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Daisy Konwar
Research Scholar,
Department of Life-Sciences,
Dibrugah University, Dibrugarh,
Assam, India

Rimen Bordoloi
Associate Professor,
Department of Zoology, D.R.
College, Golaghat, Assam, India

Niranjan Nath
Zonal Entomologists, National
Vector Borne Disease Control
Programme, Jorhat, Assam,
India

Corresponding Author:
Daisy Konwar
Research Scholar,
Department of Life-Sciences,
Dibrugah University, Dibrugarh,
Assam, India

Seasonal dynamics and abundance of mosquitoes in Sivasagar district of upper Assam, India with special reference to environmental factors

Daisy Konwar, Rimen Bordoloi and Niranjan Nath

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Abstract
The study was conducted on diversity as well as dynamic of mosquito fauna of the Sivasagar district, Assam, India for a period of one year. Total 26288 female mosquitoes belonging to 36 species under 6 genera were collected. T-test, Correlation and Regression were used to evaluate the collected data at significance level below .05 and .01. The dominant species was *Cx.quinquefasciatus Say (13.77%)* followed by *Cx.tritaeniorhynchus Giles* (11.27%), *Ar.subalbatus coquillett (8.9%)* and *Cx. vishnui Theobald (8.25).* PMHD of Anopheles (56.24), Culex (104.84), Mansonia (9.7) and Armigeres (14.55) were higher in the monsoon while Aedes (4.83) were higher in Post monsoon and Toxorhynchites (0.12) were higher in Premonsoon. Mosquito abundance were significantly positively correlated with temperature (r=0.88; $p<.01$), rainfall (r=0.91; $p<.01$) and humidity (r=0.83; $p<.01$). The Regression analysis showed strong linear relationship between mosquito abundance and environmental factors (R2= 0.788, 0.915, 0.832 for temperature, rainfall and humidity respectively).

Keywords: Mosquito, abundance, diversity, environmental factors, correlation, regression

Introduction
Documentation of species is a critically important component of biodiversity studies and has great significance in conservation of genetic resources as well as control of pests and vectors [1]. Globally, there are 3539 known species of mosquitoes belonging to 112 genera [2]. Mosquito taxonomy provides essential inputs for vector control. There were faunistic surveys carried out and published in various parts of India since the 1980s which are new addition to the mosquito fauna of India. In India total 393 mosquito species belonging to 49 genera were identified [3]. Most of the mosquito faunistic studies in India have been done in relation to the geographic location. These studies provide information on the distribution of mosquito species in different regions or states [4]. The online systematic catalog of Culicidae listed 356 species in India [5]. Mosquito species present in various places of North East India were studied morphologically and ecologically by numerous investigators and workers [6, 7, 8, 9]. The records of mosquito species include total 130 species from various sources in North East India [10]. The monograph on Indian Anophiline and Culicine published by Christopher (1933) and Barraud (1934) are the documents with coverage of the mosquitoes in some parts of Assam, India.

The eco-physiological and entomological approaches have been employed in a number of studies to explain the temporal occurrence of mosquitoes, by describing their life-cycle and addressing climatic factors. The North East (NE) India is expected to be highly prone to the consequences to climate change. Assam is very much a part of regional warming trend (data source: Assam State Disaster Management Plan, 2010). Global warming could make more areas climatologically suitable for transmission of Mosquito Borne Diseases. The distribution of mosquitoes which is also dependant on relative humidity, determines the extent of disease spread [11].

So, the aim of the present study is to investigate the abundance, seasonal variation and diversity of mosquito species as well as relationship between mosquito abundance and environmental factors in Sivasagar district of Assam.

Materials and methods
Study Area
The study was conducted in the Sivasagar district of Assam, India (Figure 1). The geographical extent of Sivasagar is 26024’0” N latitude and and 93^{0}52’ 0”E longitude with an elevation of 117 m above sea level. It is situated on upper Assam covering an area of 2668 km2. The district is bounded by the Brahmaputra River on the north, the Nagaland on the South, Dibrugarh district on the East and Jorhat district on the west side. There are some areas covering forest fringed foot hills sharing boarder with Nagaland and Arunachal Pradesh. Sivasagar district in Assam is endemic for mosquito borne diseases such as Malaria, Japanese encephalitis, Dengue and Lymphatic filariasis. The region predominantly has humid sub-tropical climate and comprises of wetlands, scattered tea meadows and forest fringed hills areas with many streams, rivers, rice fields, small irrigation canals with greasy margins to support mosquito growth and proliferation through most of the year.

