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Seasonal variation of Japanese encephalitis vector mosquito's populations in vulnerable areas of Northern parts of West Bengal, India

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Abstract
Vector control is one of the major aspects for prevention and control of Japanese encephalitis (JE). Information on population dynamics and impact of environmental factors on it are important for formulation of vector control measures. Present study was designed to study species abundance, population dynamics and impact of environmental factors on JE vectors in northern part of West Bengal. Immature and mature stages were collected on monthly basis and identified morphologically. Per dip density and man hour density were calculated and analyzed using SPSS software to determine the impact of environmental factors on JE vector. A total of 43960 mosquitoes were collected and *Culex* were predominant genus. Four JE vectors species were identified - *Cx. tritaeniorhynchus*, *Cx. gelidus*, *Cx. pseudovishnui*, and *Cx. vishnui*. Pronounced monthly variation was observed with low density in winter and high in monsoon. Abundance of JE vectors were positively correlated with temperature, relative humidity and rainfall in study areas. This study findings may serve as baseline data on population dynamics of JE vector and impact of environmental factors on it which may be useful for formulation of effective vector control strategies. Abundance of *Culex* vectors clearly indicate possible threat of JE incidence in the study areas.

Keywords: Climatic factors, India, JE vectors, Man hour density, Per dip density, West Bengal

1. Introduction
Japanese encephalitis (JE), a mosquito-borne, zoonotic, arboviral disease, is one of major public health problem in different countries of South East Asia [1]. JE is now emerged as a global public health concern due to its invasion in non-endemic areas, high epidemic potential, high case fatality rate and permanent sequelae among the survivors. Globally, an estimated 68000 clinical cases of JE and approximately 13600 – 20400 deaths were reported every year. JE transmission is endemic in 24 countries of WHO South-East Asia and Western Pacific regions and more than 3 billion people of these regions are at risk of infection [2]. JE is caused by Japanese Encephalitis virus (JEV), a member of the genus Flavivirus, family Flaviviridae [3]. JEV is maintained in a zoonotic cycle, which involves pigs as the major reservoir/amplifying host, water birds as carriers and mosquitoes as vectors.
The first confirmed case of JE in India was reported in 1955 from Vellore, Tamil Nadu. The first major outbreak was reported from Burdwan, West Bengal in 1973. Since then JE cases were reported from 171 districts of 19 states in the country [4]. Annually, an estimated 1714 – 6594 confirmed JE cases and 367 – 1665 deaths were recorded from India [5]. In 2019, West Bengal reported 82 confirmed JE cases and 11 deaths, but the state faced a large outbreak of JE cases in the northern districts with 415 cases and 78 death during 2014.
In India 16 different species of mosquitoes have been identified as potential JE vectors, out of which 10 are from *Culex*, three from *Anopheles*, and three from *Mansonia* [6]. Among these mosquitoes belonging to *Culex vishnui* subgroup i.e., *Culex tritaeniorhynchus*, *Culex vishnui* and *Culex pseudovishnui* are the major JE vector in India [7]. *Culex vishnui* subgroup is very common and widely distributed mosquitoes and breed in water bodies with luxuriant vegetation preferably in paddy field and shallow ditches and ponds [8]. It is principally zoophilic in nature with opportunistic on man. It is exophilic and orients mostly during later part (dusk) of the day [7, 9].

Apart from these primary JE vectors, *Culex gelidus* is an important secondary vector in India [10]. This vector species is widely distributed in the oriental region including India [11]. The immature stages of *Culex gelidus* are usually found in ground pools containing weed, marshy tracts, etc. [12] It is a voracious biter of humans, but also prefers to feed on large domestic animals like pigs, horse etc [13].

National Vector Borne Disease Control Programme (NVBDCP) of India formulated a National Programme on prevention and control of JE/AES which mainly depends on JE vaccination in endemic areas and vector control. Government of India launched JE vaccination with attenuated vaccine SA-14-14-2 in phased manner since 2006 particularly in endemic areas [4]. The vector control measure is one of the major aspects for prevention and control of JE by blocking the transmission of virus with limited man-vector contact. Information on several aspects of JE vectors ecology such as species abundance, feeding and resting behaviour, population dynamics, effect of environmental factors (temperature, rainfall and humidity) on population dynamics and insecticides resistance status are very essential for formulating and effective vector control strategy as well as giving an early warning signal of JE outbreak.

There are several reports on species abundance and distribution of JE vectors from different parts of India such as Uttar Pradesh [14], Karnataka [10, 15], Tamil Nadu [16], but such study from West Bengal is very limited [17, 18]. The effect of environmental factors – temperature, rainfalls, and relative humidity on population dynamics of JE vectors have been reported from different JE endemic parts of India[19, 20, 21]. But no such studies are available particularly from eastern parts of India including West Bengal. The present work was undertaken to study species abundance, population dynamics and the role of environmental factors on major JE vectors population in northern part of West Bengal, India.

2. Materials and methods

2.1 Study area: The present study was conducted in 11 villages of three northern districts of West Bengal, namely Darjeeling, Jalpaiguri, and Uttar Dinajpur. Six villages from Darjeeling district, three villages from Jalpaiguri district and two villages from Uttar Dinajpur were selected as study sites after analyzing last five years JE cases and in consultation with district health authorities. The study was conducted during January, 2019 to December, 2019. The geographical location and demography of the study villages are given in our previous report [22].

2.2 Mosquito collections and identification: During entomological survey, the insect collectors moved freely throughout the study villages, as there were no fixed sites, for collection of mosquitoes. The immature stages of mosquitoes were collected from wide ranges of naturally occurring breeding sites like paddy fields, ponds, puddles containing aquatic vegetation by spending 2-3 hours in each study villages. Different types of dippers and aquatic nets were used to examine larval habitats. For collection of larvae and pupae different sized dippers were used which includes soup ladles holding around 100-150ml water [23] or a larger 500ml dipper [24]. The collected larvae from each dip were transferred to small plastic containers (500 ml) to transport them to the laboratory. The collected immature stages were reared in the laboratory and hatched out adults were identified up to species level.

In each study villages, indoor adult mosquitoes were collected with the help of mouth aspirators (John W. Hock, USA) aided by torch lights from human dwellings and cattle sheds at monthly interval during study periods. Mosquitoes were collected for 10 min per house during morning (8.00 AM to 10.00 AM) and dusk (6.00 PM to 8.00 PM) from 150 houses of each study districts once per month by two trained insect collectors. Collected adult mosquitoes were gathered in labelled containers and transferred to laboratory for species identification.

Adult mosquitoes collected directly from the field and those hatched out in the laboratory from reared immature stages were killed by freezing and identified up to species on the basis of morphological characteristics using the taxonomic keys and description of Sirivanakarn, 1975 [25]; Reuben *et al*., 1994 [26]; and Tyagi *et al*., 2012 [6] under dissecting microscope. The number of total mosquitoes collected from each study sites were counted recorded. After identification, the total number of mosquitoes belonging to each identified species was counted and a study site wise record was maintained throughout the study period.

