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Density and Spatial Distribution of *Anopheles stephensi* in Rural Areas Surrounding Rajkot City, Gujarat, India

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Abstract
In the present study, relative to seasons, this field survey and sampling were an attempt for the first time in the rural area around Rajkot City to investigate the breeding and resting distribution preferences pattern and density of *Anopheles stephensi* species of mosquito. Species identification, larval as well as adult density, and distribution of breeding sites of the species were studied in the rural villages around the Rajkot City area. Results show that the highest density of adult *Anopheles stephensi* was recorded in the month of September, which was the peak month of the monsoon in this region, and the lowest density was recorded during the month of May, which was the lowest during the summer season in the study area. Furthermore, breeding sites of *Anopheles stephensi* were also recorded more in the monsoon and post-monsoon seasons. It was clearly indicated that climatic conditions (temperature, rainfall, humidity, etc.) play a vital role in the density and distribution of *Anopheles stephensi*.

Keywords: *Anopheles stephensi*, climatic condition, Breeding, Rajkot, Rural

Introduction
Malaria is one of the major communicable diseases that causes high mortality and morbidity among the population in India. About one million positive cases with 519 deaths were reported during the year 2012, out of which about half the cases were of Plasmodium falciparum (Pf) (Wiwanitkit 2006) [26]. This disease is transmitted by the bite of the female Anopheles mosquito species. A widespread species throughout southeast and east Asia is Anopheles *stephensi*, also it is very common mosquito species found abundantly in the study area and prevalent throughout the year. In the world, Malaria is the Third World's largest dreaded killer (Singh and Rahman, 2001) [22]. The direct costs of malaria include personal and public expenditures on both prevention and treatment of the disease, and the indirect costs of malaria are the human suffering caused by the disease. (Klein *et al*, 1995; Kwa *et al*, 2006) [13, 14] Migration of population and precipitation of drug resistance, in addition to the ecological conditions, remoteness, and inaccessibility, are contributing to the problem of malaria (William *et al*, 1998; Collins *et al*, 1997; Joshi *et al*., 2005) [30, 5, 13]
Ninety-one countries and 40% of the world's population are at risk of malaria (Matta *et al.*, 2004). The worldwide malaria incidence is estimated to be 300-500 million clinical cases every year (Matta *et al.*, 2004) [15].
An estimated 608,000 deaths occurred globally due to malaria in 2022, a mortality rate of 14·3 deaths per 100,000 populations at risk. More than 50% of all deaths occurred in just four countries Nigeria (31%), the Democratic Republic of the Congo (12%), Niger (6%), and Tanzania (4%). Around 70% of the global malaria burden is concentrated in 11 countries: Burkina Faso, Cameroon, the Democratic Republic of Congo, Ghana, India, Mali, Mozambique, Niger, Nigeria, Uganda, and Tanzania. (WHO World Malaria Report, 2023) [29]

58 species of *Anopheles* have been recorded in India (Das *et al*., 1990) [6]. Among these species, only nine species, viz. *An Stephensi, An. culicifacies, An. Varuna, An. sundaicus, An. fluviatilis, An. annularis, An. philippinensis, An. Minimus* and *An. dirus* have been known to transmit malaria. Of the nine established vectors of malaria, two species, viz. *An. culicifacies* in rural and *An. Stephensi* in urban areas are well-known vectors of malaria in our country. Millions of people die every year due to malaria. Most of the arid and semi-arid areas in western India fall in an unstable malaria zone in the country (Bhatt *et al*, 1991; Srivastava & Yadav, 2000) [3, 23].

The major tropical vector-borne diseases are typically viewed as associated with nursing environmental consequences of underdevelopment, occurring in communities (Brightmer & Fantato, 1998; Hans, J *et al*,) [4]. Local dipteron distribution and flight vary depending upon breeding surround preference, availability of the host, and resting preference. Biological invasions challenge our ability to grasp the organic phenomenon and abiotic factors that govern distribution and abundance (Steven *et al*., 2004; Douglas, E. Norris, 2004) [24, 8].