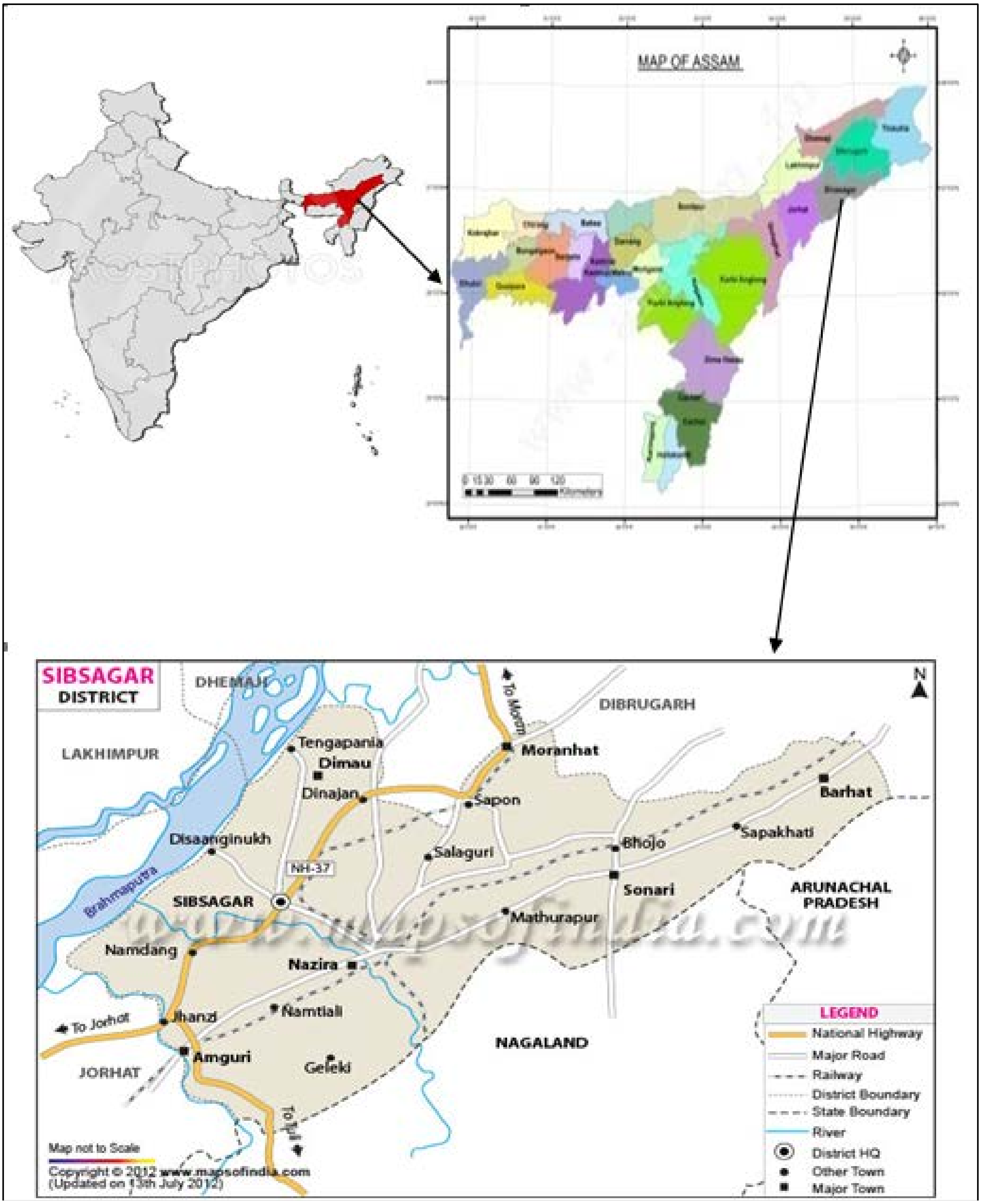


Fig 1: District map of Sivasagar, Assam (study area)

Survey
The entomological survey was carried out to study the distribution and diversity of the mosquito species found in the district from April 2018 to March 2019. Total 130 villages and 17 Tea gardens were selected randomly for mosquito collection. The total surveyed area was again divided into five types based on their geographical conditions such as Urban, Semi urban, Rural, Tea Garden (TE) and forest fringe areas. Among the selected village and TEs, 24 were identified as sentinel sites and rest was random sites.

