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Abhishek Gupta
Department of Zoology, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India

Alka Rani
ICMR National Institute of Malaria Research, Sector-8, Dwarka, New Delhi, India

Anushrita
Saphin Consulting LLC, USA

BN Nagpal
World Health Organisation, Country Office for India, New Delhi, India

Corresponding Author:
Abhishek Gupta
Department of Zoology, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India

Effect of changing climatic variables on abundance of *Anopheles* mosquitoes (Diptera: Culicidae) in urbanizing Ghaziabad district, Uttar Pradesh, India

Abhishek Gupta, Alka Rani, Anushrita and BN Nagpal

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Abstract
Climatic variables like temperature, rainfall and humidity plays an important role in dynamics of mosquitoes. But the effect of climate change during urbanization on Anopheles mosquitoes has not been studied. The objective of present study was to explore the effects through the changes in climate variables of urbanizing Ghaziabad District, India on malaria vector *Anopheles* sp., particularly *Anopheles stephensi* (Liston) and *An. culicifacies* sensu lato (Diptera: Culicidae). The study was conducted in urban, rural and peri-urban parts seasonally from April 2014 to October 2016. Association between climate variables, malaria and Anopheles abundance was studied through Pearson's correlation and Principal Component Analysis (PCA). Results showed influence of rainfall, humidity and temperature on *An. culicifacies* and *An. stephensi. An.culicifacies* found to be highly associated with rainfall. Due to low rainfall their abundance (lesser density) has decreased in rural and peri-urban areas. Pollution in natural water bodies has further decreased their density. In urban and peri-urban areas rainfall has lesser influence but provides breeding sites to *An. stephensi.* This species breeds throughout the year easily and hence increased in all areas. Humidity supports both the vectors. The role of temperature was found to be uncertain, although minimum temperature found to influence vector density as indicated by cluster analysis in the present study.

Keywords: Climate changes, urbanization, malaria vector, *Anopheles culicifacies*, *Anopheles stephensi*

Introduction
Mosquitoes have been evolving progressively with the changes in surrounding mainly due to anthropogenic modifications during urbanization process [1, 2]. Mosquitoes belonging to the genera *Anopheles* include many species as vectors of mosquito-borne disease, are most invasive and adapted to man-made environments [3, 4]. Primary vectors reported in India are *An. culicifacies, An. stephensi, An. minimus, An. fluviatis, An. sundaicus* and *An. baimaii. An. annularis and An. nigerrimus* are secondary vectors while *An. subpictus* is a non-vector [5]. *An. culicifacies* is a vector of malaria which is responsible for transmitting *Plasmodium* parasite in rural and peri-urban India causing large number of cases and deaths [6]. It also has become resistant to many insecticides including DDT and Pyrethroid [7, 8, 9, 10]. *An. stephensi* is a vector of urban India causing malaria in cities. It has become resistant to various insecticides and is also expanding its range as reported in India and other parts of world [11, 12, 13, 14, 15]. Recently, non-vector *An. subpictus* reported of having *Plasmodium* sp. infection thus acting as a vector of malaria in few states of India [16, 17, 18, 19].

Besides the human-induced transformations, climate changes have also become major factor in different adaptations for mosquitoes [20]. Vector abundance and their presence, human-vector contact and, ultimately, malaria intensity is associated with the ecosystem. The abundance of Anophelines is the most common entomological measure used to describe the relationship between vectors and the incidence of malaria. Several studies have shown that climate change influences the pattern of malaria by affecting *Anopheles* mosquito life cycle as well as *Plasmodium* parasite [21].

It has been proven that there is reduced sporogony of *Plasmodium* parasite in *Anopheles* mosquito with a rise in temperature from 20 °C to 25 °C [22]. Moreover, with a rise in temperature from 32 °C to 39 °C, excessive mortality among mosquitoes is reported in India [23].

An understanding of factors that influence the abundance and diversity of Anopheline species in the Ghaziabad District of India provides an opportunity to better understand the dynamics of malaria transmission in different ecosystems. Ghaziabad is a rapidly urbanizing district of Uttar Pradesh with *An. culicifacies* and *An. stephensi* as the vectors of malaria. As per ISRO, the urban built up has increased from 47% to 67% while agricultural and rural land has decreased from 45% to 31% [13]. The present study has attempted to understand the association of Anopheles Vectors *An. culicifacies* and *An. stephensi* with climate change in urbanizing Ghaziabad District, India. This study highlighted the influence of climate change on Anopheles in its breeding, distribution and abundance. This will be helpful in designing appropriate malaria control strategies.

Materials and methods

Study area and data acquisition

Ghaziabad District has recently witnessed urbanization with high density of people. It lies at the latitude 28°40'north and longitude 77°25'east. It is a dry area with a rainy season from June to September, having an average rainfall of 732 mm and an average temperature of 20 ºC to 42 ºC. This district contains rural, urban and peri-urban parts (as per census characteristics) thus can be compared for the urbanization, Anopheles species and climate in a small region [24]. Three weather variables were utilized here i.e. average rainfall, temperature (maximum, minimum and average) and humidity. Monthly rainfall data was obtained from Indian Meteorological Department (IMD) [25], India from 2014 to 2016, while temperature and relative humidity data was extracted from World Weather Online for each day and transformed to month data from 2014 to 2016. Humidity from 2014 to 2016 was converted to daily (as an average of eight values), monthly and yearly data from World Weather Online [26]. Malaria cases data was obtained from Primary Health Centres of Ghaziabad District.

Mosquito collection

Anopheles mosquitoes were collected from rural, urban and peri-urban parts of Ghaziabad District from April 2014 to October 2016 (Figure 1). The collection was made for 6 days in each season i.e. pre-monsoon, monsoon and post monsoon. The habitats were identified and investigated for presence or absence of larvae. For huge water bodies like rivers and ponds, the presence of *Anopheles* was determined after taking 5-10 dips at different spots. For small water bodies about 15 dips were taken, while for suspected containers 5 dips were taken. Pipette was used for collection of larvae from very small containers. Indoor resting adult Anopheles mosquitoes were collected from human dwellings, cattle-sheds, and mixed dwellings using hand catch collection method. Man hour density of collected Anopheles mosquitoes was measured for finding abundance of each species. Adult Anopheles mosquitoes and emerged adults were identified to respective species under a compound microscope using keys of Nagpal and Kalra (1997) [27].

$$\text{MHD} = \frac{\text{No. of mosquitoes collected}}{\text{Time spent in minutes}} \text{X Number of persons involved in the collection}$$