Our study aimed to find out the density, distribution, and seasonal variation in the population of selected areas in rural areas for *Anopheles stephensi* in different habitats for the first time. This work also describes mosquitoes' active habitats for breeding, which are significant for vector management and useful control strategies in selected areas of Rajkot rural.

Materials and Methods : The Study area is situated near Rajkot (22°20'3"N 70°45'56" E), in the semi-arid tropical region of Gujarat, where the climate is mostly hot and dry. A total of seven villages around Rajkot city were selected for investigation on the distribution and density of *Anopheles stephensi* in outskirt and domestic shelters. All seven selected villages have having network of canal irrigation drainage system. The seepage and waterlogging from the canal create an ideal habitat for the mosquitoes.

The villages are Madhapar, Naranka, Sokhda, Kalipat, Metoda, Ronki, and Kankot, were ideal for the assessment and have a semi-arid type of climate with three distinct seasons, viz. summer (March to June), monsoon (July to September), and winter (October to February) months. The study area also has two major seasonal rivers, Aji and Nyari, and further having four major dams have been built for irrigation purposes in the study area. (Fig.1)

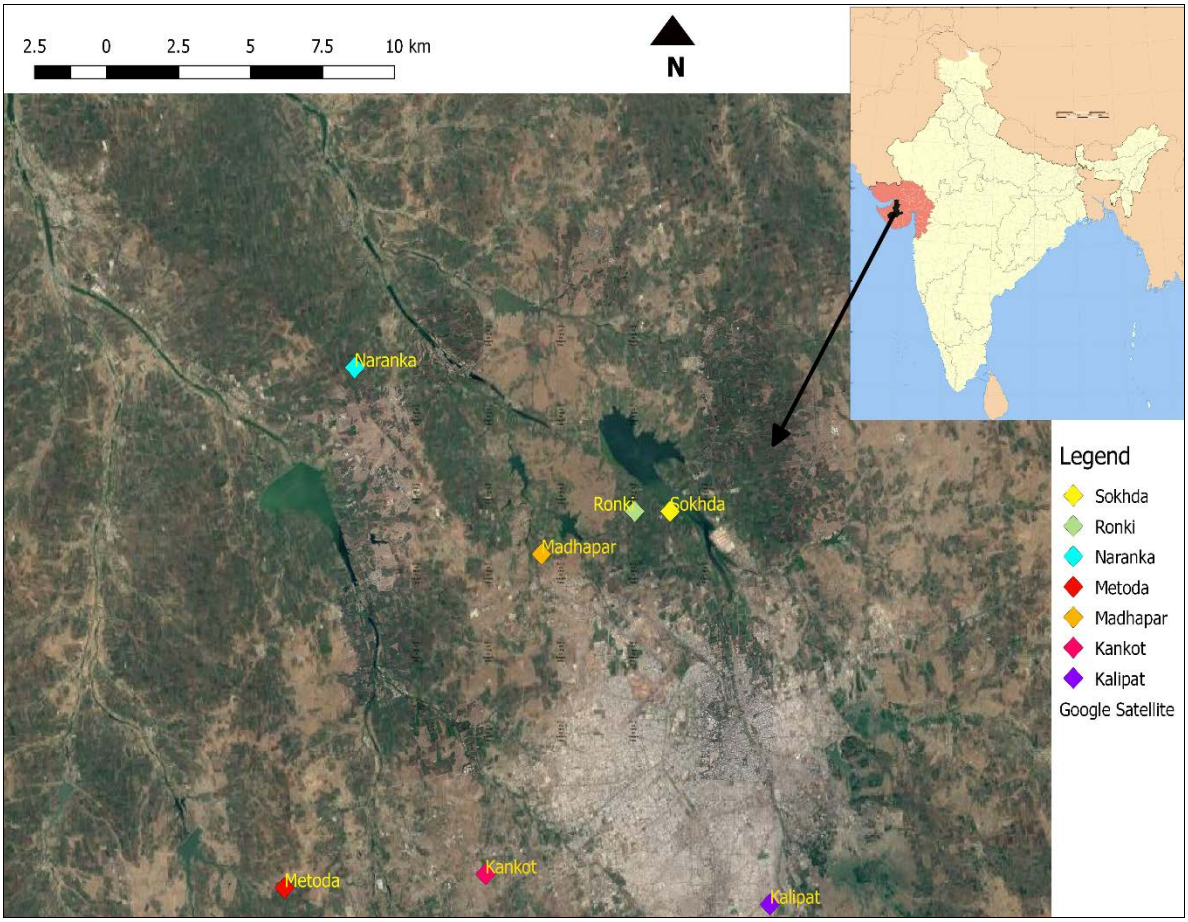

Fig 1: Showing study sites in the rural areas around Rajkot city (Source: Google Satellite image)

The study was conducted from 1st January 2006 to 31st December 2007. The dipping method was used as described by WHO (1975) for the Mosquitoes and larva collection from the residence area (domestic area) and the surrounding of the residence area (outskirt area) in the morning between 6 am to 8 am. References Key was used for the identification of mosquitoes and this larva (Roy and Brown, 1971; Patel, 2002; Sumit) [21, 18]. Breeding sites and resting sites of the mosquito were categorized based on observations during the sample collection of specimens. During the study period, every month, larvae and adult specimens were collected from each and every type of domestic and the outskirts of the selected villages. These samples were further analyzed to determine the status of larvae/adult and identify their presence/absence in the study area.