Adult and larval collection
During Dusk (6-8 pm) and morning (4-6 am) hours adult mosquitoes resting both inside and outside were collected from human and domestic animal habitats of sentinel and random sites. Visited sentinel sites on weekly basis. Hand collection with suction tube and total catch method were used to collect adult mosquitoes. After collection mosquitoes were kept in the test tube with proper labeling. Mosquito larvae were collected from all types of possible water bodies of the study area following standard dipping methods. Collected larvae were kept in plastic containers and reared up to adult. Mosquitoes were anesthetized by chloroform and identified using microscope with standard mosquito identification keys and nomenclature [12, 13, 14, 15, 16, 17].
The study period was divided into four seasons as Pre monsoon (March , April, May), Monsoon (June, July , August) , Post-monsoon(September, October, November) and Winter (December, January , February) in a year to understand the seasonal variation of mosquito diversity. During collection period environmental variables (Temperature, Rainfall, Humidity) were recorded from www.worldweatheronline.com and Department of Agrometerology, Assam Agriculture University, Jorhat, Assam.

Data analysis
The collected mosquitoes were standardized to numbers per man – hour density (PMHD). The number of mosquito collecting per man in per hour (PMHD) for each species was calculated as per the following formula:
PMHD = Total number of mosquitoes collected /Number of person * Time spent in hours.
Statistical calculations were carried out using Microsoft excel and SPSS .Statistical techniques used in our study to examine the interrelationship between different variables were t-test , Pearson's correlation, multiple regression analysis using linear, quadratic combinations of variables. T-test is done to assess the data between mosquitoes collected in different seasons. Correlation is checked to estimate the relationship among mosquito abundances and weather factors. Multiple regression analysis was done for understanding the role of environmental factors in seasonal abundance of different mosquitoes [18, 19]. A linear Regression line with equation was prepared from the Regression analysis.

Results and Discussions
Over the course of 12 months study, total 26288 female mosquitoes belonging to 36 species under 6 genera were collected from the study area. Total collected mosquito species are tabulated in table 1. During the study Culex is the most dominant genus (54.7%) comprises of 12 species followed by Anopheles (28.4%) with 19 species , Armigeres (9%) with only 1 species, Mansonia (5.2%) with 2 species , Aedes (2.7%) with 2 species and the least found is Toxorhynchites (0.07%) with 1 species . Figure 2 shows the abundance of collected mosquitoes in all genus. Similarly, the predominant species is *Cx.quinquefasciatus Say (13.77%)* followed by *Cx. tritaeniorhynchus Giles* (11.27%), *Ar. subalbatus Coquillett (8.9%)* and *Cx. vishnui Theobald (8.25%*). Other abundant species are *Cx. gelidus Theobald, Cx. bitaeniorhynchus Giles, Mn. uniformis, An. culicifacies Giles (gr.), An. fluviatalis James (gr.), An. hyrcanus, and Cx. fuscocephala.*
The seasonal abundance of mosquitoes in Sivasagar is high. Studies on mosquito abundance in different geographical locations in India were carried out to understand the bionomics of mosquitoes by many researchers. A checklist of 64 mosquito species were recorded in Pondichery[1] , total 119 species of mosquitoes were recorded in Nilgiri hills [20] and survey for mosquito species in West Bengal conducted in Jalpaiguri [21,22] and described mosquitoes from coastal districts of Orissa. The biodiversity of Anophelines with 33 species were recorded [23] in Meghalaya. Similarly, diversity of malaria vector study was done in Assam [24] , Nagaland [25] and Mizoram [26] in earlier. The habitat preferences of both larva and adult mosquitoes are found with broad ranges in the district. Culex and Mansonia prefers agricultural lands and also their habitat are diverse. The dominant species of our study Cx. *quinquefasciatus Say* breeds in stagnant and organic polluted water [27]. *Cx tritaeniorhynchus Giles* act as primary vector for Japanese encephalitis in Assam. The larvae of *Cx tritaeniorhynchus Giles* mostly prefer rural settings in nursery paddy beds, puddle and paddy fields, ponds with aquatic vegetation and the adults are found mostly in cattle sheds, pig sties and with other domestic animals. *Cx vishnui* and *Cx pseudovishnui* larvae are also associated with paddy cultivation [18]. *Cx. gelidus* larva are found abundantly in marshy water collections and ground pools. *Cx. vishnui* adults were observed in both rural and urban settings and the vicinity of the houses in urban areas. It has been found that Anopheles prefers to breed in slow flowing streams with grassy margins while few prefer forest areas. *An minimus* and *An fluvitalis* are targeted as vector for Malaria in NE [28]. *An minimus* is a species of ecotone zone being considered as major vector for Malaria in NE india [29]. Breeding sites of mosquito larvae prefers slow running water bodies such as small irrigation canal or drainage, paddy field, seepage water streams, ponds etc. [30]. Water collection in forest fringe hills and artificial water containers, small canals in TE are good sources of larval breeding grounds. All types of possible habitat of larvae including tree holes, leaf axils and bamboo shoots were searched during the study.
The adult mosquito density is expressed in Max per man hour density (MPHD) and about 190 man hour were spent for collection of mosquitoes during the period. High variation is observed in the PMHD during the study. Table 2 shows the PMHD of Anopheles (56.24), Culex (104.84), Mansonia (9.7) and Armigeres (14.55) were higher in the monsoon season while Aedes (4.83) are higher in Post monsoon and Toxorhynchites (0.12) are higher in Premonsoon. The t-values resulted that mosquitoes are statistically significant in seasonal differences at .05 level. Figure 3 illustrates the mosquito abundances are high in monsoon and as illustrated by Figure 4, mosquito abundance gradually increases from the month of March and reached the peak in August and then declined gradually. PMHD recorded high in the month of June and August and are very low in December and January (Figure 5).
Figure 6 explains the abundance of mosquitoes with the trend of weather parameters of Sivasagar during the study period. The annual average temperature was 18.85^0 C min. and 28.25º