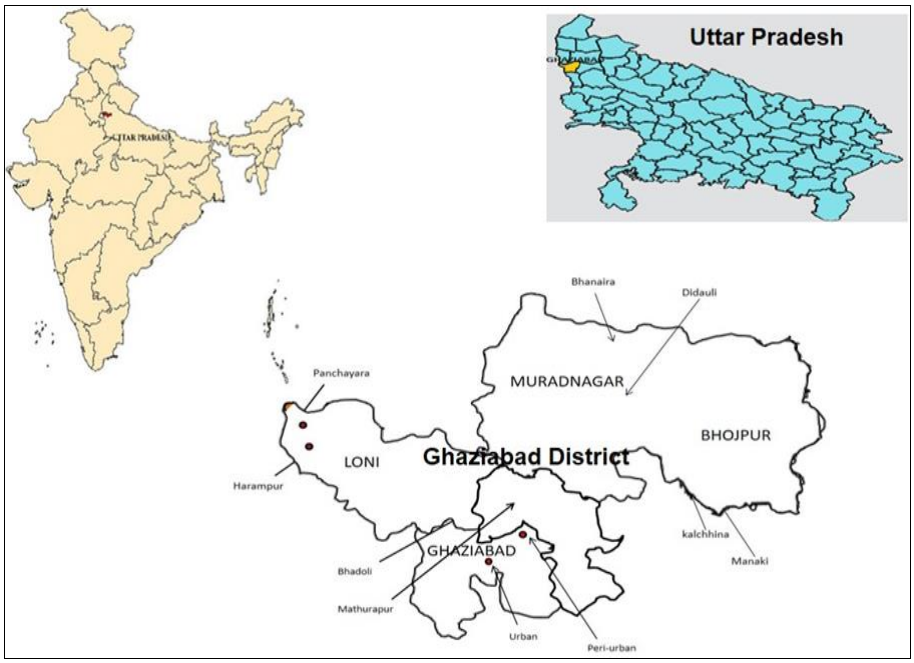

Fig 1: Map showing sites selected for collection of Anopheles mosquitoes

Urban = Ghaziabad PHC (Vaishali: residential, Red mall: commercial, Nandi Park: cattleshed)

Rural=Loni PHC (Panchayara and Harampur villages), Muradnagar (Bhanaira and Didauli villages), Razapur PHC (Bhadoli and Mathurapur villages), Bhojpur PHC (Manaki and Kalchhina villages)

Peri-urban=Ghaziabad PHC (Duhai and Sadarpur villages)

Statistical analyses

The association between monthly malaria cases, climatic variables and Anopheles abundance was studied graphically. Malaria vectors *An. culicifacies* and *An. stephensi* of Ghaziabad were examined with climatic variables. These were investigated for normality using Shapiro-Wilk test at alpha =0.05% and transformed when necessary. Descriptive statistics was generated for variables of urbanization and Anopheles vector (means, standard error, standard deviations at 95% confidence interval) using Microsoft Excel 2013 (15.0.5172.1000), where $p \leq 1$ was used at every step to find statistical significance. For the association of malaria vectors (mosquito breeding sites and adult abundance) with

meteorological variables Pearson's correlation analysis was conducted on data for the period of 2014 to 2016. Hierarchical Cluster Analysis was conducted on the relationship between meteorological variables like maximum temperature, minimum temperature, average temperature, average rainfall and humidity with Anopheles abundance. Comparison of adult Anopheles abundance with climatic variables was done to find any association using Principal Component Analysis. As per Shapiro-Wilk test, all data including Anopheles abundance, climate variables and cases shows normal distribution at alpha = 0.05% with p-value ranging from 0.01 to 1 at 95% confidence interval except *P. falciparum* data (Supplementary table 1).

Results

Habitat diversity and mosquito collection

Breeding of anopheles in diverse habitats in urbanizing study area

Habitats in selected study sites were grouped into eleven types which includes drains, tanks, containers, OHTs, tube-wells, rivers, canals, pools, pits, rice fields and miscellaneous. Highest number of habitats were artificial type in urban in all seasons followed by peri-urban and rural (Figure 2). About 19.27 of habitats were found positive with *Anopheles* mosquito breeding. Tanks (48.26%) and pits 41.97% were most popular breeding sites among all study sites and simultaneously contributes 15.2% to the positive breeding sites (supplementary table 2). Pits with maximum positivity (of Anopheles) in urban as water-logged areas and rural have pits in deserted areas, and construction sites of brick-kilns and houses. The pits present in peri-urban were mostly polluted. Tanks in urban area were mainly found as underground tanks in recreational areas (Mahamaya stadium) and in residential areas (Vaishali and Kavi Nagar). Curing tanks in urban were identified in Kavi nagar colony and nurseries and rarely in Viklang colony. Rural has mainly curing tanks as positive habitats in Panchayara (Loni PHC), Manaki and Kalchhina villages (Bhojpur PHC) and Mathurapur (Razapur PHC). In peri-urban some underground tanks were inaccessible, but those checked had breeding of Anopheles. Rural has breeding of Anopheles in canals mainly with low density in rivers and ponds (sometimes if unpolluted). Other habitats were drains, containers, OHTs, tube-wells, rice fields (in rural only) and miscellaneous like a swimming pool, discards, pots etc. (Figure 3).

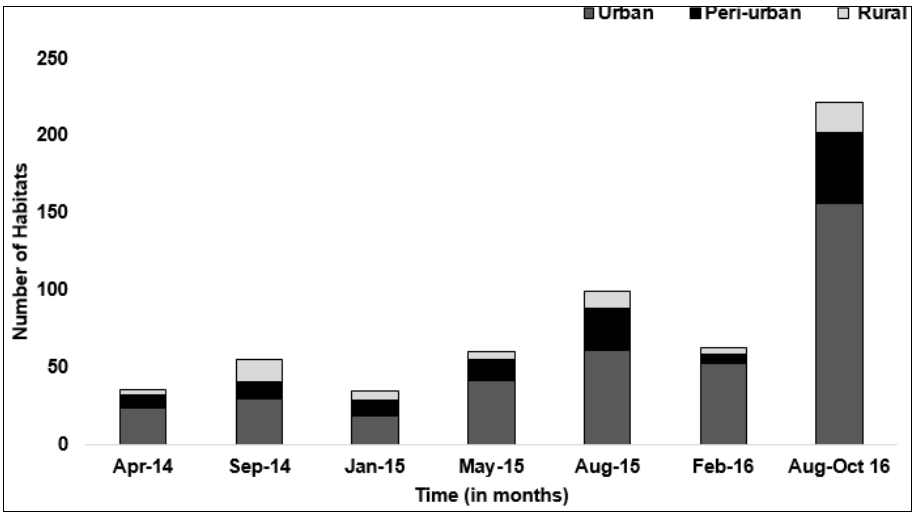

Fig 2: Diversity of habitats during collection of Anopheles mosquito from April 2014-October 2016 (March-May=Pre monsoon season, June-September=Monsoon Season, October-Feb=Post Monsoon Season)