During the survey, the dipping method was used to collect the larvae specimens, and the collected samples were kept in standard plastic tubes and brought into the laboratory for identification and rearing. After that, the collected larvae specimens were reared in standard plastic jars that were covered with cotton cloth containing the feed mixed with yeast and biscuit powder mixture according to references (Joseph *et al.*, 2004; Das *et al.*, 2003; Helge *et al.*, 2002) [32, 7, 33]. All the collected specimens were brought to the laboratory for species identification (Jagdish and Jagbir; Joshi, 2005) [12]. Morphological identification was done up to the species level with the help of standard references such

as Roy and Brown (1971), Patel (2002), and Sumit [21, 18]. Collected data were analyzed to calculate the Larval Density (LD), average monthly and village-wise larval and adult densities by using the formula LD = number of larvae collected/ number of dips made, while adult density was calculated as Man per Hour Density (MHD) by the formula MHD = Total number of mosquitoes collected / Total time of collection.

Results

Breeding and resting preferences: The results of the study showed that the breeding of *the Anopheles stephensi* species of mosquitoes was found during field collection at the different breeding places of study sites in the rural areas around Rajkot. *Anopheles stephensi* was predominantly a domestic area breeder and found maximum numbers as larvae (50%) from ground tanks. They were mainly preferred clean domestic purposes use water as a breeding habitat that has an ample amount of algae, followed by barrels (18%), small vessels (08%), earth pots (4%), and cattle shed (03%). Larvae of this species were also recorded from outskirt area water, like drainage ditches (7%), and stagnant wastewater (4%) and pots (4%), except rain pools and small rivers. (Tab.1)

Table 1: Mosquito larvae breeding preference (in percent) and Day time resting habitat preference (in percent) of adult mosquito species at various habitats in the rural areas around Rajkot city during the year 2006-07.

Breeding Habitats -*Anopheles stephensi*				**Resting Habitats**	
Domestic		**Outskirt**		**Human Dwellings Houses without cattle**	*Anopheles stephensi*
Ground tanks	50%	Chekdam	-	Cemented Houses	10%
Overhead tanks	06%	Rivers	-	clay Houses	12%
Earth pots	04%	Rain Pools	-	Huts	21%
Cattle shed with home	03%	Stagnant waste water	04%	**Mixed Dwellings Houses with Cattle**	*Anopheles stephensi*
Underground	-	Drainage***	07%	Cemented Houses	11%
Barrels of plastic and metal	18%			Clay Houses	30%
Small vessels	08%			Huts	16%
		***Surface ditches drainage water			

