

7 Petri dishes of equal size and depth were taken containing the material mentioned above. These materials were kept in the cage individually in separate Petri dishes covered with a thin film of water. 10 pairs of mosquitoes were released in the cage containing the Petri dishes. Mosquitoes were provided with glucose solution. The numbers of eggs laid by *A. stephensi* in each of the Petri dish were counted. The experiments were performed 5 times and mean readings were taken for interpretation. The number of eggs laid on different surfaces is given below:

Different Surface	No. of eggs laid
Tyre	52.6
Cement	40.4
Earthen Pot	31.2
Wood	19.4
Steel	15.6
Aluminium	11.4
Glass	8.4

The female *A. stephensi* mosquitoes are also selective for the surfaces on which they lay eggs. It was found that rough and black surfaces were more attracted to the females than the smooth and white ones.

3) Vessels with Coloured bottoms

Method of Treatment : Contact
Number of pairs : 10
Temperature and Humidity : 28 ±1° C, 70 -80 % RH
Dark: Light Period : 14: 10 hours

Facto: Colored vessels (red, black, green, blue, yellow, white)

6 Petri dishes of equal size and depth painted with different colors on outside surface containing tap water were placed in the cage. 10 pairs of *A. stephensi* mosquitoes were released in the cage containing the Petri dishes. Mosquitoes were provided with glucose solution. The numbers of eggs laid in each of the Petri dish were counted. The experiments were performed 5 times and mean readings were taken for interpretation. The numbers of eggs laid on different colored vessels is given below:

Different Colors Vessels	No. of eggs laid
Black	57.0
Red	48.8
Green	39.0
Blue	28.4
Yellow	19.2
White	11.6

The female *A. stephensi* prefers certain colours for oviposition. They avoid the brighter shades. The darker shades are quite appealing to them. It was found that maximum numbers of eggs were laid on the black colored vessels indicating the order of preference for certain colors. Red color was preferred at the second place. Minimum numbers of eggs were laid on the white colored vessel indicating that the bright colors were not found attractive by the females for oviposition.

4) Different kinds of Water

Method of Treatment : Contact
Number of pairs : 10
Temperature and Humidity : 28 ±1° C, 70 -80 % RH
Dark: Light Period : 14: 10 hours

Factors: Different water samples (rain water, pond water, fresh standing water, standing water with algae, sandy water, water with organic material, distilled water, tap water)

8 Petri dishes of equal size and depth containing different water samples were placed in the cage. 10 pairs of *A. stephensi* mosquitoes were released in the cage containing the Petri dishes. Mosquitoes were provided with glucose solution. The numbers of eggs laid in each of the Petri dish were counted and the mean readings were taken for interpretation. The experiments were performed 5 times and mean readings were taken for interpretation. The number of eggs laid on different kinds of water is given below:

Different kinds of Water	No. of eggs laid
Fresh Standing water	57.4
Sandy water	56.6
Standing water with algae	52.8
Distilled water	40.2
Sewage Water	31.4
Pond water	26.0
Tap water	19.4
Rain water	11.0

The female *A. stephensi* are also specific in the selection of different kinds of water for oviposition. It was found that fresh standing water was highly preferred by the female anophelines. Tap and rain water were not preferred for egg laying.

C. Population dynamics

Different experiments were conducted to determine the effect on the population of mosquitoes at,

- Different heights
- Different light intensities
- Different temperatures

1) Population dynamics at different heights

Temperature and Humidity : 28 ±1° C, 70 -80 % RH
Factor : Different height (5and 10ft)

The experiment was conducted mainly to find out the density of *A. stephensi* mosquitoes at different heights. For this slum houses of varying heights were considered. The numbers of mosquitoes found at varying heights were counted after a definite interval of time. The experiments were performed 5 times and mean readings were taken for interpretation. The numbers of mosquitoes collected at different heights of temporary accommodations are given below:

Height of a building (Ft)	No. of mosquitoes collected
5	36.4
10	20.2

The population of mosquitoes also varies with the height. Maximum numbers of mosquitoes were found on the bottom moist sandy floor than at the higher heights. The population of the mosquitoes is likely to increase at the higher heights in residential colonies provided they are able to find proper hideouts or shelters, suitable host for feeding and a proper place for egg laying *i.e.*, the standing water source. If man-made sources of stored water are available like open water tanks, empty flower pots retaining water, water vessels kept for birds to drink water provide breeding place to the mosquitoes. Therefore, it is seen that the houses which are spacious with good inflow of sunlight and clean surroundings have fewer mosquitoes even at the ground level and at the subsequent higher storeys as compared to dark and damp houses surrounded by tree canopies and open water sources.