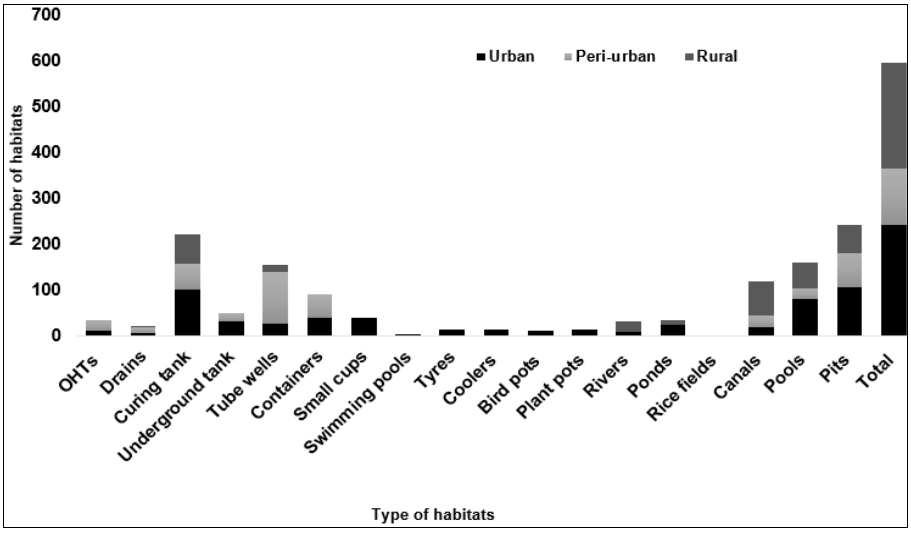

Fig 3: Breeding habitats in Ghaziabad district (rural, urban and peri-urban) with *Anopheles* breeding

During study period, major Anopheles species in Ghaziabad district were *An. culicifacies*, *An. annularis*, *An. subpictus*, and *An. stephensi*. *An. culicifacies* earlier reported as a primary vector of malaria in Ghaziabad used to breed in all

natural water bodies of rural. Most of these water bodies were polluted (rivers and ponds) with organic waste, hence during surveillance their breeding sites were pits, pools and canals. This rural vector during study was breeding only in all natural water bodies without pollution with maximum contribution of canals and pits. *An. stephensi* an urban malaria vector earlier reported to be in very low density in urban only breeding in man-made habitats like tanks, containers, etc. During study, it was found breeding in all parts (rural, urban and peri-urban) of Ghaziabad in tanks and drums. *An. subpictus* was the major breeder in polluted ponds, pools and pits as well as muddy water of brick kilns and pits of construction sites. Other *Anopheles* species also found breeding were *An. pulcherrimus* (rice fields), *An. nigerrimus* (polluted pits), and *An. annularis* (ponds).

Anopheles abundance

Total *Anopheles* mosquitoes collected during study i.e. 2014-2016 were 1701 (excluding insectary reared mosquitoes). Of the total *Anopheles* collected *An. subpictus* was the maximum in abundance (50.5%) followed by *An. annularis* (19.5%), *An. stephensi* (15.7%) and *An. culicifacies* (13.52%). *An. culicifacies* contributed 7.2% in rural, 3.63% in urban and 2.52% in peri-urban. Among malaria vectors *An. stephensi* was the most abundant. *An. subpictus* was higher in rural in 2014-15 and 2015-16 and *An. annularis* was followed by *An. subpictus* while highest in rural in 2016. *An. subpictus* in peri-urban is next in contribution to density after rural followed by urban. *An. subpictus* is recently found to support in malaria cases in this district although it was deemed as a non-vector earlier while *An. annularis* is a secondary vector. Although *An. culicifacies* and *An. stephensi* have very low density throughout yet, they contributed to maximum cases of malaria in Ghaziabad hence are the main vectors. In rural, *An. stephensi* was predominant during dry (November to June) as well as wet season (July-October) during whole study period in tanks, containers, drums etc. *An. culicifacies* has higher density preceding rains, although rarely in pools, pits, ponds, rivers and canals in rural; pools and pits in peri-urban and urban (Figure 4).

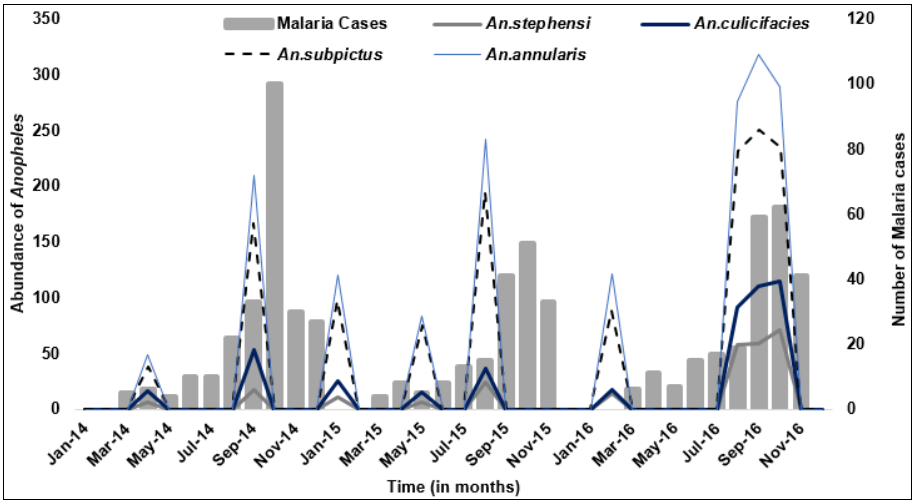

Fig 4: Abundance of *Anopheles* collected from Ghaziabad in urban, peri-urban and rural

Climate Change, malaria cases and mosquito abundance during 2014-2016

Malaria cases in PHCs of Ghaziabad district were compared with climate change. It was found that Ghaziabad PHC contributes to malaria cases whole year regardless of rainfall (except winters with temperature lower than 25 °C). Urban and peri-urban PHC of Ghaziabad has Anopheles breeding in man-made breeding sites, largely. Other PHCs (rural) contributed only during monsoon every year during survey. In 2014, Bhojpur and Loni has malaria cases during February to March which is due to rains during that year from January to March creating breeding sites for Anopheles (Figure 5).