Adults of the species were recorded from all types of houses throughout the year. Among the domestic habitat of human dwelling houses with cattle- Clay houses (30%) were recorded more adults the clay houses (18%) huts (16%) and cemented houses (11%) (Table; 1) and human dwelling houses without cattle- huts (21%), clay houses (12%) cemented houses (10%)

Larval density- Outskirt area

Outskirt habitat recorded maximum average larval density of *Anopheles stephensi* during September 2006 (1.65) and August 2007 (1.77), while the lowest was during March 2006 (0.37) and the month of May 2007 (0.22). (Fig.2)
Village-wise results show that the Naranka and Madhapar villages have the maximum average larval density of the species was recorded in the month of September-2006&07 while village Kankot and Metoda have the minimum average larval density of the species recorded during Apr-06(0.0) and Metoda Mar-07 years. (Fig.2)

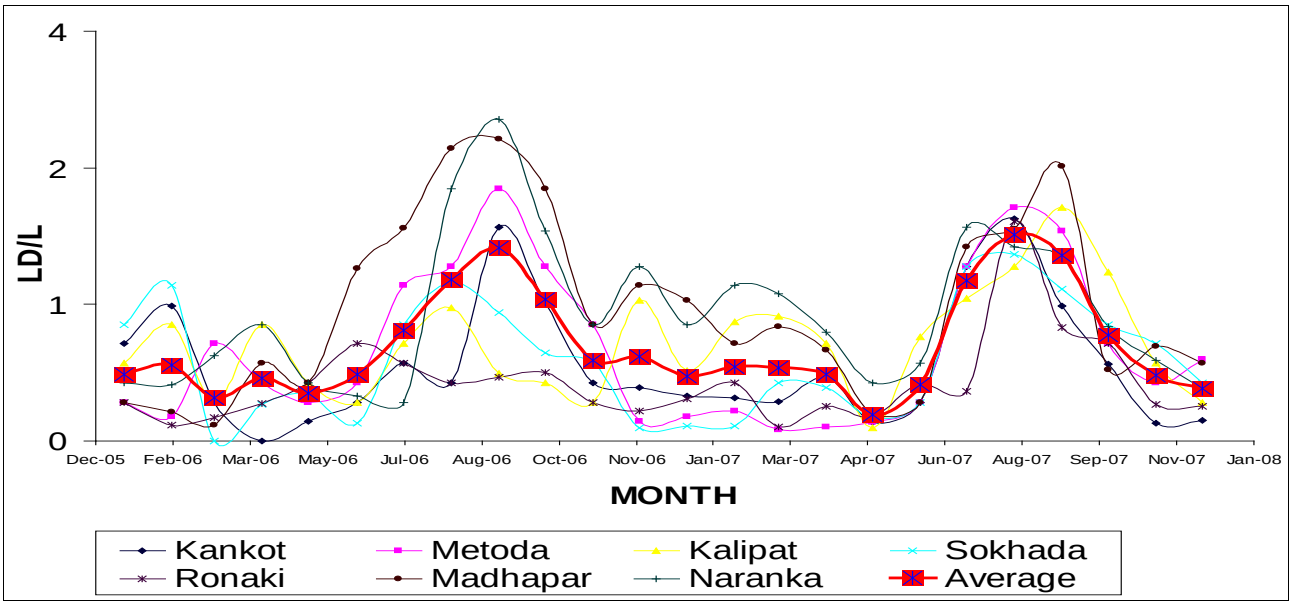

Fig 2: Month wise outskirt larval density (LD) per litre of sample water of mosquito *Anopheles stephensi* of villages surveyed around Rajkot city during 2006-07(n=24/village/month).