2) Different Light intensities

Method of Treatment : Contact
Number of pairs : 10
Factor: Varying light intensities emitted by 25W, 40W, 60W and 100 W bulbs)

10 pairs of *A. stephensi* mosquitoes were taken. It was made sure that the female was well fed with blood and already mated. The mosquitoes were released in big glass jars filled 1/4th with water. The jars covered with white muslin cloth were placed under different light intensities till egg laying. The number of eggs laid in each of the jars was counted. The eggs laid were kept under the same of light till hatching. The numbers of larvae hatched were also counted. The experiments were performed 5 times and mean readings were taken for interpretation. A separate control was also run simultaneously for comparison. The observations recorded at different light intensities are given below:

Light Intensity	No. of eggs laid	% Egg hatching
25 watt	53.6	63.43
40 watt	8.7	45.97
60 watt	Nil	Nil
100 watt	Nil	Nil
Control	105.0	93.33

The higher light intensities are repelling to the mosquitoes. The mosquitoes prefer the low light intensities and dark places for resting and egg laying.

3) Different temperatures

Method of Treatment : Contact
Number of pairs : 10
Factors: Varying temperatures (0, 10, 20, 28, 30, 40 and 50°C)

10 pairs of *A. stephensi* mosquitoes were taken for study. It was made sure that the sure was well fed with the blood and already mated. The mosquitoes were then release in big glass jars filled 1/4th with water. The jars were covered with white muslin cloth were placed under varying condition of temperatures using Room air conditioners and room heaters. The number of eggs laid in each of the jars was counted. The eggs laid were kept under the same temperatures till hatching. The numbers of larvae hatched were also counted. A separate control was also run simultaneously for comparison.

The following observations are recorded at different temperatures:

Temperature (C)	No. of eggs laid	% egg hatching	% corrected sterility
0	Nil	Nil	Nil
10	Nil	Nil	Nil
20	53.4	59.92	35.79
30	98.0	91.83	1.60
40	Nil	Nil	Nil
50	Nil	Nil	Nil
control	105.0	98.33	Nil

Both high and low temperatures were not favorable to the *A. stephensi* mosquitoes. The mosquitoes died at very high temperatures due to desiccation as they do not possess any sufficient mechanism to prevent or avoid desiccation caused due to water loss at high temperatures. At low temperatures the adults survive only for a few days.

D. Reproductive activity during Day and Night

Method of Treatment : Contact
Number of pairs : 10

Temperature and Humidity : 28 ±1° C, 70 -80 % RH

Factors: 24 hours of total darkness, 24 hours of total light, 14: 10 hours of dark and light.

10 pairs of *A. stephensi* mosquitoes were taken in a cage and kept under total darkness till egg laying and hatching. The males were provided glucose solution and the females were provided human blood feed. Laboratory tray filled with water was also provided in the cage for egg laying. In the next set of experiment 10 other pairs of mosquitoes were kept under continuous light as provided by a 25W bulb till egg laying and hatching. The number of eggs laid in each of the sets was counted. The eggs laid were kept under the same condition of darkness and light and the larvae hatched were counted. A separate control of 14:10 hours of darkness and light was also run simultaneously for comparison. The observations recorded under the different light conditions are given below:

Light conditions	No. of eggs laid	% Egg hatching	% corrected sterility
Dark (24 : 0)	86.5	43.93	52.93
Light (0 :24)	54.0	68.51	26.59
Control (14 : 10)	105.0	93.33	-

The female *A. stephensi* mosquitoes were found to reproduce only at a particular time of the day. It was seen that the mosquitoes formed swarms and had copulation during the dusk time. It was in the later hours of the night or early morning hours before dawn that the females prefer to lay eggs. If the adults were exposed to continuous light they adapted themselves to that pattern and laid eggs successfully but the number laid was not satisfactory as compared to that of control. When the mosquitoes were subjected to continuous dark only few of the eggs laid managed to hatch indicating the necessity of appropriate light for egg hatching.