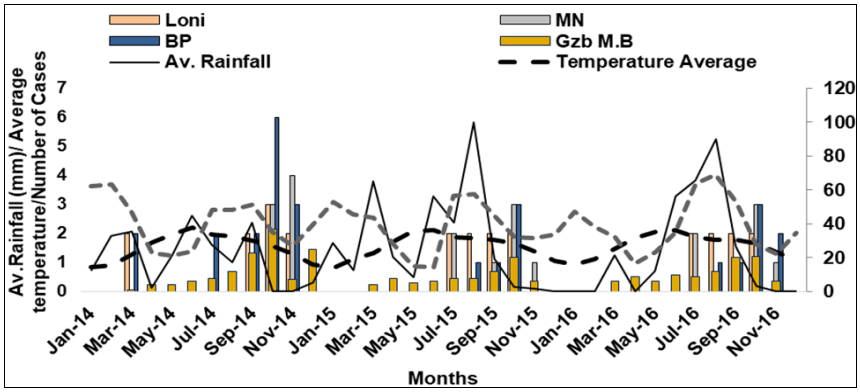

Fig 5: Association of Climate variables with malaria cases in different primary health centres of Ghaziabad District (MN=Muradnagar, BP=Bhojpur)

An. culicifacies and *An. stephensi* were plotted with meteorological data (humidity, Average temperature and Average rainfall) to find relationship between these variables. *An. stephensi* abundance is supported by humidity (Figure 6A). This vector is present even if there is no rainfall and their density becomes even higher with rains, hence showing

relationship with rainfall (Figure 6B). *An. culicifacies* is influenced by humidity and completely depends on rainfall for breeding (Figure 6A and 6B).

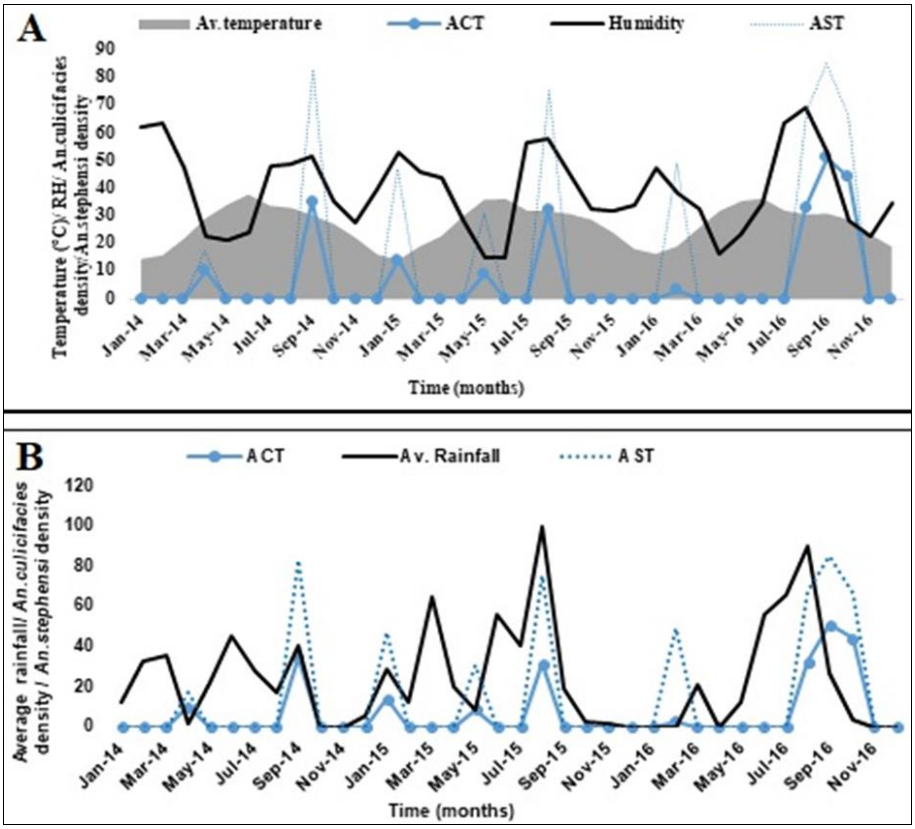

Fig 6: (A-B) Monthly collection of *Anopheles* vectors (ACT = *An. culicifacies* Total, AST = *An. stephensi* Total) in relation to average temperature (Source: WWO 2014-2016), Humidity (Source: WWO 2014-2016) and Average rainfall (Source: IMD 2014-2016) for the study period (2014-2016) in Ghaziabad District

Statistical analysis for relationship of climate with *Anopheles* mosquito

Correlation analysis

Table 1 shows Pearson's correlation analysis of *Anopheles* vector of Ghaziabad with meteorological variables. Correlation analysis shows *An. culicifacies* to be influenced by rainfall and humidity positively in Ghaziabad, although their impact was low as other factors are also involved. Average rainfall and humidity influence this vector in rural (humidity = 0.308, p = 0.034, average rainfall = 0.376, p = 0.012) and peri-urban (Humidity, r = 0.250, p = 0.071, average rainfall, r = 0.377, p = 0.012) but not in urban. Overall in Ghaziabad district *An. culicifacies* shows positive correlation of r = 0.277, p = 0.051 with humidity and r = 0.310, p=0.033 with average rainfall. *An. stephensi* also shows positive correlation with humidity (r = 0.255, p = 0.067) and average rainfall (r = 0.256, p=.066) in rural while in urban and peri-urban this vector although shows positive correlation (although less with average rainfall r = 0.205, p=.116 in peri-urban and humidity r=0.173, p = 0.156 in urban). Overall this urban vector in Ghaziabad district is positively correlated with average rainfall (r=0.245, p=0.075) and humidity (r=0.246, p=.074). *An. culicifacies* shows positive correlation with natural breeding sites (r=0.260, p>1) while negative correlation with artificial breeding sites (r=-0.220, *p*>0.01). *An. stephensi* shows positive correlation with artificial breeding sites (r=0.420, *p*>0.01) but no correlation with natural breeding sites. Temperature shows very low positive correlation hence requires further study to find its influence on *Anopheles*. This analysis showed anopheles vectors in Ghaziabad are more influenced by average rainfall, humidity.

Table 1: Correlation analysis to find relationship between climate variables with abundance of *Anopheles* and breeding sites.