C max., average annual rainfall was about 207.08 mm and the relative humidity was about 79.28% in average. The results of Pearson correlation analysis are tabulated in table 3. The results indicate that the abundance of mosquitoes are significantly positively correlated with temperature (r=0.88; $p < .01$), rainfall (r=0.91; $p < .01$) and humidity (r=0.83; $p < .01$). Among them the correlation is high with rainfall. The Regression analysis shows strong linear relationship between mosquito abundance and environmental factors in table 4 (R^2= 0.788, 0.915, 0.832 for temperature, rainfall and humidity respectively). The linear regression equations are found and described by regression lines in Figures 7A, 7B and 7C. It has been found from the study that one unit increase in temperature, rainfall and humidity, the abundance of mosquito increases by 552.67, 10.44 and 315.82 units. Therefore, 79%, 91% and 83% variability could be explained by temperature, rainfall and humidity respectively along with time.

The effect of environmental factors on the seasonal abundance and diversity of mosquito varies significantly. The effect of weather parameters on mosquito abundance were reported from across the globe [31, 32, 33, 34, 35]. In Assam, mosquito density appeared rising with increasing temperatures and rainfall beginning from March and peak density was reported in April till August [36]. High correlation between Malaria incidence and weather parameters were found in Sonitpur district of Assam [37, 38]. The temporal variation in mosquito population is driven by a complex interplay of biotic and abiotic factors in India [39]. A strong correlation was found between vectors of Japanese encephalitis and meteorological factors like rainfall and humidity in different ecological habitats of Chandigarh [40]. Increase or decrease in weather parameters effects mosquito population [23, 41, 42]. In our study, two peaks were observed in the year in species abundance, one in June and another in August. Moderate rainfall can be beneficial for mosquitoes, while heavy can flush out mosquito larvae [43]. Few parts of Sivasagar become flooded during heavy rainfall months. So we may assume that the flush out and destroy of larvae from flooded area which may lower the abundance in mosquitoes in peak rainy months sometimes. The temporal change in abundance of potential Japanese encephalitis vector species was surveyed in Dibrugarh, Assam [19] using multiple regressions and accounted for 25-98% of variation .Similarly, 32% of variations in weather variables were reported in Bareilly district, UP [44].