E. Preference for different food materials

Method of Treatment : Contact
Number of male mosquitoes : 50
Temperature and Humidity : 28 ±1° C, 70 -80 % RH
Dark: Light period : 14:10 (hours)
Factors : Different food material

7 Petri dishes of equal size and depth were covered by thin and small pieces of food materials like fresh peeled sugar cane, soaked raisins, apple slice, *Calotropis* leaves, *Ocimum*, green grass and flowers. The Petri dishes were kept in a cage and the adult *A. stephensi* mosquitoes were released in that cage. The cage was kept under optimum condition of light and humidity. The number of mosquitoes attracted to different food materials was counted after definite interval of time. The experiment was repeated 5 times and the mean readings were taken for interpretation. The percent adults found feeding on different food materials is given below:

Food materials	% adults attracted to food materials
Soaked raisins	12.4
Peeled sugarcane	11.6
Slices of apple	5.2
Calotropis leaves (cut)	2.0
Flowers (periwinkle)	1.2
Green grass	0.4
Ocimum leaves (cut)	0.0

The male *A. stephensi* mosquitoes were attracted mainly to the food materials which were easily penetrable and had a rich supply of glucose. The maximum percentages of adults were found attracted to soaked raisins. They preferred peeled sugarcane on the second place. Slices of apple attracted very few mosquitoes. Leaves and flowers were generally not preferred by the mosquitoes. Green grass and *Ocimum* leaves did not appear attractive to them.

F. Study of the behavior activity – attraction to human male and female sweat

Method of Treatment : Contact
Number of mosquitoes : 50
Temperature and Humidity : 28 ±1° C, 70 -80 % RH
Dark: Light period : 14:10 (hours)
Factors :Human male and female sweat

50 *A. stephensi* mosquitoes were collected at random and released in the cage containing human male and female sweat clothing. The sweat-soaked clothing was hung separately at equal distance from the center of the cage. The number of mosquitoes attracted to the sweat cloth was recorded from time to time. The experiment was repeated 5 times and the mean readings were taken for interpretation. The percent adults attracted to the sweat clothing is given below:

Sweat clothing	% adults attracted to sweat clothing
Male sweat	12.0
Female sweat	20.0

The adult mosquitoes were found susceptible to different odours, moisture content and the amount of carbon-dioxide emitted due to sweating. The human female sweat clothing attracted more mosquitoes than the male sweat clothing.

4. Discussions

The behaviour, development and population of mosquitoes are strongly influenced by climate. Temperature, rainfall and humidity all affect mosquito population. The relationship between these climatic factors and the mosquitoes is highly complex and varies from country to country and from species to species. Increased rainfall and higher temperature provide more breeding pools for mosquitoes and quicken the development of mosquito larvae into adult. However, where mosquitoes breed in large open pools or rivers, increased rainfall may flush out breeding sites and actually reduce the mosquito population. In other areas where mosquitoes breed in

small puddles or pools, increased rainfall may provide more breeding sites and therefore increase mosquito populations. Climatic factors play an important role in the transmission of malaria. Mosquitoes are highly sensitive to rising temperature, which accelerate their metabolic rate, biting rates and feeding frequency. Temperature also affects their reproduction and growth.

The adult *A. stephensi* collected at random were released in the cage containing various coloured cloth stripes of same size and texture hung at equal distances from the centre of the cage. The color preference of the mosquitoes was assessed on the basis of the number of mosquitoes resting on each colored strip. White and green colors were the last preference. Several studies have been made of the response of mosquitoes to the light of different wavelengths but in only a few cases has the relative brightness of the different colors been taken into account. The rate at which caged mosquitoes landed on discs of different colors had been measured. When all the discs produced 40-ft candles of reflected light the attractiveness of the colours was in the following order, the groupings showing differences significant at the five percent level:

Aedes aegypti – Yellow > Orange and Red > Green, Violet, Black, Blue and White.

Aedes taeniorhynchus – White > Orange, Green, Yellow and Blue > Violet, Red and Black.

Aedes sollicitans – Black, Blue, Orange and Green > Yellow, Violet, Red and Yellow.