Larval density- domestic area

Domestic habitat was also recorded maximum average larval density of *Anopheles stephensi* during September in both years (2006-07), while the lowest was recorded during May-06 (0.14) and Dec- (0.14). The density was recorded minimum (Fig.3) Village-wise results show that the Naranka and Metoda villages have the maximum average larval density of the species was recorded in the months of

sept-06 and spet-07 while village Kankot, Metoda and Naraka have a minimum average larval density of the species that was recorded in the month of May in both years (2006-07). (Fig.3)

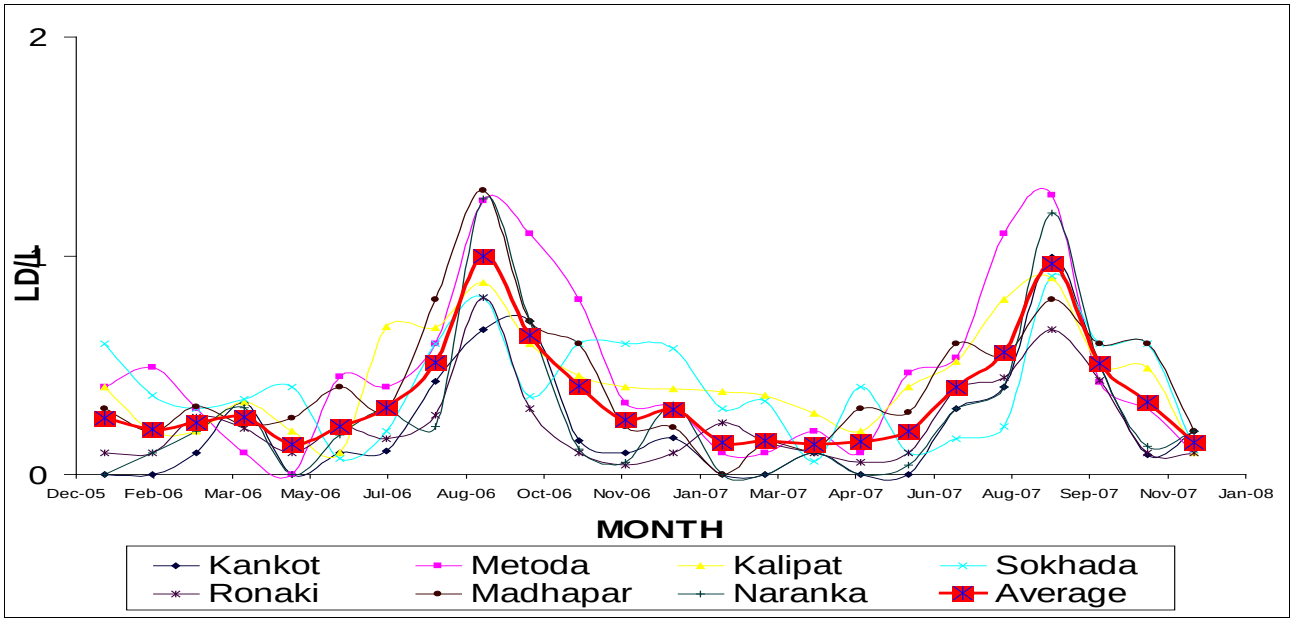

Fig 3: Month wise larval density (LD) per litre of sample water stored in tanks for domestic use of mosquito *Anopheles stephensi* of villages surveyed around Rajkot city during 2006-07(n=40/village/month).

Adult density (MHD): Results of the *Anopheles stephensi* average adult density was maximum recorded during in the month of Sept-06 and Sept-07 (03.62) and (03.40) respectively (Fig.4) and during this month total rainfall was 13.40cm and 4.10cm and average temperature was 28 °C and 28 °C respectively (Fig.5) and density was minimum recorded during May in both the years (2006-07) (00.91) (00.80) (Figure-3). During this month, the average temperature was 33 °C and 34 °C and with no rainfall. (Fig.5) Whereas the village-wise maximum average adult density of *Anopheles stephensi* was recorded from Metoda in the month of August of both years, while minimum density was observed in Kankot and Naranka village in the month of May in the years 2006 -07(00.60) (00.40). (Fig.4)