Variable	Malaria cases	Temp Max	Temp Min	Temp Average	Humidity	Average Rainfall	Natural	Artificial
ACR	.287* (.045)	.120 (.243)	.194 (.128)	.161 (.174)	.308* (.034)	.376* (.012)	NA	NA
ACP	.230 (.089)	.147 (.195)	.199 (.122)	.176 (.152)	.250 (.071)	.377* (.012)	NA	NA
ACU	.442** (.003)	.112 (.258)	.163 (.171)	.140 (.207)	.188 (.137)	.118 (.247)	NA	NA
ACT	.356* (.017)	.132 (.222)	.199 (.123)	.168 (.163)	.277 (.051)	.310* (.033)	0.260	-0.220
ASR	.358* (.016)	.083 (.316)	.152 (.188)	.120 (.242)	.255 (.067)	.256 (.066)	NA	NA
ASP	.406** (.007)	.091 (.299)	.151 (.189)	.124 (.236)	.219 (.100)	.178 (.149)	NA	NA
ASU	.267 (.058)	.123 (.237)	.151 (.189)	.139 (.209)	.173 (.156)	.205 (.116)	NA	NA
AST	.366* (.014)	.095 (.291)	.159 (.177)	.130 (.225)	.246 (.074)	.245 (.075)	-0.076	0.420

Where ACR = *An. culicifacies* rural, ACP = *An. culicifacies* peri-urban, ACU = *An. culicifacies* urban, ACT=*An. culicifacies* total, ASR = *An. stephensi* rural, ASP = *An. stephensi* peri-urban, ASU = *An. stephensi* urban, AST = *An. stephensi* total, NA= Data not applied, Temp=Temperature, Max=Maximum, Min=Minimum, Figures in parenthesis indicates p value.

Hierarchical Analysis

For further interpretation of relationship between climate variables and *Anopheles* vector hierarchical analysis was done. The factors taken were temperature (maximum, minimum and average), humidity, rainfall (average), and abundance of *Anopheles* vector (*An. culicifacies* and *An. stephensi*). The data was recorded for two years (January 2014 to November 2016) for different season i.e. pre-monsoon, monsoon and post monsoon. The technique of cluster analysis is a multivariate analysis which is used to classify the factors or variables into clusters of groups possessing high homogeneity level within each cluster and high level of heterogeneity between the clusters.

The relationship of *Anopheles* vector species with different climatic factors were determined using cluster analysis and the results are expressed using a dendrogram as indicated in figure 7. The dendrogram displays a picture of the clusters of months based on the breeding sites data of *Anopheles* vector species. The months which have linkage closer to each other mark a stronger relationship between sample/variables or cluster of sampling site/variable. Here wet months (June to September) were in cluster 2 and dry months (October to May) i.e. pre-monsoon and post-monsoon in cluster 1. The further analysis of the clusters is done using principal component analysis as discussed in the next section.

Principal component analysis (PCA)

This method is frequently used as a method to extract smaller set of factors. In the present analysis our purpose is to extract the factors which are responsible for causing malaria in a given time period i.e. 2014 to 2016. Criteria having an eigenvalue of 1.0 or more were considered significant while the criteria having the highest eigenvalues were the most significant. The scree test is illustrated in supplementary figure 1. Table 2 implied the presence of three factors which accounts for approximately 87.5% of total variance. Hence, three components were extracted from the data. The communalities given in table 3 explains the proportion of each factors variance by the retained factors. We have utilized varimax rotation, which is basically an orthogonal rotation method that minimizes the number of variables that have high loading on each factor. The criteria having correlation more than 0.75 are considered as strong and indicate high proportion of its variance explained by the criteria, between 0.50 and 0.75 is considered as moderate loading while 0.30-0.50 as weak significant factor loading, indicating much of that attribute's variance remains unexplained and it is less important. We did hierarchical analysis to find the factor which has highest influence on *Anopheles* vector for each cluster. The three extracted principal factors are revealed in Table 4. The criteria having highest factor loading are highlighted for each component. It can be seen that for component 1 we have Malaria Case, ACR, ACP, ASR, ASP, and ASU. In case of component 2, the criteria, the highest factor loading is by Temp Max, Temp Min and Temp Average. Humidity and Average Rainfall form the part of component 3.

Analysis for Cluster 2

Similar steps are performed for cluster 2. It can be seen from the scree plot given in figure 8, that we have three factors in this cluster. Here, we also extracted three factors from the data. The Table 5 elaborates upon the variance explained by each factors along with the cumulative variance explained by all factors. It is seen that the third factor constitutes 86.54% of the variance. The communalities given in table 6 explains the proportion of each factors variance by the retained factors. Table 7 gives the rotated component matrix of the data where three extracted principal factors are shown. It can be seen that for component 1 we have Humidity, Average rainfall, ACU, ASR, ASP. In case of component 2, the criteria, the highest factor loading is by Temp Max, Temp Min and Temp Average. Malaria case, ACR, ACP and ASU form the part of component 3. All Factors plotted in rotated factor space is presented in figure 8.

Table 2: Variance table for cluster 1

S. No.	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	5.978	49.813	49.813	5.978	49.813	49.813	5.697	47.476	47.476
2	3.286	27.380	77.192	3.286	27.380	77.192	3.429	28.578	76.054
3	1.242	10.346	87.538	1.242	10.346	87.538	1.378	11.484	87.538
4	.820	6.834	94.373						
5	.195	1.627	95.999						
6	.169	1.408	97.408						
7	.136	1.137	98.544						
8	.095	.793	99.337						
9	.065	.540	99.877						
10	.012	.104	99.981						
11	.002	.018	99.999						
12	.000	.001	100.000						

Extraction Method: Principal Component Analysis

Table 3: Communalities for Cluster 1

Communalities	Initial	Extraction
Malaria Cases	1.000	.414
Temperature Maximum	1.000	.906
Temperature Minimum	1.000	.949
Temperature Average	1.000	.968
Humidity	1.000	.841
Average Rainfall	1.000	.950

ACR	1.000	.942
ACP	1.000	.838
ACU	1.000	.943
ASR	1.000	.948
ASP	1.000	.975
ASU	1.000	.830

Extraction Method: Principal Component Analysis.
Note: ACR =*An. culicifacies* rural, ACP =*An. culicifacies* peri-urban, ACU =*An. culicifacies* urban, ASR =*An. stephensi* rural, ASP =*An. stephensi* peri-urban ASU =*An. stephensi* urban

Table 4: Rotated Component Matrix for Cluster 1

	Component		
	1	**2**	**3**
Malaria Cases	.429	.232	-.419
Temperature Maximum	.114	.945	-.028
Temperature Minimum	.173	.959	-.003
Temperature Average	.149	.973	.000
Humidity	.158	-.760	.488
Average Rainfall	.109	-.016	.968
ACR	.962	.078	.107
ACP	.909	.108	-.013
ACU	.967	.085	.039
ASR	.972	.042	-.045
ASP	.983	.066	.075
ASU	.903	.093	-.075