Using a brightness of 20 ft-c the relative attractiveness of certain colors differed from that found at 40 ft-c to a significant extent in each species. Brett (1938) measured a landing rate of females of *Aedes aegypti* and found that although the mosquitoes generally preferred dark surfaces, red was more attractive than several colors which reflected more light whereas blue was more repellent than several colors which reflected less light. These experiments showed a differential response by mosquitoes to different colors of the different wavelengths. The results have not proved that mosquitoes are capable of color discrimination. Studies by Suzuki (1961) on the optomotor responses of female *Culex pipiens* var. pallens to moving colored stripes have confirmed that colors reflecting equal amounts of light may have strikingly different physiological intensities to the mosquito and that this plays an important part in the apparent discrimination of color.

The attractiveness of different colored surfaces for female *Aedes aegypti* is an inverse function of the intensity of reflected light between 4750 and 6250 A; no correlation with intensity is found outside these limits (Brown, 1954). This indicates an insensitivity to the deeper shades of red, to infra – red and to the violet and ultraviolet light.

From the data recorded the color preference of the adult anophelines comes to Red > Black > Blue > Yellow > White > Green.

The adult *A. stephensi* collected at random were released in the cage containing human male and female sweat clothing hung at equal distance from the centre of the cage. The attraction of the mosquitoes to the particular sweat clothing was assessed on the basis of the number of mosquitoes resting on the particular sweat clothing. The anophelines were attracted in large numbers to the female sweat. Sweat, blood, and urine have been investigated as sources of odour. Some workers have obtained negative results with sweat (Howlett, 1910; Rudolfs, 1923; 1925) but others have obtained clear responses to it. They have also found that *Aedes aegypti* accumulated on the side of the cage and probed when human armpit sweat was held a short distance away in still air, responding slightly but significantly more than to moisture alone. Brown (1951) found that air blown through a solution of armpit sweat, when diluted so that the product of one armpit was contained in 60 ml. of water; it attracted a significantly larger number of *Aedes aegypti* but when it was 10 times more concentrated the sweat was slightly repellent. Dummies soaked with human sweat with armpits and general body surfaces were significantly more attractive to *Aedes* in the field than controls with equally moist clothing (Brown 1951, 1958). These results indicate that mosquitoes are attracted by human apocrine sweat. The protein of apocrine sweat makes it a good medium for bacteria and the decomposition products of this sweat give rise to the characteristic smell of the human adult. Individual variations in odour may reflect chemical differences in apocrine sweat or differences in the bacterial flora or both.

Human beings differ markedly from one another in their attractiveness to mosquitoes. When *Aedes aegypti* were able to choose between streams of warm, moist air which had passed over the hands or over the armpits of two individuals they would frequently show a strong selection of those from one individual. In tests with 4 men and 3 women, examine in pairs, it was found that individual attractiveness varied from one experiment to another and that individual of one sex might or might not differ significantly from one another in their attractiveness, but the men were almost invariably much more attractive than the women. When *Anopheles stephensi* were offered two air streams which had passed over the skin of different individuals and which differed only in odour they made a significantly stronger response to one air stream (Brouwer, 1960a; 1960b).

A. stephensi were found to be more attracted to the female sweat than the male and sat in larger number on the sweat soaked clothing of the female.

The gravid female *A. stephensi* were provided waters having different pH for oviposition. The preference for oviposition was assessed on the basis of the number of eggs laid on the different pH. The female preferred both the acidic and alkaline media for egg laying. Observations of mosquito behaviour suggest that the females use their tarsal sense organs in selecting an oviposition site. These organs are sensitive to a number of inorganic ions, and females of *Aedes aegypti* select certain concentrations of sodium chloride from others for oviposition (Wallis, 1954; Hudson, 1956). The distribution of larvae in

natural waters of varying pH shows that many species are able to live under both alkaline and acid conditions. The observations such as these and the knowledge that female mosquitoes are highly selective in nature in choice of oviposition site have prompted the suggestion that the distribution of larvae is controlled not by survival in suitable and extinction in unsuitable habitats but by the discrimination of the ovipostion female (Beattie, 1932; Beklemishev and Mitrofanova, 1926). Laboratory experiments in enclosed cages suffer from a few serious draw-backs, one of which is that the females are forced to lay eggs, even if the water is unsuitable, and secondly the gradients of humidity, temperatures, odours, etc. get rather blurred in the enclosed space.