2.3 Entomological indices: Per dip density is the measurement parameter of immature stage concentration in different habitats and in different time periods of a year. Per dip density was calculated for estimating the concentration of larvae and pupae in different breeding habitats throughout the year. Per dip density was calculated as follows –

$$\text{Per dip density} = \frac{\text{Total no. of larve and pupae collected from a habitat}}{\text{Total no. of dips performed}}$$

For calculation of per dip density of JE vector immature stages, site wise month wise collected larvae were reared in the laboratory and allowed to hatched into adults. After hatching JE vectors were identified and counted to calculate the per dip density of JE vectors larvae. The no. of non-JE vector mosquitoes were excluded from this calculation.

Man hour density (MHD) is the measurement parameter of adult mosquitoes density in different habitats collected by mouth aspirator. MHD for the mouth aspirator collected mosquitoes is calculated as follows –

$$\text{MHD} = \frac{\text{Total no. of mosquitoes collected}}{\text{Total time spent in hours} \times \text{No. of insect collectors}}$$

2.4 Meteorological data collection: During the study period, weather data including maximum and minimum temperature (^{0}C) and relative humidity (%) were recorded on the day of entomological with the help of digital thermometer and hygrometer from different study villages. The data of monthly average rainfall of each study sites were collected from the website (https:/www.worldweather.com).

2.5 Ethical statement: Before initiation of the entomological collection, a village level informational meeting was organized with the villagers in presence of local health and administrative personnel. The aim and objectives of the study were explained and individuals were requested to assist the study team members during the study period. Before collection of the mosquitoes, permission was taken from the owners of the privates houses and lands. No endangered and

protected species were involved in the present study. The study protocol was approved by the Institutional Ethics Committee of Calcutta School of Tropical Medicine, Kolkata, India. During the study both adults and immature stages of mosquitoes were collected following standard collection methods.

2.6 Data analysis: The number of larvae and pupae collected and number of dips performed and adult mosquitoes collected by mouth aspirator during survey were entered into an excel worksheet according to the months of collection and study sites. From these collected data, per dip density and man hour density were calculated for larvae for indoor collected adult mosquitoes, respectively. The cumulative month wise distribution of JE vectors, breeding site positivity rate, per dip density of JE vector immature stages and man hour density of total JE vectors in all three study districts were analyzed by t-test, whereas species wise man hour density of adult JE vectors was analyzed by one-way ANOVA. The impact of climatological factors (maximum, minimum, average temperature, relative humidity and average rainfall) on population dynamics of JE vectors (indoor collection) were determined by Spearman's correlation and univariate negative binomial regression model analysis. All the statistical analysis was performed by SPSS software (version 21.0).

3. Results
3.1 Species composition: Over the course of 12 months study period, a total of 43960 female mosquitoes belonging to 13 species under five genera were collected from the study areas. *Culex* was the most predominant genus (74.48%) followed by *Anopheles* (13.28%), *Armigeres* (5.49%), *Aedes* (4.67%), and *Mansonia* (2.07%). Five different species under the genus *Culex* were identified and their order of predominance was *Cx. quinquefasciatus* (25.73%), *Cx. tritaeniorhynchus* (21.00%), *Cx. gelidus* (20.45%), *Cx. pseudovishnui* (4.88%), and *Cx. vishnui* (2.43%). Five species under the genus *Anopheles*; *An. ahomi* (4.05%), *An. dirus* (3.66%), *An. subpictus* (3.05%), and *An. stephensi* (2.51%) were collected. Two species of *Aedes* (*Ae. albopictus* 3.63%, *Ae. aegypti* 1.04%) and one species each of *Armigeres* (*Am. subalbatus*5.49%) and *Mansonia* (*Ma. uniformis* 2.07%) were identified (Fig. 1).

3.2 Major JE vectors in the study area: Out of 43960 collected mosquitoes, 21434 (48.76%) belonged to four different species of JE vectors. *Cx. tritaeniorhynchus* (43.08%) and *Cx. gelidus* (41.94%) were the predominant species followed by *Cx. pseudovishnui* (10.01%) and *Cx. vishnui* (4.98%) (Fig. 2). The overall abundance of *Cx. tritaeniorhynchus* was significantly higher than *Cx. pseudovishnui* (p = 0.013) and *Cx. vishnui* (p = 0.005). Similarly, *Cx. gelidus* was significantly higher than *Cx. pseudovishnui* (p = 0.044) and *Cx. vishnui* (p = 0.023). But no significant difference was found between the abundance of *Cx. tritaeniorhynchus* and *Cx. gelidus* (p = 0.949); and *Cx. pseudovishnui*, *Cx. vishnui* (p = 0.182).

3.3 Larval and pupal survey for major JE vectors: During the study period, a total of 2101 potential breeding sites were surveyed. The common larval habitats were ponds, unused well, paddy fields, cess pool, cess pit, cement tank, culvert, drain, fallow field, hoof print, irrigation channel, mud pool,

pond, river bed pool, rain water pool, slow flow stream, etc. The site positivity rate for immature mosquito stages were almost similar in all three study districts: 69.79% (737/1056) in Darjeeling, 61.30% (407/664) and 65.35% (249/381) in Jalpaiguri and Uttar Dinajpur respectively. No significant difference was recorded in breeding site positivity rate between Darjeeling and Jalpaiguri (p = 0.597), Darjeeling and Uttar Dinajpur (p = 0.621) and Jalpaiguri and Uttar Dinajpur (p = 0.967). The breeding site positivity rate was very low during winter months (November to February) which gradually increased during summer and reached to its peak in rainy season. In Darjeeling, highest breeding site positivity rate (98.4%) was recorded in the month of September, whereas in Jalpaiguri (94.8%) and Uttar Dinajpur (93%) in August.

A total of 15045 immature stages of mosquitoes were collected, of which 8098 (53.83%) were identified as JE vectors. Per dip density was very low during winter months (November to February) which gradually increased during summer and reached to its peak in rainy season. The highest per dip density of JE vector was 7.1, 6.6 and 8.0 in Darjeeling, Jalpaiguri and Uttar Dinajpur, respectively in the month of August (Fig. 3). No significant difference in per dip density between Darjeeling and Jalpaiguri (p = 0.442), Darjeeling and Uttar Dinajpur (p = 0.751) and Jalpaiguri and Uttar Dinajpur (p = 0.677) was observed.