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
Rotation converged in 4 iterations.
Note: ACR =*An. culicifacies* rural, ACP =*An. culicifacies* peri-urban, ACU =*An. culicifacies* urban, ASR =*An. stephensi* rural, ASP =*An. stephensi* peri-urban ASU =*An. stephensi* urban

Table 5: Variance table for cluster 2

Total Variance Explained									
S. No.	**Initial Eigenvalues**			**Extraction Sums of Squared Loadings**			**Rotation Sums of Squared Loadings**		
	Total	**% of Variance**	**Cumulative %**	**Total**	**% of Variance**	**Cumulative %**	**Total**	**% of Variance**	**Cumulative %**
1	6.233	51.938	51.938	6.233	51.938	51.938	3.733	31.111	31.111
2	2.679	22.326	74.265	2.679	22.326	74.265	3.481	29.007	60.118
3	1.474	12.280	86.545	1.474	12.280	86.545	3.171	26.427	86.545
4	.923	7.688	94.234						
5	.527	4.394	98.628						
6	.130	1.079	99.707						
7	.031	.258	99.965						
8	.004	.035	100.000						

Extraction Method: Principal Component Analysis

Table 6: Communalities for Cluster 2

Component	**Initial**	**Extraction**
Malaria Cases	1.000	.837
Temperature Maximum	1.000	.980
Temperature Minimum	1.000	.910
Temperature Average	1.000	.991
Humidity	1.000	.537
Average Rainfall	1.000	.678
ACR	1.000	.952
ACP	1.000	.789
ACU	1.000	.860
ASR	1.000	.939
ASP	1.000	.971
ASU	1.000	.941

Extraction Method: Principal Component Analysis
Note: ACR=*An. culicifacies s.l* rural, ACP= *An. culicifacies s.l* peri-urban, ACU=*An. culicifacies s.l* urban, ASR= *An. stephensi* L rural, ASP=*An. stephensi L* peri-urban ASU= *An. stephensi L* urban

Table 7: Rotated Component Matrix for Cluster 2

Rotated Component Matrix[a]			
	Component		
	1	2	3
Malaria Cases	-.037	.913	.056
Temperature Maximum	-.228	-.090	.959
Temperature Minimum	.020	-.013	.954
Temperature Average	-.102	-.052	.989
Humidity	.389	.376	-.495
Average Rainfall	.801	.003	-.191
ACR	.508	.821	-.141
ACP	.371	.797	-.129
ACU	.825	.414	-.094
ASR	.935	.245	-.068
ASP	.893	.406	-.091
ASU	.358	.889	-.148

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
a. Rotation converged in 5 iterations.
Note: ACR=*An. culicifacies* rural, ACP=*An. culicifacies* peri-urban, ACU=*An. culicifacies* urban, ASR=*An. stephensi* rural, ASP=*An. stephensi* peri-urban ASU=*An. stephensi* urban