Table 1: Mosquito diversity in Sivasagar

Genus	Sl. No.	Species	Total	RA (%)	PMHD
Anopheles	1	*An aconitus*	560	2.13	8.8
	2	*An. aiitkanii(gr.)*	95	0.36	1.5
	3	*An. Annularis*	415	1.58	6.6
	4	*An. Nivipes*	245	0.93	3.9
	5	*An. phillipinensis*	250	0.95	3.9
	6	*An. barbirostris(gr.)*	572	2.18	9.0
	7	*An. culicifacies Giles (gr.)*	1053	4.01	16.6
	8	*An. fluviatalis James (gr.)*	943	3.59	14.9
	9	*An. Gigas*	353	1.34	5.6
	10	*An. Hyrcanus*	939	3.57	14.8
	11	*An. maculatus Theobald*	261	0.99	4.1
	12	*An. Majidi*	84	0.32	1.3
	13	*An. Minimus*	132	0.50	2.1
	14	*An. Pallidus*	187	0.71	3.0
	15	*An. Riperi*	87	0.33	1.4
	16	*An.stephensi Liston (gr).*	295	1.12	4.7
	17	*An. subpictus Grassi*	442	1.68	7.0
	18	*An. Vagus*	342	1.30	5.4
	19	*An. Varuna*	201	0.76	3.2
Culex	20	*Cx. bitaeniorhynchus Giles*	1027	3.91	16.2
	21	*Cx. Cornetes*	86	0.33	1.4
	22	*Cx. Edwardsi*	93	0.35	1.5
	23	*Cx. fuscocephala*	863	3.28	13.6
	24	*Cx. gelidus Theobald*	1644	6.25	26.0
	25	*Cx. Infula*	220	0.84	3.5
	26	*Cx. pseudivishnui*	785	2.99	12.4
	27	*Cx.quinquefasciatus Say*	3620	13.77	57.2
	28	*Cx. tritaeniorhynchus Giles*	2962	11.27	46.8
	29	*Cx. Univittatus*	204	0.78	3.2
	30	*Cx. vishnui Theobald*	2170	8.25	34.3
	31	*Cx. Whitmorei*	719	2.74	11.4
Mansonia	32	*Mn. Annulifera*	236	0.90	3.7
	33	*Mn. Uniformis*	1132	4.31	17.9
Armigeres	34	*Ar. subalbatus Coquillett*	2339	8.90	36.9
Aedes	35	*Ae. albopictus Skuse*	522	1.99	8.2
	36	*Ae. aegypti Linnaeus*	192	0.73	3.0
Toxorhynchites	37	*Tx. Splendens*	18	0.07	0.3

*Total mosquito collected 26288 (n=26288)

* RA- relative abundance

Table 2: Season wise variation of mosquitoes

Genus	Season	Mosquito collected	PMHD	Mean	SD	Se	t-test	Sig t-value
Anopheles	Winter	198	3.12	10.42	16.29	3.73	2.257	0.109
	Pre Monsoon	725	15.2	38.16	26.26	6.02		
	Monsoon	3562	56.24	187.47	158.93	36.46		
	Post monsoon	2971	46.9	156.37	122.87	28.18		
Culex	Winter	508	8.02	42.33	102.9	29.7	2.281	0.107
	Pre Monsoon	1272	20.08	106.00	106.27	30.68		
	Monsoon	6640	104.84	553.33	512.87	148.05		
	Post monsoon	5973	94.31	497.75	518.14	149.57		
Mansonia	Winter	64	1.01	32	31.113	22.0	2.34	0.101
	Pre Monsoon	116	1.8	58	12.72	9.0		
	Monsoon	615	9.7	307	287.792	203.50		
	Post monsoon	573	9.04	286	301.93	213.5		
Armigeres	Winter	296	4.67	296	0	0	3.418	0.042
	Pre Monsoon	284	4.48	284	0	0		
	Monsoon	922	14.55	922	0	0		
	Post monsoon	837	13.2	837	0	0		
Aedes	Winter	39	.61	19.5	13.43	9.5	2.726	0.072
	Pre Monsoon	96	1.51	48.0	38.18	27		
	Monsoon	273	4.3	136.50	98.28	69.5		
	Post monsoon	306	4.83	153.0	83.43	59.0		
Toxorhynchites	Winter	0	0	0	0	0	2.635	0.078
	Pre Monsoon	8	.12	8	0	0		
	Monsoon	6	.09	6	0	0		
	Post monsoon	4	.06	4	0	0		