The females of *A. stephensi* were found laying eggs on both alkaline and acid media. Acid medium was much preferred than the alkaline medium. The gravid female *A. stephensi* were provided different surface test materials plates for oviposition. The preference for different surfaces was assessed on the basis of the number of eggs laid on that particular test material. The females were found laying eggs on rough black surface as compared to other surfaces. Many aedine species lay their eggs on a moist surface just above the edge of a body of water and the color and texture of the surface effect the number of eggs laid. All species tested in the laboratory preferred a dark surface to a light one and a rough surface to a smooth one (Beckel, 1955; O'Gower, 1955, 1957, 1958) but under natural conditions *Aedes aegypti* showed no significant preference for a black surface over a whit one. The importance of surface texture is shown by the preference of *Aedes scutellaris* and *Aedes australis* in the laboratory for a grey rough surface over a black smooth one according to O'Gower (1955, 1958). Wallis (1954) observed that *Aedes polynesienis* in nature lays eggs in cracks and crevices and in the laboratory it search for angles and corners to lay its eggs.

The females of *Anopheles stephensi* laid eggs on different surfaces in the following order – Scooter tyre > Cement plate > Earthen pot > Wooden pot > Steel plate > Aluminum plate > Glass plate.

The female *A. stephensi* were offered different colored vessels for oviposition. The preference for egg laying on different colored vessels was assessed on the basis of the number of eggs laid on the particular colored vessel. The females were attracted mainly to the black and red shades. Attraction of gravid females of *Anopheles*, *Aedes* and *Culex* from a distance in the laboratory has only been obtained by displaying surfaces which are dark in the contrast to their surroundings; in nature a water surface which is shaded appears darker than its surroundings when seen at night from near the ground. (Kennedy, 1942). The mosquitoes find water by flying towards areas which appear darker than their surroundings. Sites which are shaded, whether in the field or in the laboratory, are more attractive to gravid females than those which are not, shade being important at night as well as by day (Jobling, 1935; De Zulueta and Bates, 1948). According to Russell and Rao (1942a) *Anopheles* culicifacies appears to be unusual in being neither attracted nor repelled by shade background color is generally important. Jobling (1935); Bates (1940); Lund (1942); O'Gower (1957) observed that a dark background is preferred to a light one. According to (Wilton and Fay, 1971) in laboratory studies *A. stephensi* females were found to show some important preferences for certain colors. Using monochromatic light they found strong positive reactions to the middle ultraviolet wavelength of 290 mu and also towards 355 mu. near the ultraviolet. Colours of the blue green portion of the spectrum were comparatively unattractive. One study in Salem Town in Tamil Nadu revealed that at night the female *A. stephensi* get easily disturbed by the yellowish light thrown by flash lights. But when the flash lights are covered by a red cellophane paper, the adults are not disturbed at all.

The female anophelines preferred the different colored vessels for oviposition in the following order – Black > Red > Green > Blue > Yellow > White.

The female *A. stephensi* were provided different water samples for egg laying. The preference for the particular water was assessed on the basis of the number of eggs laid on a particular water sample. Maximum numbers of eggs were laid on fresh standing water. Field studies by Muirhead Thomson (1942) on a number of Anophelines species have revealed that in nature where a number of different habitats are close together eggs are found only in their normal habitat or with a very slight overlap. The presence of organic matter, whether sterile or putrid, renders water highly attractive for the oviposition to a number of species. (Manefield, 1951) found that *Culex pipiens fatigans*, *Aedes polynesienis* and *Aedes aegypti* prefer such water to fresh water, the strength of the preference reflecting the extent of contamination normally found in natural larval habitats. On the contrary according to Muirhead Thomson, (1942) *Anopheles minimus*, a stream breeder, will avoid even slightly polluted water in the laboratory. Laboratory experimental studies on preference for egg laying of *Anopheles culicifacies* were made by Pal (1945). In Petri – dishes kept in small cages, the number of eggs found in different types of water were irrigation water (452), tap water (426), muddy pond water (274) and stagnant water (243). These experiments indicate that no difference exists with regard to attractiveness of the different types of waters. However, such cage experiments suffer from well-known limitation in that the females do not have a chance to exercise a clear choice because of the proximity of the Petri dishes and the merging together of the "influences" of the different types of water which do not occur in natural conditions as in open air.