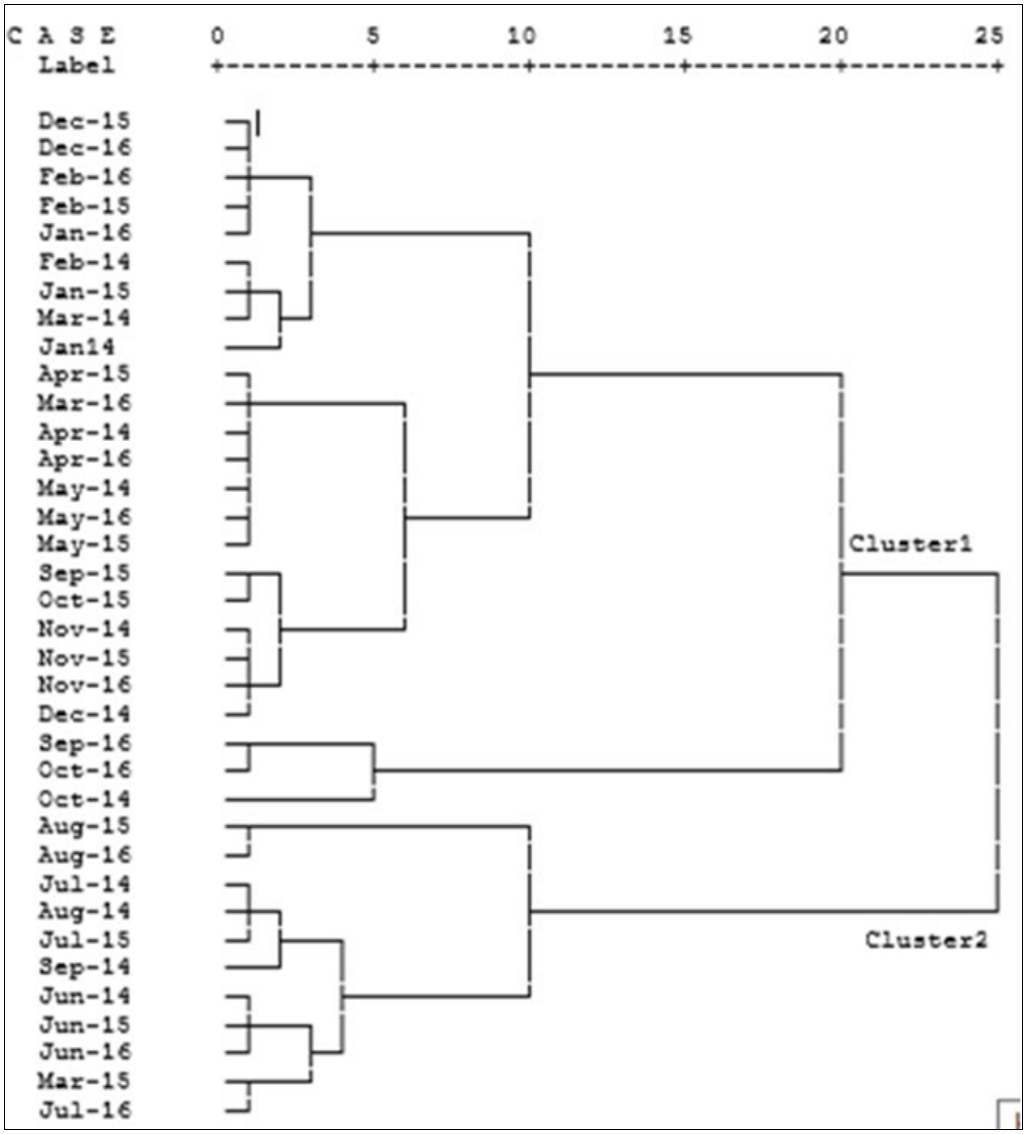

Fig 7: Dendogram showing cluster 1 and cluster 2

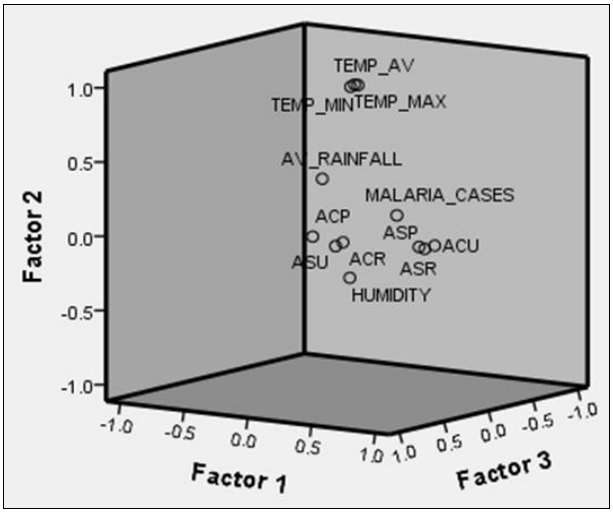

Fig 8: Factor plot in rotated factor space

Discussion

The climate variability played an important role in supporting breeding and distribution of Anopheles vectors and hence malaria. Meteorological records from 2005 to 2016 shows that climate warming has taken place in Ghaziabad with a rise in temperature and humidity while reduction in rainfall. Average rainfall in last 11 years has dropped while average temperature has increased. The average temperature had increased from 19.89 °C in 2005 to 26.8 °C in 2016, while average rainfall had declined from 175.9 mm in 2005 to 60.7 mm 2016. The malaria cases have been fluctuating from 931 in 2005 to 20 in 2021, which shows an overall reduction (supplementary data fig 3B). The reason being anthropogenic activities in urbanizing Ghaziabad with a decrease in agricultural land (supplementary figure 3A) and increase in construction activities [28].

Peaks of Anopheline abundance can be observed preceding rainfall by one to two months while humidity coincides. Temperature shows peaks two to three months preceding to peak abundance of Anopheles, hence it may be affecting Anopheles (Figure 6). Many studies confirmed that urbanization tends to increase in breeding sites for mosquitoes due to various reasons as shown in supplementary figure 3B [29, 30, 31, 32]. Ghaziabad District is rich in natural water bodies used to serve as the breeding sites for rural vector *An. culicifacies*. In the process of urbanization, these breeding sites have become polluted. Now artificial breeding sites have ample numbers of *An. stephensi* throughout the year with low density [13]. Peaks of *An. culicifacies* and *An. stephensi* were observed during monsoon season i.e. June-September (Figure 6).

In this study, it was found that *An. stephensi* was supported by humidity and rainfall significantly by increasing their density by providing more breeding sites [13, 33]. *An. culicifacies* is highly influenced by humidity as well as rainfall (Figure 6). We did not find any significant relationship between *Anopheles* vector and temperature by correlation (Pearson's). Similarly, a study in Bangladesh demonstrated no association of temperature with mosquito density and malaria incidence as suitable temperature required for mosquito breeding and development is always present. Correlation analysis shows that *An. culicifacies* as well as *An. stephensi* influenced by rainfall and humidity positively in Ghaziabad District. These vectors when compared in all areas of Ghaziabad District were very less impacted in urban by rainfall and humidity when plotted. Although in rural and peri-urban relationship similar to correlation was found as both the vectors are highly influenced by rainfall and humidity. But a study in public province of china found that relative humidity has more influence than rainfall and temperature on malaria. *An. culicifacies* shows positive correlation with natural breeding sites while negative correlation with artificial breeding sites. *An. stephensi* shows positive correlation with artificial breeding sites but no correlation with natural breeding sites (Table 1).

Cluster analysis shows that malaria is highly influenced by minimum temperature and rainfall in both the clusters. Minimum temperature plays role in transmission of malaria by increasing continuity of *Anopheles* and malaria parasite even during cooler months. *An. stephensi* has major impact on malaria in peri-urban and urban followed by *An. culicifacies* in rural and urban in Ghaziabad District. Role of temperature on malaria cases was demonstrated by a study conducted in Jinan, northern China. According to this, both maximum and minimum temperature when increased by 1 ºC are responsible for an increase in malaria cases [34]. Rainfall plays an important role in malaria epidemiology, as in moderation it provides sites for breeding of aquatic stages of mosquitoes and also increases the relative humidity. Even though with the increase in temperature (minimum) and decrease in rainfall, *An. stephensi* which breeds in artificial sites like stored water have got an advantage over *An. culicifacies* yet breeding of both the vector is supported by rainfall with a lag of one month in Ghaziabad district. In Dehradun, rainfall and malaria show high correlation with a lag of one month like our study where malaria cases and *Anopheles* vector have moderate positive correlation with rainfall. This one-month lag period is required for the development of a new generation of female *Anopheles* with malaria parasite infection [35]. In another study in a forest-fringed village of Assam this lag period was observed to be of two-week between rainfall and vector abundance [36]. This study demonstrates that malaria vector is highly related with climate variables in a multifaceted manner. In Ghaziabad, rainfall seems to perform more important role in malaria transmission compared to temperature and humidity. Rainfall provides more breeding sites for both the vectors while humidity and temperature provides ambient conditions for their development.

Conclusion

The climatic variables influence malaria and its vectors in a complex manner. This study revealed that rainfall, relative humidity and temperature have influenced the abundance of studied Anopheles vector in urbanizing Ghaziabad District, India in urban, rural and peri-urban in different ways. However, this study also illustrated the role of rainfall and minimum temperature as the major factor yet the study on microclimate of Anopheles vectors, their control and malaria transmission dynamics is needed to assess risk factors.