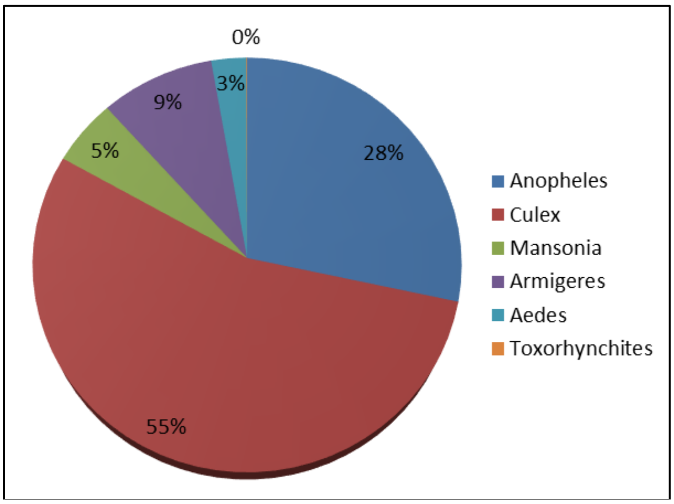


Fig 2: Abundance of mosquitoes in different Genus

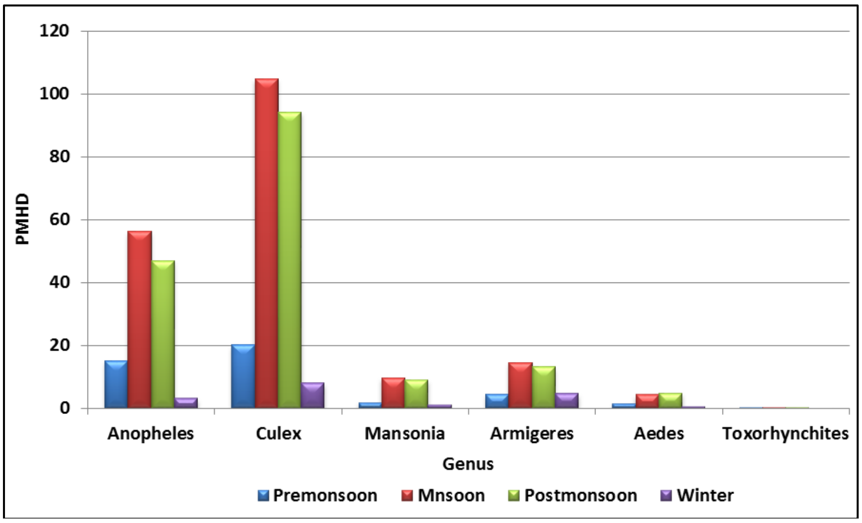


Fig 3: Seasonal variation of mosquitoes

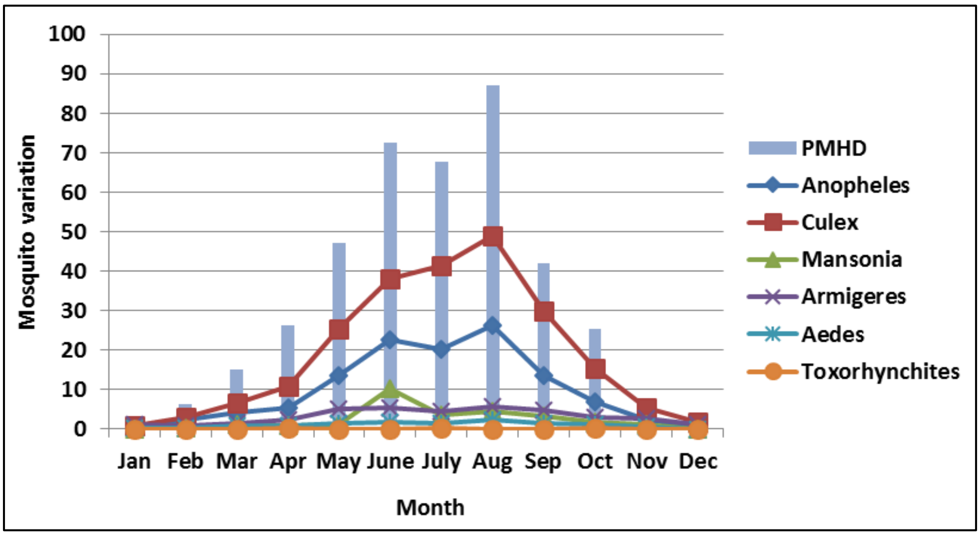


Fig 4: Monthwise prevalence of mosquitoes.