3.4 Adult mosquito survey and man hour density of major JE vectors: During the study period a total of 13336 adult JE vector were collected by mouth aspirator. Month wise man hour density of JE vectors in three study districts is given in Fig. 4. The man hour density of JE vectors were very low during winter months (January to February) which gradually increased from the month of March and reached the peak in August then declined gradually in Darjeeling and Jalpaiguri districts. In Uttar Dinajpur, two peaks were recorded, one in the month of April and another in August. The highest man hour density of total JE vectors was recorded in the month of August in Darjeeling (85.9), Jalpaiguri (105.6) and Uttar Dinajpur (168.0).There was no significant difference between the man hour density of total JE vectors in Darjeeling and Jalpaiguri (p = 0.662), Darjeeling and Uttar Dinajpur (p = 0.112) and Jalpaiguri and Uttar Dinajpur (p = 0.222). Significant difference was observed in man hour density of four different JE vector species: *Cx. tritaeniorhynchus, Cx. vishnui, Cx. pseudovishnui, Cx. gelidus* in Darjeeling (F = 5.17, p = 0.004), Jalpaiguri (F = 5.65, p =0.002) and Uttar Dinajpur (F = 6.46, p =0.001) (Table 1).

3.5 Impact of environmental factors on JE vector populations dynamics: The abundance of JE vectors in Darjeeling and Jalpaiguri was highest in the month of August and lowest in the month of January. The abundance of JE vectors increased gradually from March to November. But in Uttar Dinajpur, the highest abundance of major JE vectors was recorded in August and lowest in December. Two different peaks in major JE vectors abundance were observed - one relatively small peak in April and another relatively large peak in August. Spearman's correlation analysis revealed that abundance of JE vectors were significantly positively correlated with maximum temperature, minimum temperature, average temperature, relative humidity and rainfall in Darjeeling, Jalpaiguri and Uttar Dinajpur; except

maximum temperature in Uttar Dinajpur (Table 2). Univariate negative binomial regression model analysis resulted that every one unit increase in maximum, minimum, average temperature, relative humidity and rainfall, the rate of JE vectors abundance increased significantly by 1.001 to 1.428 in all three study districts, except rainfall in Uttar Dinajpur (Table 2) (Fig. 5, 6, 7).

4. Discussion
Japanese Encephalitis (JE) is a re-emerging zoonotic disease which has much public health importance due to its high epidemic potential, high case fatality rate (CFR) and sequelae among survivors. It is endemic in several parts of India including West Bengal. The main risk factors involved in the transmission of JE virus are rainfall, temperature, relative humidity, virus infection from reservoirs, and socio-economic conditions etc [27]. Vector mosquitoes are the key player for the transmission of JE virus. JE prevention and control programme for JE is depends on vector control measures. Formulation and implementation of effective vector control strategies require extensive knowledge on population dynamics of vectors.

In the present study species abundance, population dynamics and effect of weather factors on major JE vector population was studied over a 12-months period in 2019. Thirteen different species of mosquitoes were identified from the study areas which was almost similar with the findings of Mariappan *et al*., in 2011-2012 [18]. *Culex* were the most abundant genus (74.48%) in the study areas like others in different parts of India [10, 18, 28, 29, 30, 31]. Presence of suitable breeding habitat for these mosquitoes such agricultural field, stagnant polluted water containing decayed organic matter, would be the reasons behind this[32]. Five different species of *Culex* mosquitoes were collected from the study areas, of which four species - *Cx. tritaeniorhynchus*, *Cx. pseudovishnui*, *Cx. Vishnui*and *Cx. gelidus*were designated as JE vectors and included in the list of 16 mosquitoes from which JE virus has been isolated in India [6]. Three species of *Cx. vishnui* subgroup and *Cx. gelidus* were the major JE vectors in the study areas, similar observations were also recorded from different parts of the country [10, 18, 28, 29, 30, 31].

In the present study two density peaks were observed for the total mosquito population, one short peak in the month of March to April due to high abundance of *Cx. quinquefasciatus* and *Cx. tritaeniorhynchus*. The population of *Cx. quinquefasciatus* increased during these months due to availability of favourable breeding habitat containing stagnant water polluted with decayed organic matter and sudden rise in mean air temperature. The second peak was recorded in the months of August and September, due to high prevalence of *Cx. tritaeniorhynchus* and *Cx. gelidus*. The abundance of *Cx. tritaeniorhynchus* is closely associated with paddy cultivation as nursery paddy beds, puddles and paddy fields are preferred breeding sites for this species [33]. In norther part of West

Bengal, a single rice crop is grown per year, which might be the reason for single JE vector peak in the study areas except in Uttar Dinajpur where two peaks were noticed due to two rice crops are cultivated as observed in south India, two high peaks of JE vectors has been recorded as paddy crop is grown twice per year [15, 16, 34]. The occurrence of JE in the study areas was closely associated with this seasonal peak of JE vectors. So, period from May to October is considered as JE transmission period. The results of present study were found in accordance with the study conducted in different parts of India [14, 35, 36].

During post monsoon period from September to October, *Cx. gelidus* was found abundant due to the presence of marshy water pools [20], as this species breeds profusely in paddy fields and ground pools as well as in fresh and dirty water i.e. cow dung pit, ground pools, marshes and waste water canal containing much weeds and high concentration of organic matter[37, 38]. *Cx. pseudovishnui* and *Cx. vishnui* was the third and fourth most abundant species of JE vectors recorded during the JE transmission period. These species are considered to be an important vector of JE in India and Sri Lanka [39, 40]. The abundance of these two species is also associated with paddy cultivation as rice field is their preferred breeding sites.

During the study period, different larval habitats such as ponds, unused well, paddy fields, cess pool, cess pit, cement tank, culvert, drain, fallow field, hoof print, irrigation channel, mud pool, pond, river bed pool, rain water pool, etc were examined for JE vector immature stages. In all three study districts it was found that temperature (maximum, minimum, and average) and rainfall were strongly positively correlated with per dip density of JE vector larvae but insignificantly correlated with relative humidity.

The man hour density of JE vectors were very low during winter months (November to February) which gradually increased during rainy months starting from April to October in all three study districts. The man hour density of total JE vectors was positively correlated with temperature (maximum, minimum and average) rainfall and relative humidity in all three study districts except maximum temperature in Uttar Dinajpur.

Temperature and humidity are most important climatological factors for mosquito occurrence [41]. In the present study, weather variables including rainfall, average temperature, average relative humidity were tested to analyse its effect on JE vector abundance and found to have very important effect on mosquito dynamics. The higher temperature during early spring accelerates the breeding season and increases the density [42]. Further, at lower temperatures (8–10°C), the mosquito population decrease, as only few eggs are capable of hatching at this temperature [43]; and the temperature-dependencies will not be same among the stages, leading to non-linearities in population responses to temperature [44, 45].