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References

1. Kamdem C, Fossog BT, Simard F, Etouna J, Ndo C, Kengne P, *et al*. Anthropogenic habitat disturbance and ecological divergence between incipient species of the malaria mosquito *Anopheles gambiae*. PloS one. 2012; 7:76-82.
2. Rochlin I, Faraji A, Ninivaggi DV, Barker CM, Kilpatrick AM. Anthropogenic impacts on mosquito populations in North America over the past century. Nature Comm. 2016; 7:1-14.
3. Sharma N. Emerging and re-emerging infectious diseases in South East Asia. Indian J Derma. 2017; 62:451-456.
4. Gopalakrishnan R, Veer V. Bio ecology, Insecticide Susceptibility and Management of Culex quinquefasciatus Say, 1823: A Major Vector of Lymphatic Filariasis in India. In Lymp Filariasis. 2018; 4:199-210.
5. Subbarao SK, Nanda N, Rahi M, Raghavendra K. Biology and bionomics of malaria vectors in India: existing information and what more needs to be known for strategizing elimination of malaria. Malaria Journal. 2019;18 (1):396.
6. Sharma SN, Shukla RP, Mittal PK, Adak T, Subbarao SK. Insecticide resistance in malaria vector *Anopheles culicifacies* in some tribal districts of Chhattisgarh, India. Current Science. 2017; 23:1280-1282.
7. Singh OP, Raghavendra K, Nanda N, Mittal PK, Subbarao SK. Pyrethroid resistance in *Anopheles culicifacies* in Surat district, Gujarat, west India. Curr Sci. 2002; 12:547-550.
8. Davari B, Vatandoost H, Oshaghi MA, Ladonni H, Enayati AA, Shaeghi M, *et al*. Selection of *Anopheles stephensi* with DDT and dieldrin and cross-resistance spectrum to pyrethroids and fipronil. Pestic Biochem Phys. 2007; 89:97-103.
9. Mishra AK, Chand SK, Barik TK, Dua VK, Raghavendra K. Insecticide resistance status in *Anopheles culicifacies* in Madhya Pradesh, central India. J Vector Borne Dis. 2012; 49:39-45.
10. Raghavendra K, Barik TK, Sharma SK, Das MK, Dua VK, Pandey A, *et al*. A note on the insecticide susceptibility status of principal malaria vector *Anopheles culicifacies* in four states of India. J Vector Borne Dis. 2014; 50:230-239.
11. Martens P, Hall L. Malaria on the move: human population movement and malaria transmission. Emerg Infect Dis. 2007; 6:103-108.
12. Ramasamy R, Surendran SN. Mosquito vectors developing in atypical anthropogenic habitats: Global overview of recent observations, mechanisms and impact on disease transmission. J Vector Borne Dis. 2016; 52:91-97.
13. Rani A, Gupta A, Sinha S, Nagpal BN, Singh H, Vikram K, Saxena R. Malaria epidemiology in changing scenario and anopheles vector in Ghaziabad district, Uttar Pradesh, India. Int J Mosq Res. 2017; 28:77-75.
14. Sinka ME, Pironon S, Massey NC, Longbottom, J, Hemingway J, Moyes CL, *et al*. A new malaria vector in Africa: Predicting the expansion range of *Anopheles stephensi* and identifying the urban populations at risk. Proceedings of the National Academy of Sciences. 2020; 117 (40):24900-24908.
15. Surendran SN, Sivabalakrishnan K, Sivasingham A, Jayadas TT, Karvannan K, Santhirasegaram S, *et al*. Anthropogenic factors driving recent range expansion of the malaria vector *Anopheles stephensi*. Front. Public Health. 2019; 7:67-78.
16. Chatterjee S, Chandra G. Role of Anopheles subpictus as a primary vector of malaria in an area in India. J Trop Med Hyg. 2000; 28:177-181.
17. Kumar A, Hosmani R, Jadhav S, de Sousa T, Mohanty A, Naik M, *et al*. *Anopheles subpictus* carry human malaria parasites in an urban area of Western India and may facilitate perennial malaria transmission. Malaria J. 2016; 15:121-124.
18. Rani A, Nagpal BN, Singh H, Mehta SS, Srivastava A, Saxena R. Potential role of Anopheles subpictus as a malaria vector in Ghaziabad District, Uttar Pradesh, India. International Journal of Tropical Insect Science. 2020; 23:1-11.
19. Singh RK, Kumar G, Mittal PK, Dhiman RC. Bionomics and vector potential of Anopheles subpictus as a malaria vector in India: An overview. Int J Mosq Res. 2014; 1:29-37.
20. Roberts DR, Andre RG. Insecticide resistance issues in vector-borne disease control. Am J Trop Med Hyg. 1994; 50:21-34.
21. Becker N. Influence of climate change on mosquito development and mosquito-borne diseases in Europe. Parasitol Res. 2008; 103:19-28.
22. Dash AP, Bhatia R, Sunyoto T, Mourya DT. Emerging and re-emerging arboviral diseases in Southeast Asia. J Vector Borne Dis. 2013; 50:77-84.
23. Dhiman RC, Pahwa S, Dash AP. Climate change and malaria in India: Interplay between temperature and mosquitoes. Health for Journal. 2008; 12:27-37.
24. Indian Meteorological Department: https://mausam.imd.gov.in/imd_.php accessed on December, 2017.
25. Census of India; c1971. Available at: http://censusindia.gov.in/2011census/dchb/ghazibad, accessed on 31, October 2019.
26. World Weather online, https://www.worldweatheronline.com, accessed on December, 2017.
27. Nagpal BN, Kalra N. Malaria vectors of India. J Parasit Dis. 1997; 21:105-112.
28. Guo G, Wu Z, Xiao R, Chen Y, Liu X, Zhang X. Impacts of urban biophysical composition on land surface temperature in urban heat island clusters. Landscape and Urban Planning. 2015; 135:1-10. https: //doi.org/10.1016/j.landurbplan.2014.11.007.
29. Easton ER. Urbanization and its effects on the ecology of mosquitoes in Macau, Southeast Asia. J Am Mosquito Contr. 1994; 10:540-544.
30. Oyewole IO, Awolola TS. Impact of urbanisation on bionomics and distribution of malaria vectors in Lagos, southwestern Nigeria. J Vector Borne Dis. 2006; 43:173-179.
31. Malhotra G, Yadav A, Dudeja P. Knowledge, awareness and practices regarding dengue among rural and slum communities in North Indian city, India. Int J Med Sci Public Health. 2014; 3:295-299.
32. Li Y, Kamara F, Zhou G, Puthiyakunnon S, Li Liu Y, Zhou Y, *et al*. Urbanization increases Aedes albopictus larval habitats and accelerates mosquito development and survivorship. PLoS Neglec. Trop Dis. 2014; 8:23-34.

33. Rani A, Gupta A, Nagpal BN, Mehta SS. Mosquito borne diseases and Sanitation in Ghaziabad district, Uttar Pradesh, India. Int J Mosq Res. 2018; 5:25-30.
34. Zhang Y, Bi P, Hiller JE. Meteorological variables and malaria in a Chinese temperate city: a twenty-year time-series data analysis. Environ Int. 2010; 36:439-445.
35. Devi NP, Jauhari R. Meteorological variables and malaria cases based on 12 years data analysis in Dehradun (Uttarakhand) India. Eur J. Exp Bio. 2013; 3:28-37.
36. Prakash A, Bhattacharyya DR, Mohapatra PK, Mahanta J. Seasonal prevalence of Anopheles dirus and malaria transmission in a forest fringed village of Assam, India. Indian J Malariol. 1997; 34:117-25.