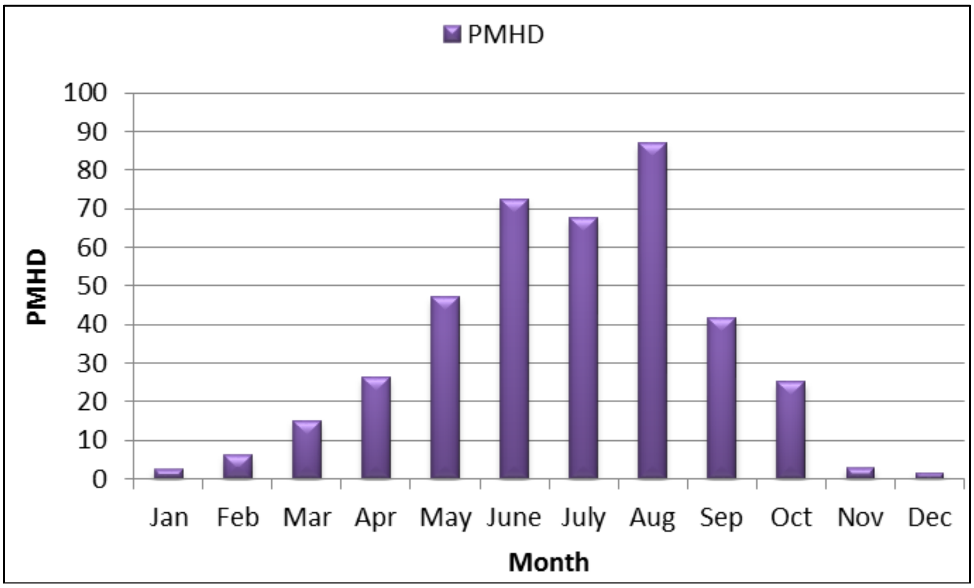


Fig 5: Monthly PMHD of mosquito abundance

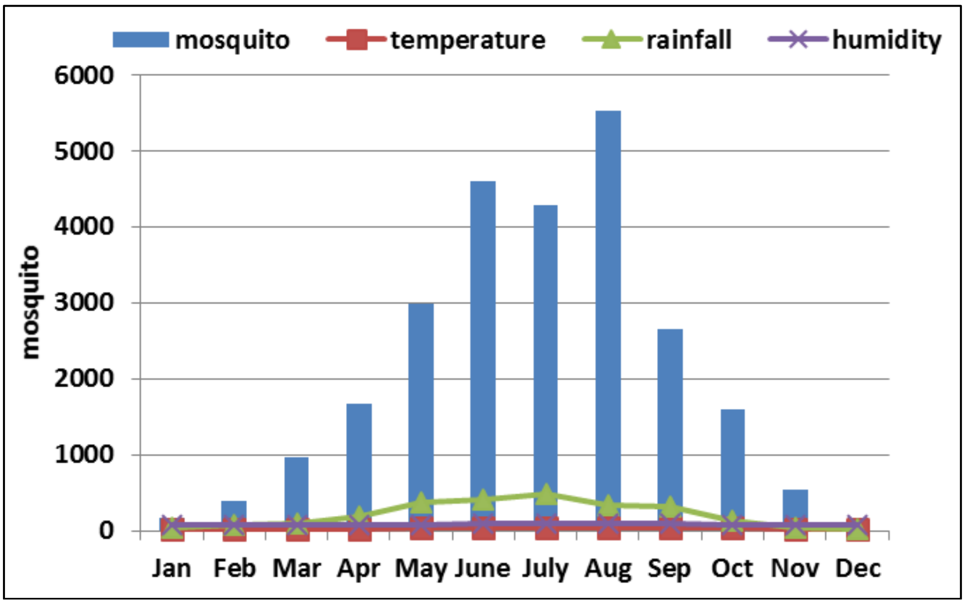


Fig 6: Association between environmental parameters (temperature, rainfall and humidity) and mosquito abundance

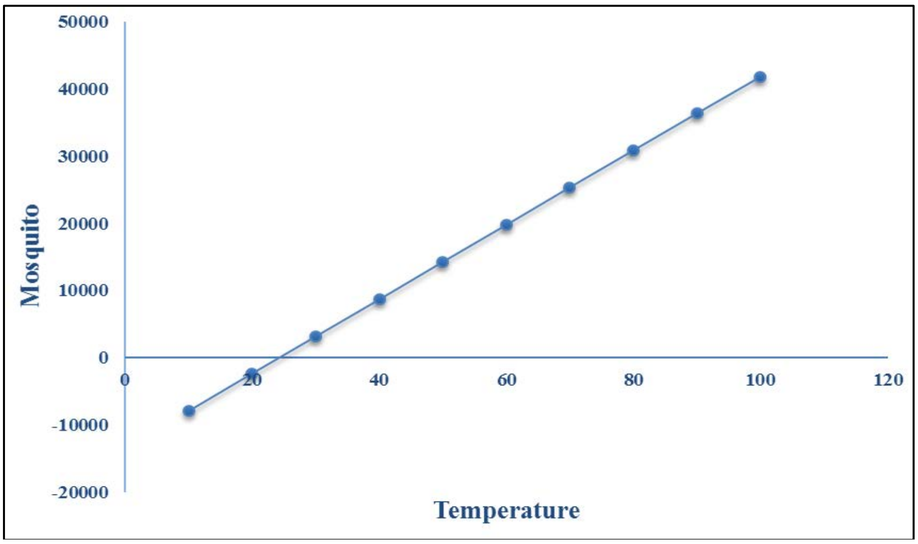
Table 3: Correlation between Mosquito and environmental factors