Table 1: Statistical analysis of month wise species wise man hour density of four JE vectors in study areas

Study sites	Species*	Basic statistics of month wise MHD			One-way ANOVA
		Mean ± SD	Range	95% CI	
Darjeeling	Ct[a]	12.43±12.55	0.10-34.70	5.46-21.40	F = 5.17 p = 0.004
	Cv[c]	1.39±2.22	0-6.10	0.64-2.81	
	Cp[bc]	3.15±3.57	0-9.90	1.03-5.42	
	Cg[ab]	12.31±13.39	0.60-35.90	3.87-20.82	
Jalpaiguri	Ct[p]	16.08±14.17	0.90-42.30	4.09-25.08	F = 5.65

	Cv[r]	1.53±1.98	0-5.70	0.57-2.79	p = 0.002
	Cp[qr]	3.8±4.04	0-10.90	1.17-6.37	
	Cg[pq]	14.88±15.91	0.50-47.60	4.59-24.99	
Uttar Dinajpur	Ct[x]	25.85±19.51	0.80-58.40	5.63-38.25	F = 6.46 p = 0.001
	Cv[y]	3.95±3.60	0-11.20	1.04-6.24	
	Cp[y]	3.37±2.82	0-8.40	0.81-5.16	
	Cg[x]	25.68±28.45	1.60-77.80	8.21-43.76	

Ct = Cx. tritaeniorhynchus, Cv = Cx. vishnui, Cp = Cx. pseudovishnui, Cg = Cx. gelidus, Total = Total JE vectors
*Means that do not share a letter are significantly different by Tukey Test

Table 2: Analysis of impact of maximum, minimum, average temperature, relative humidity and rainfall on abundance of JE vectors by correlation and univariate negative binomial regression model