From the experiments conducted the females mosquitoes were found laying eggs in maximum number on fresh standing water. The order of preference comes to be: fresh standing water > Sand water > standing water with algae > distilled water > water with organic material > pond water > tap water > rain water.

The mosquitoes were collected at random from different height temporary accommodations. The population of mosquitoes most frequently found at different heights was assessed by counting the number collected. Pal (1945) observed that the un-plastered walls of a room yielded large number of *A.culicifacies* than room with the walls plastered. Many observations have also been on the relative frequency of resting on surfaces such as those of cloths, umbrellas, furniture, fire wood, etc. (Muirhead Thomson, 1941a; Ariarathnam, 1955) but critical evaluation of the attractiveness of such surfaces have not been made. Even regarding the preferential heights above the ground selected for day time resting there has been no general agreement. Rao (1981) while studying the preferential heights above ground for day time resting observed that while no part of the wall is free, there is general preference for the roofs when they are at the normal height of 2.4 to 3.6 meters above ground. In a study in Maharashtra stare about 70% of *A. culicifacies* were found resting on the underside of roof of village houses while only 30% rested on the vertical wall and surfaces of furniture, vessels, grain bins, etc. In general, it may be stared that tough the resting females and males of the species many be found in the darkest corners of house, they are not averse to resting even in the moderately lighted portions of s a small hut. In Sri Lanka Ariarathnam (1955) found that the species preferred to rest on wall below 6 feet.

In villages around Delhi, 77% *A. culicifacieis* rested on the ceiling on the walls above 6 feet from the floor and that hanging objects, shelves and objects on the floors served only as incidental resting places. The species avoided really dark places. Cobwebs hanging from the ceiling were a favourite place of resting. The distribution of *A.funestue* inside a small iron roof huts varied with temperature. When the temperature was maximum near the roof the mosquitoes were distributed or near the floor and when the temperature was same at both the sites *i.e*. near the roof and the floor, the mosquitoes were found near the roof. In the studies conducted the mosquitoes were generally found resting on the ceiling and corners of the accommodation. There number gradually decreased with increase in height.

The preference of the adults to different temperature was assessed on the basis of the number of eggs laid and percent hatching at different temperature. The gravid female *A. stephensi* were subjected to different temperatures by contact method. Water was also provided for oviposition. High and low temperatures were not favourable to the mosquitoes and they died. Laboratory studies carried out by Pal (1945) provided some information on the longevity. Females exposed to constant temperature of 40° C did not survive for more than 24 hours irrespective of changes in the relative humidity. However, at 35° C with relative humidity 20 – 100%, they lived for 4 – 10 days but very low or very high relative humidity at these temperatures was not favourable for the life of Anophelines. Temperatures below freezing point were lethal to the adults and 41° C was considered to be the thermal death point for the adults.

Muirhead Thomson, 1941b reported that most of the newly emerged females avoided he higher temperatures when subjected to 25° C. They showed little choice below that temperature. Gorged and gravid females avoided the higher temperatures above the 25° C and hungry females still showed some

Adult male *A. stephensi* were released in the cage containing different food materials. Their preference for specific food was assessed on the basis of the number of adults feeding on that specific food material. The male anophelines were mainly found feeding on food rich in sugar content. The males hovered away from the food which was not of their liking and sat on the preferred material. Mosquitoes do not possess an efficient mechanism for preventing water loss and depend for survival upon meals of blood and nectar. It is well known that the principal food of female mosquito was blood; male and female mosquitoes both feed exclusively on nectar. More than two-third flower visiting mosquitoes are species of *Aedes*, but *Anopheles, Culex, Mansonia, Psorophora* and *Toxorhynchites* have also been found feeding on nectar. It has been reported that sugar alone was sufficient to maintain adult life and females are able to live for several weeks in the laboratory on sugar without undue reduction of tissue nitrogen. It has also been reported that the most important food for female is blood.

The order of preference for different food materials come out to be in the following order – Soaked raisins > Peeled sugar cane > Apple slices > *Calotropis* leaves > Periwinkle flower > Green grass > Green *Ocimum* leaves.