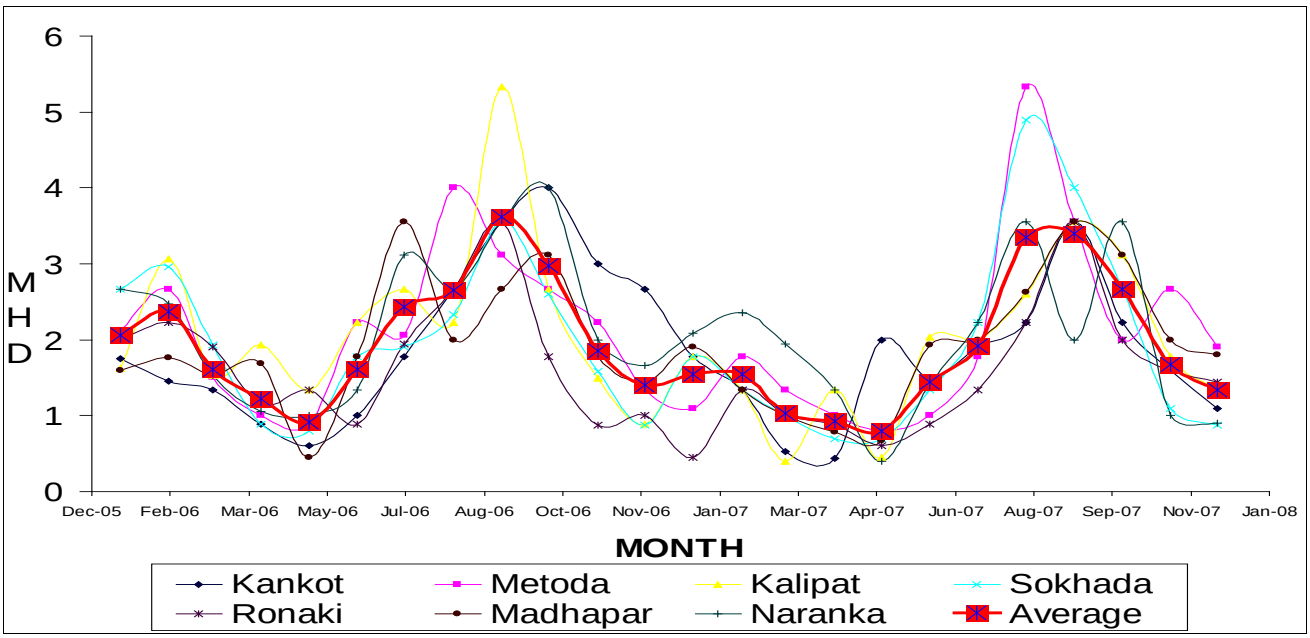

Fig 4: Month wise adult MHD (Man hour density) of *Anopheles stephensi* of villages surveyed around Rajkot city during 2006-07(n=9/village/month).

Certain factors have been affecting the mosquito's distribution and prevalence, like season, temperature, water table, and environmental conditions; therefore, we have compared the average adult density with rainfall and temperature in the present study. Therefore, it was concluded from the study results that the seasonal conditions and environmental factors positively affect the adult mosquito density.