Study sites	Climatic factor	Spearman's correlation analysis		univariate negative binomial regression model analysis	
		ρ	p value	Incidence rate ration (95% CI)	p value
Darjeeling	Max. temp	0.771	0.003	1.424(1.192-1.700)	<0.0001
	Min. temp	0.846	0.001	1.416(1.209-1.658)	<0.0001
	Avg. temp	0.811	0.001	1.428(1.207-1.690)	<0.0001
	RH	0.872	<0.0001	1.071(1.036-1.108)	<0.0001
	Rainfall	0.895	<0.0001	1.001(1.000-1.003)	0.016
Jalpaiguri	Max. temp	0.622	0.031	1.352(1.050-1.742)	0.020
	Min. temp	0.881	<0.0001	1.349(1.152-1.580)	0.001
	Avg. temp	0.762	0.004	1.387(1.141-1.686)	0.001
	RH	0.872	<0.0001	1.074(1.038-1.113)	0.009
	Rainfall	0.690	0.013	1.001(1.000-1.003)	0.013
Uttar Dinajpur	Max. temp	0.559	0.059	1.232(1.006-1.509)	0.043
	Min. temp	0.748	0.005	1.292(1.097-1.527)	0.002
	Avg. temp	0.629	0.028	1.282(1.069-1.537)	0.007
	RH	0.699	0.011	1.045(1.008-1.085)	0.024
	Rainfall	0.821	0.001	1.004(0.999-1.010)	0.118
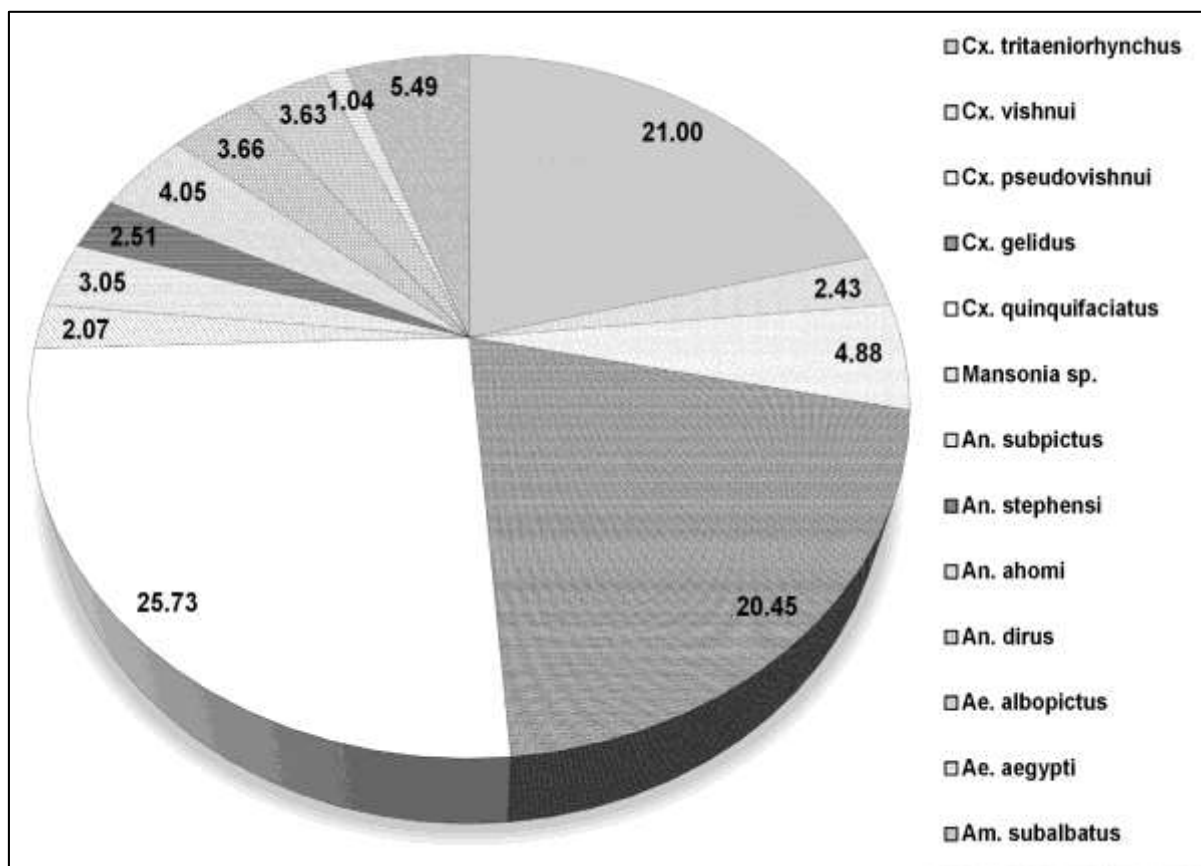
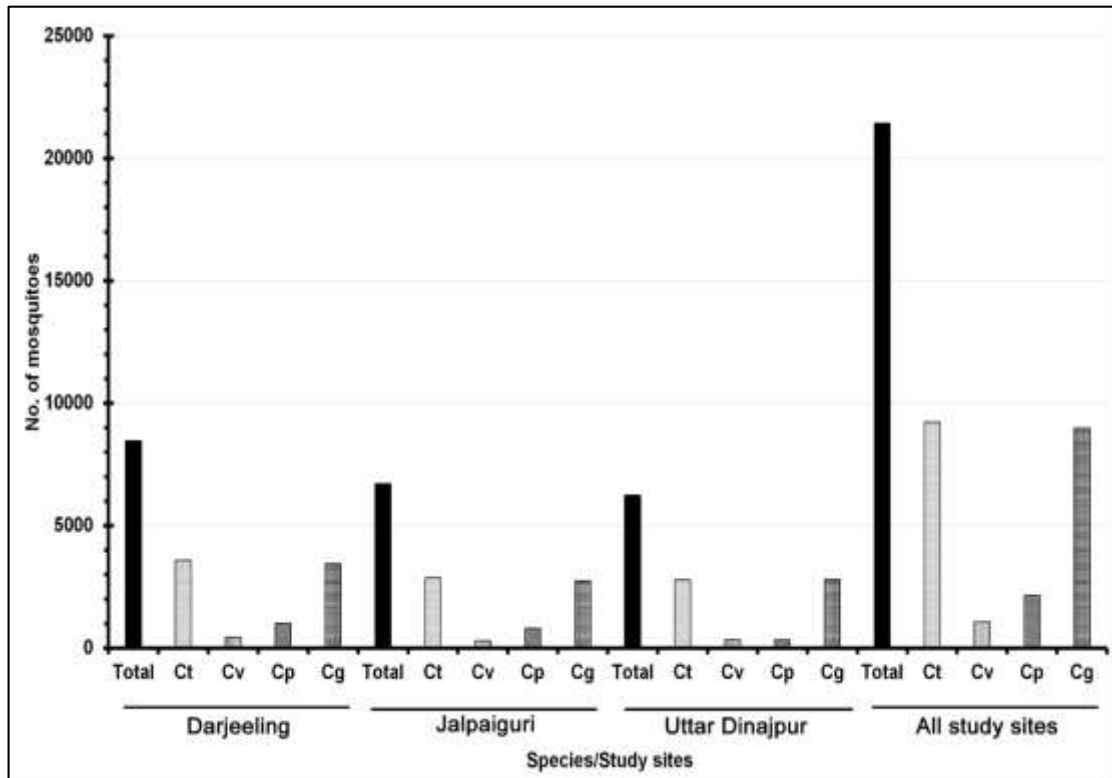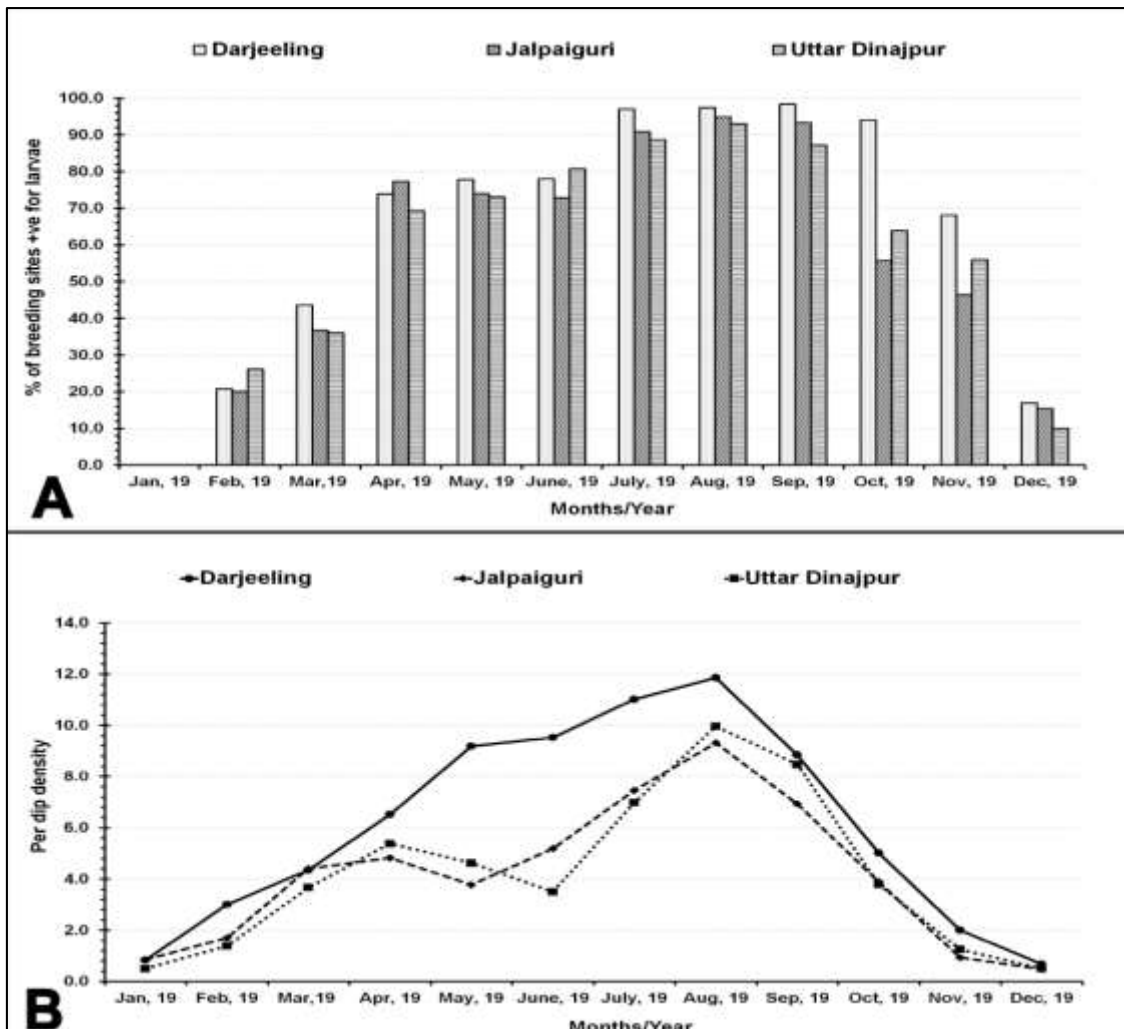**Fig 1:** Species composition of mosquitoes collected during the study period