The adult *A. stephensi* were exposed to different light intensities. Water was also provided oviposition. The preference for different light intensities was assessed on the basis of the number of adults surviving at different intensities, number of eggs laid, and egg hatching at particular light intensities. Very high light intensities were unfavorable to the adults and they died within 24 hours. The low intensities were favorable and the adults managed to lay eggs successfully with considerable egg hatching. Little is known of the reaction of mosquitoes to the light under experimental conditions. Female Anopheles quadrimaculates were repelled by bright daylight but showed no response to differences of light intensity of 500 ft-c, whereas females in a choice chamber at light intensity of 2-3ft-c showed no significant preference for a background of any color Platt (1957). They further remarked that the influence of light on choice of oviposition site is more variable than has been assumed. When several laboratory strains of *Aedes aegypti* were given the choice between ovipositing at 0.02 lumen/ft^2 and 3.5- 6.5 lumen/ft^2 certain strains showed a strong tendency to lay at the darker site, other strains at the brighter site.

From the distribution of *Anopheles minimus* inside houses it was concluded that light was the most important factor in choice of resting place and the light intensity in the most frequented resting places was usually less than 0.01 ft-c (Muirhead Thomson, 1941a). *Anopheles sacharovi* and

Anopheles maculipennies were found to prefer light intensities of 1-5 ft-c for their resting places, avoiding higher and lower intensities. Below 23°C a stronger light intensity was tolerated than at higher temperatures and very high temperatures caused the mosquitoes to seek darker quarters than usual. The mosquitoes are quite alert during night and they are responsive to stimuli very readily. Even a beam from torch light, which does not affect them during day time, disturbs them at night and they start flying. They reported further that as the light intensity increases the anophelines are repelled by strong light (negative phototaxy to strong light) and fly towards any dark objects which come into their field of vision. A dark area, a bush, a tree or a hole becomes its target but in urban areas, it is the dark, windows, doors etc. towards which they fly. As light becomes stronger they go deeper into the shelters. When the light is really strong, they begin to become inactive presumably because of the depressant effect of strong light and also perhaps partly due to a diurnal rhythm.

Kennedy (1942) conducted experiments on *Aedes aegypti* and *Culex molestus* and found that when disturbed the mosquitoes moved towards the dark objects. All these experimental observations showed that dark objects were attractive to the mosquitoes indicating negative phototaxy to strong light.

In the present investigation the adults of *A. stephensi* behaved normally and laid eggs at the lower light intensities only. The eggs laid managed to hatch although hatching was only 60.0 percent. At the higher intensities the mosquitoes showed excitability and flew here and there in search of dark corners. They ultimately died either due to the depressant effect of strong light or due to the raised temperature at such high light intensity. The mosquitoes avoided feeding on glucose solution under the impact of strong light and ultimately died indicating that the strong light was not preferred by the mosquitoes. The order of preference for strong light was as follows – Light intensity as produced by different watt bulbs – 25 watt > 40 watt > 60 watt = 100 watt.

Adult *A. stephensi* were released in the cage provided with water for the egg laying. The reproductive activity of the mosquito during day and night was observed on the basis of the number of eggs laid in the dark period and light period and their subsequent hatching. It was observed that the adults when exposed to a period of continuous darkness laid eggs satisfactorily but egg hatching was considerably reduced. When the adults were subjected to a period of continuous light, there was considerable reduction in egg laying but egg hatching was satisfactory. These observations indicate that the female preferred to lay eggs in darkness but light is necessary for their successful hatching. The male mosquitoes were found dead in the Petri dish on which eggs had been laid. Russell and Rao (1942b) found that though *A.culicifacies* laid eggs throughout night the greatest number was laid in first third of the night under natural conditions. Pal (1945) in his laboratory experiments in Northern India and Muirhead Thomson (1940) with *A. minimus* in Assam also recorded a similar habit of egg laying. It has been found that the mosquitoes which have been kept throughout life in constant dark lay irregularly but when transferred to regime of 12 hrs light and 12 hrs dark per day they immediately start to lay rhythmically and the rhythm is maintained for at least 3 days after return to constant darkness, temperature and humidity, suggesting that it is circadian in nature. The female Anophelines preferred to lay eggs in the dark but light is essential for egg hatching.

5. Conclusion

This paper presented an overview on Breeding Biology and Ecology of *Anopheles Stephensi* in Kota Region, Rajasthan

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