	Temperature	Rainfall	Humidity
Mosquito	.888**	.915**	.832**
Temperature	1	.817**	.885**
Rainfall		1	.781**
Humidity			1

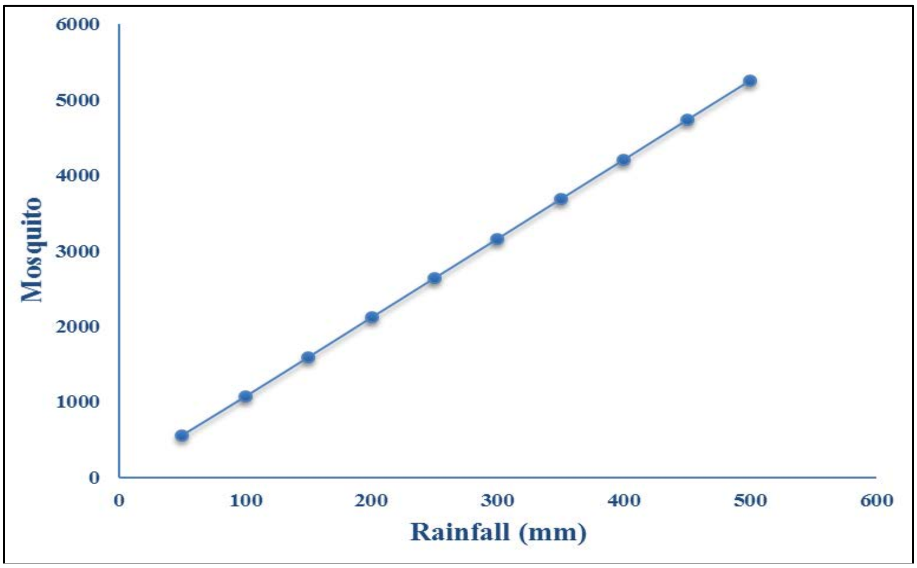
** Correlation is significant at the 0.01 level (2-tailed).

Table 4: Result of Regression analysis

	Co-efficients	R^2	Y
Constant	-13422.302	0.788	-13422.302+ 552.671X
Temperature	552.671		
Constant	27.156	0.915	27.156+ 10.448X
Rainfall	10.448		
Constant	-22834.194	0.832	-22834.194 + 315.822X
Humidity	315.822		



A.



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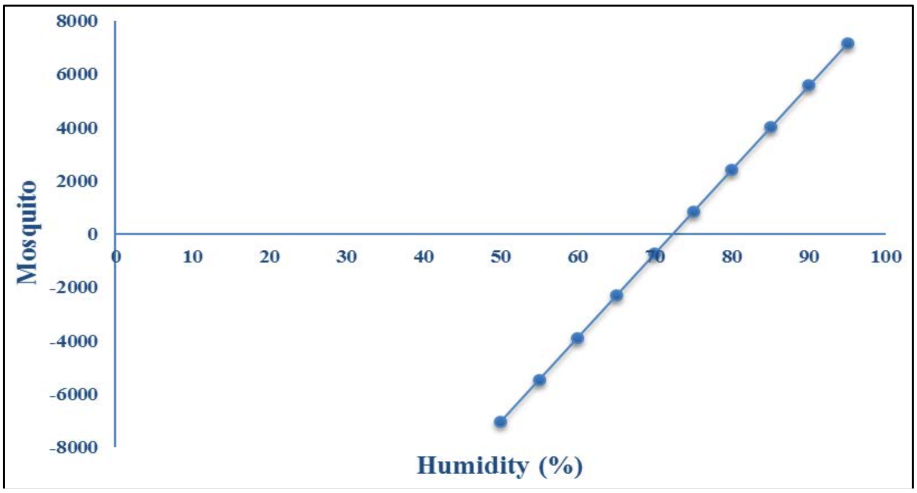

C.

Fig 7: Regression lines showing the relationship between mosquito abundance and environmental factors. (A) Regression line where Y (mosquito) = -13422.302 + 552.671X (temperature), (B) Regression line where Y (mosquito) = 27.156 + 10.448 X (rainfall), (C) Regression line where Y (mosquito) = -22838.194+ 315.822X (humidity).

Conclusion

Mosquito surveys in various parts of the globe have been carried out during the past two decades to study the occurrence, distribution pattern, composition of species and identification of vectors. The comprehensive recent information on distribution of mosquito species is very meages. There is an urgent need for exploration and documentation of species diversity in the regions for management of vector borne diseases.

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Conflict of interest & funding

There is no conflict of Interest. The authors have not received any funding or benefits from elsewhere to conduct the study.

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