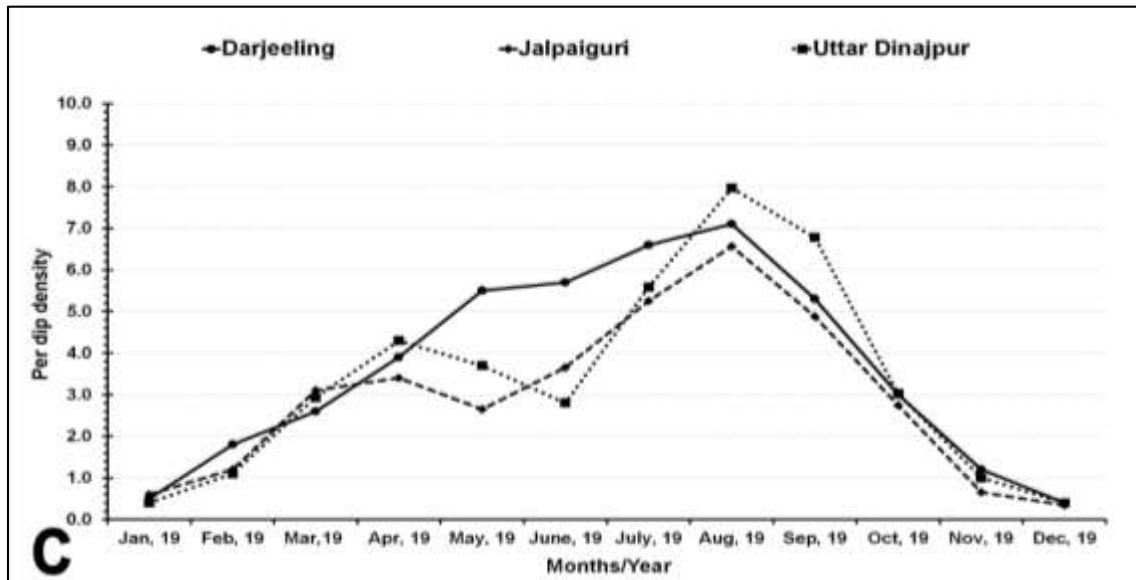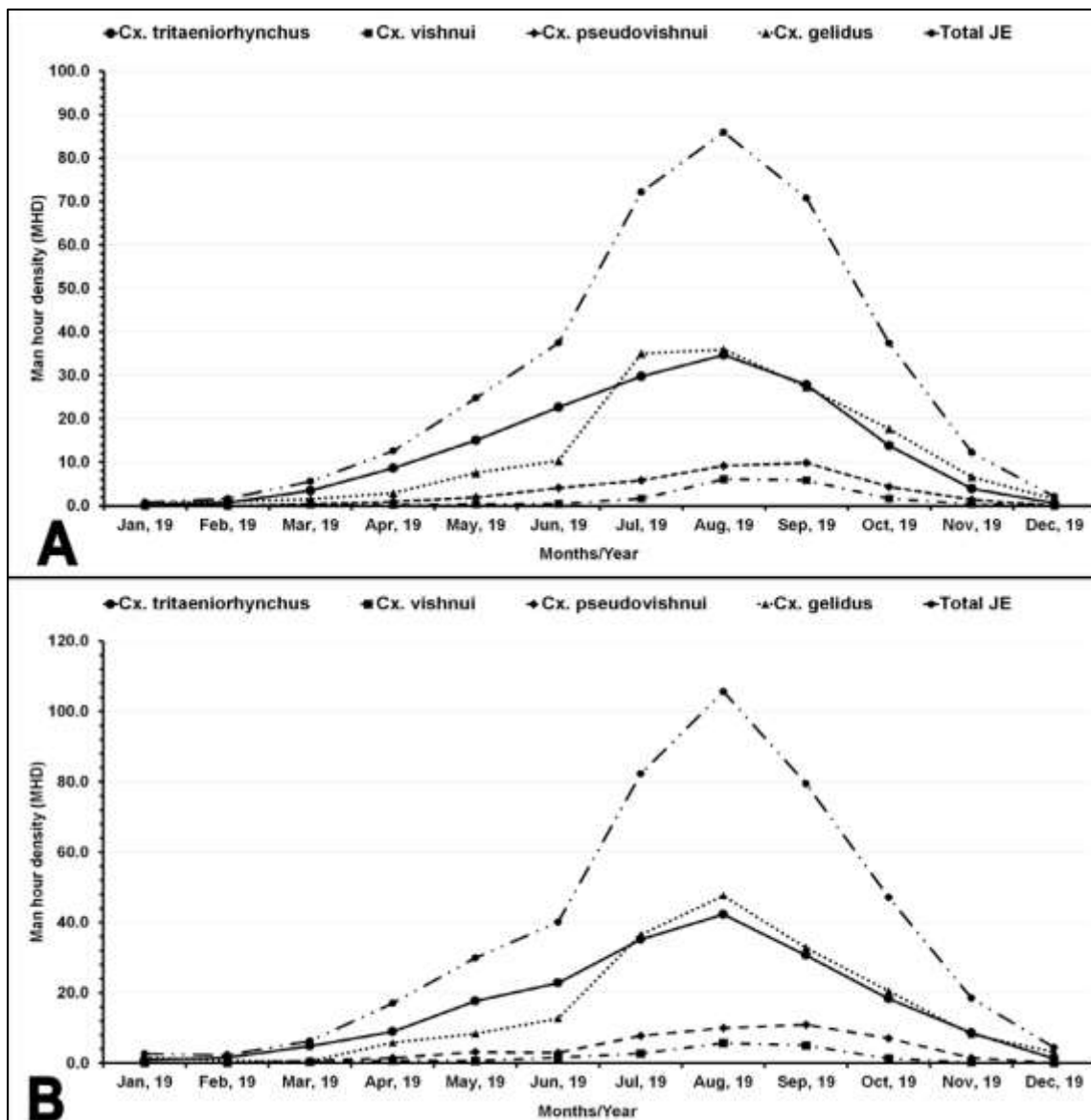**Fig 2:** Abundance of JE vector mosquitoes in study sites