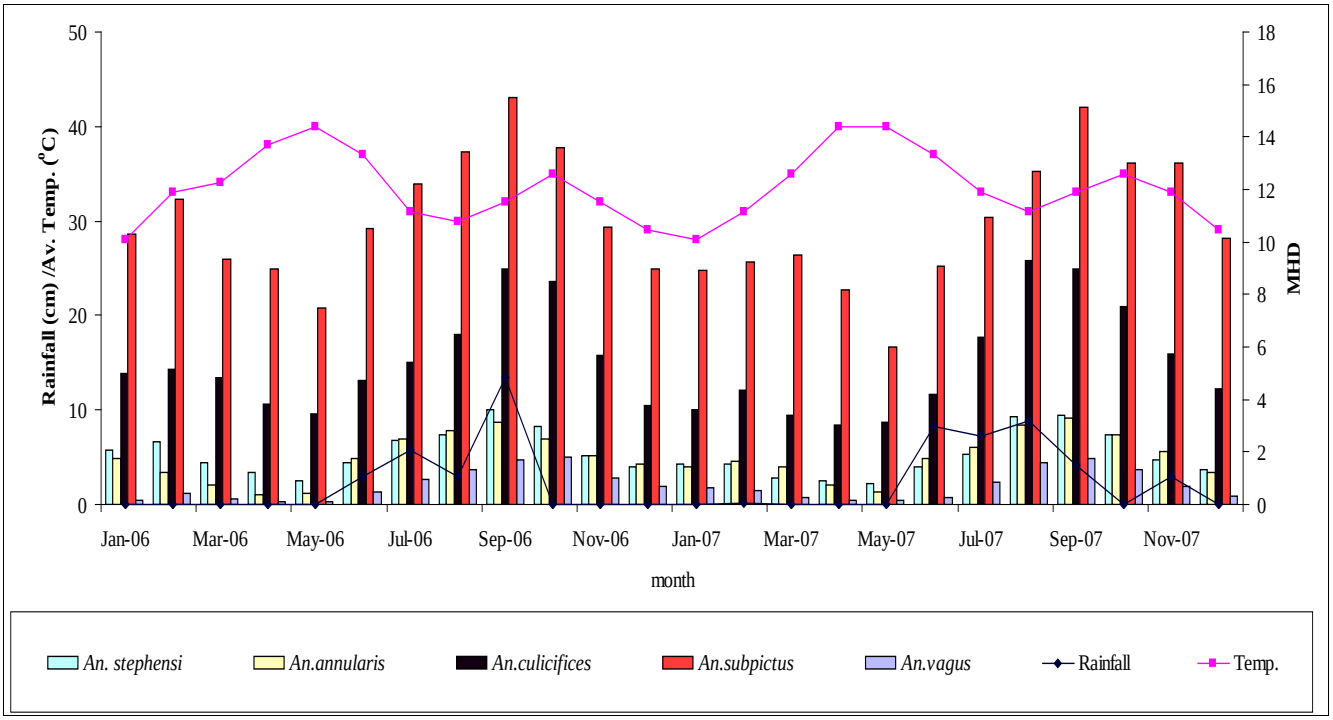

Fig 5: Month wise adult MHD (Man hour density) of Anopheles species / Total Rainfall (cm) / Average Temp. (ºC) of in the rural area around Rajkot city during 2006-07.

Discussion

The present study results clearly show that the micro habitats (all village areas) provide ideal breeding conditions for the *Anopheles stephensi*. One of the important observations made during the study was that the water storage tanks built under the staircase provided an ideal environment for mosquitoes to breed, due to the low sunlight penetration and the controlled conditions. (Michael *et al*, Mittal *et al*, 2003) [17]. Aquatic vegetation in larval habitats may also affect the abundance of mosquito larvae by providing protection (Rajnikant *et al.*, 1996) [20], while during the present study, we have also observed that the mosquito larvae were found more in the peripheral regions having vegetation. Many studies suggest and record that environmental factors like rainfall and temperature are considered important factors for the breeding of mosquitoes and for disease transmission. (Alicia *et al.*, Joseph *et al.*, 2004, Pemola and Jauhari, 2006. Viroj *et al.*, 2006) [32, 19, 26] and our present study results were also recorded similarly.

The dependency of larval density and adult mosquitoes is upon the availability of breeding grounds and resting habitats in the area. (Yadav *et al.*1989) [31] The higher the number of breeding and resting sites, are more the density and mosquitoes and vice versa. For example, more human-populated areas mean that they provide additional blood meal opportunities and more breeding habitats (Batra *et al.*, 1995; Joseph *et al.*, 2004) [32, 2].

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Author's Contribution

Suresh Chovatiya conceived the idea and initially wrote the manuscript. Dhiraj R. Parmar and Mukeshkumar Mori extended the initial manuscript and formatted it according to the needs of the topic under discussion. V. C. Soni has provided Continuous Guidance and Support.

Declarations

Competing interests: the authors have no competing interests to declare that are relevant to the content of this article.

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Data Availability

All data underlying the results are available as part of the article, and no additional source data are required.

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