Fig 3: Month wise percentage of breeding site positive (A), per dip density of all species (B) and JE vectors (C) larvae and pupae in the study area
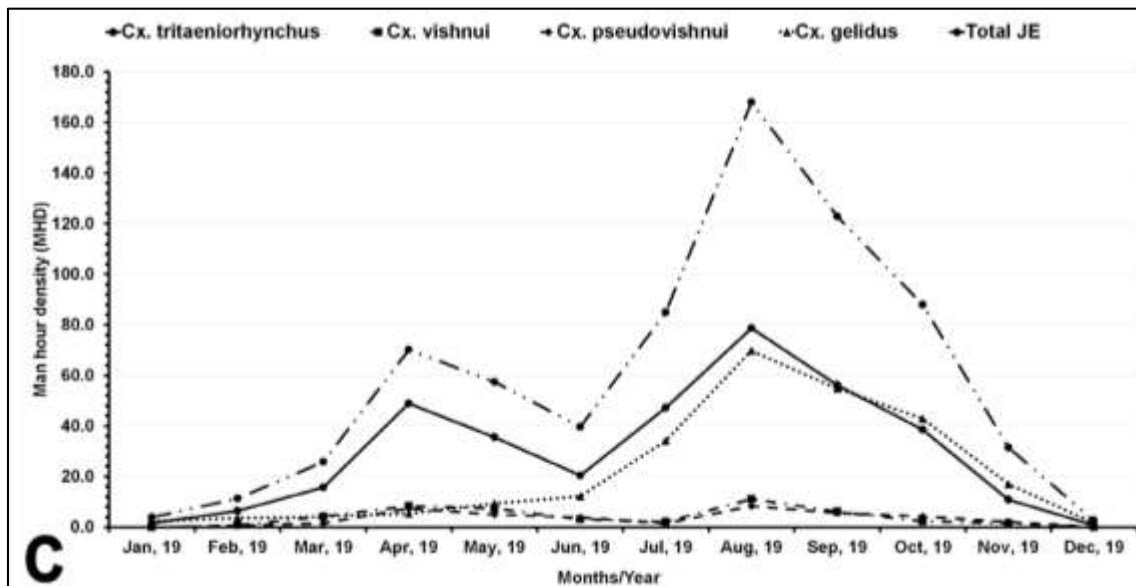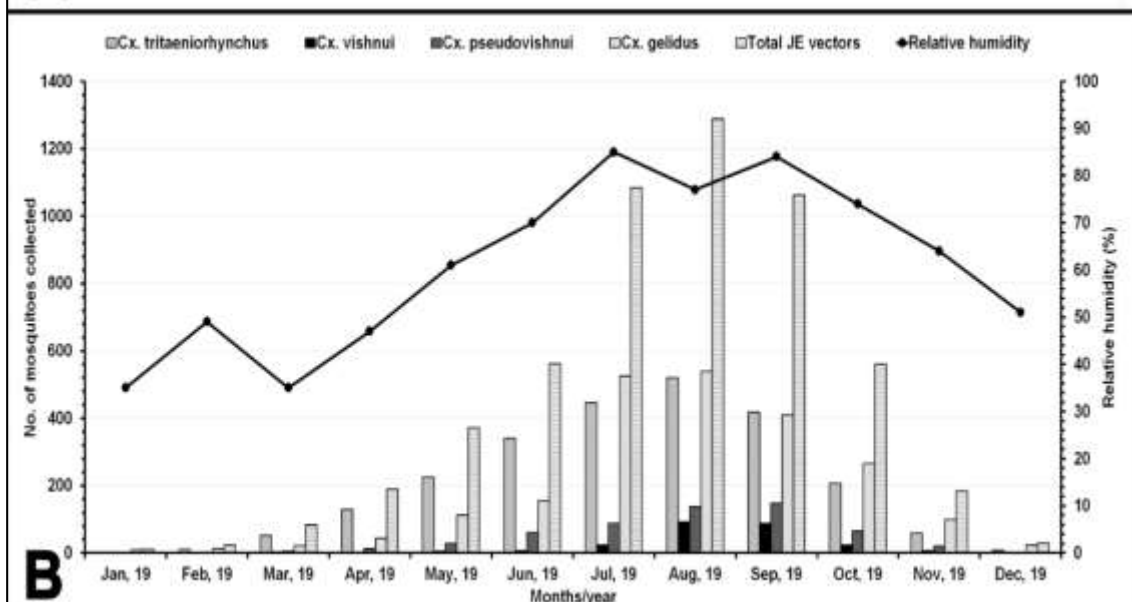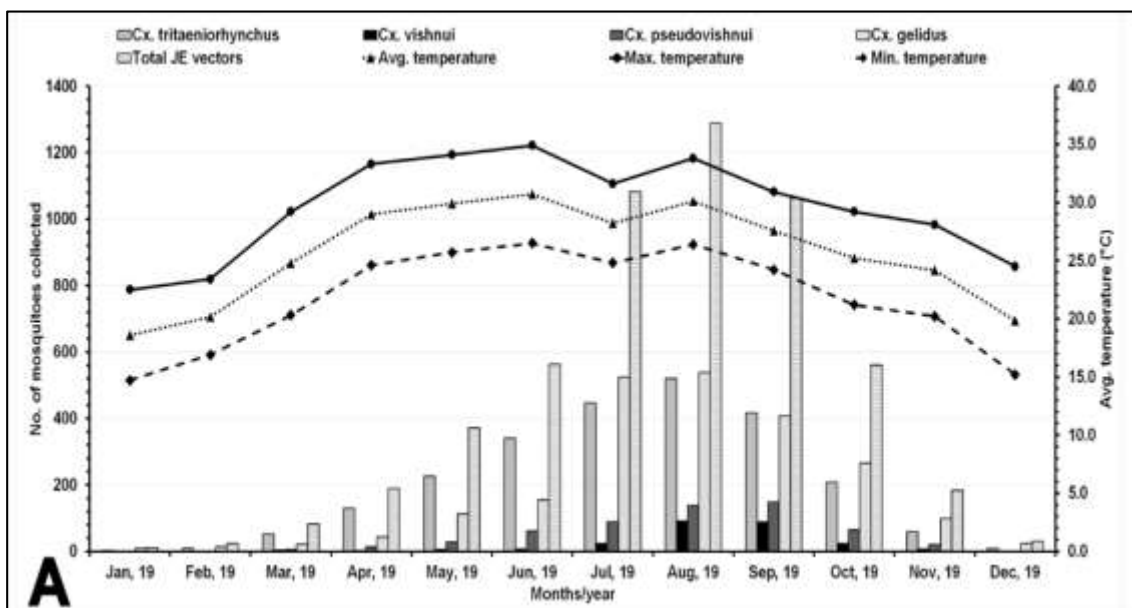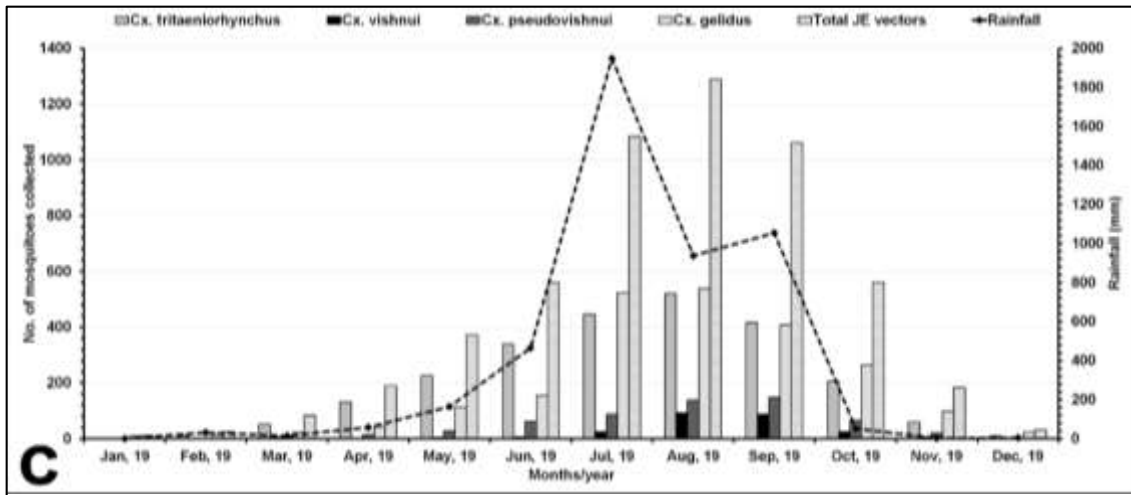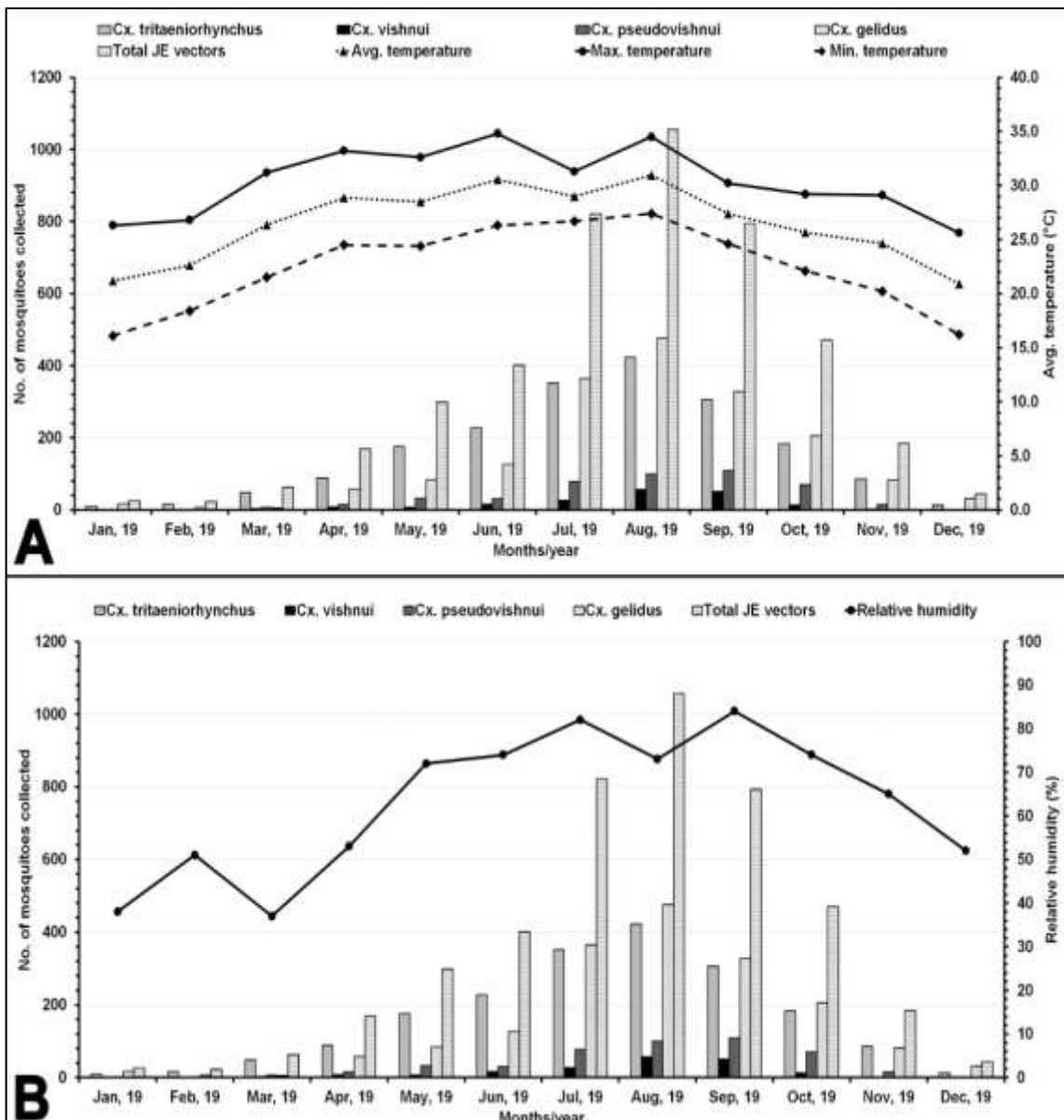
Fig 4: Month wise man hour density of major JE vector in Darjeeling (A), Jalpaiguri (B) and Uttar Dinajpur (C)
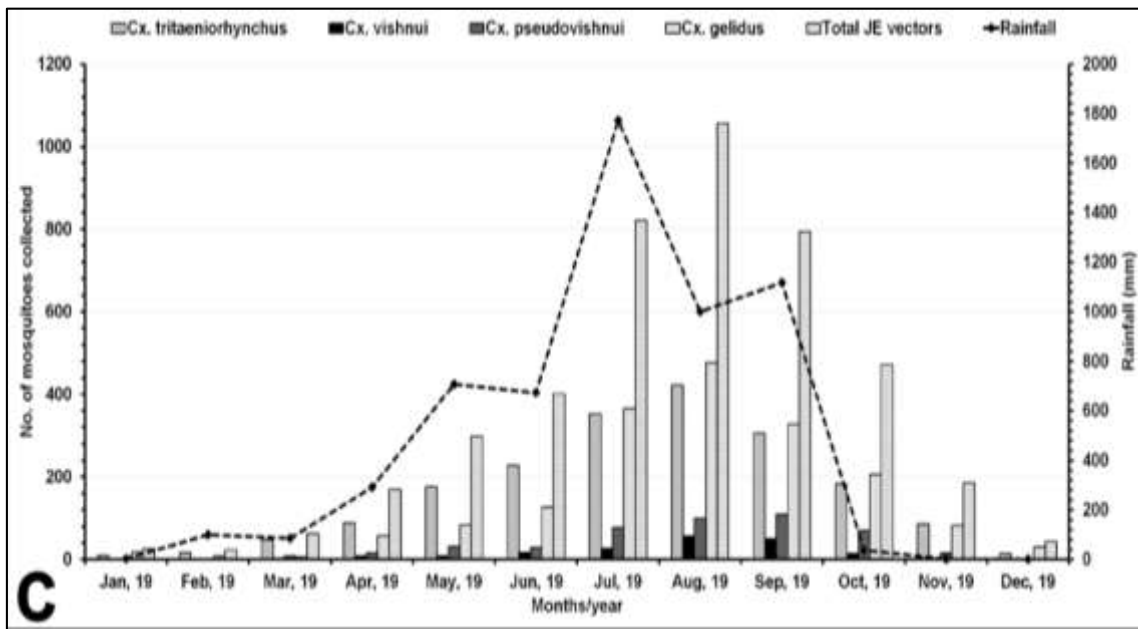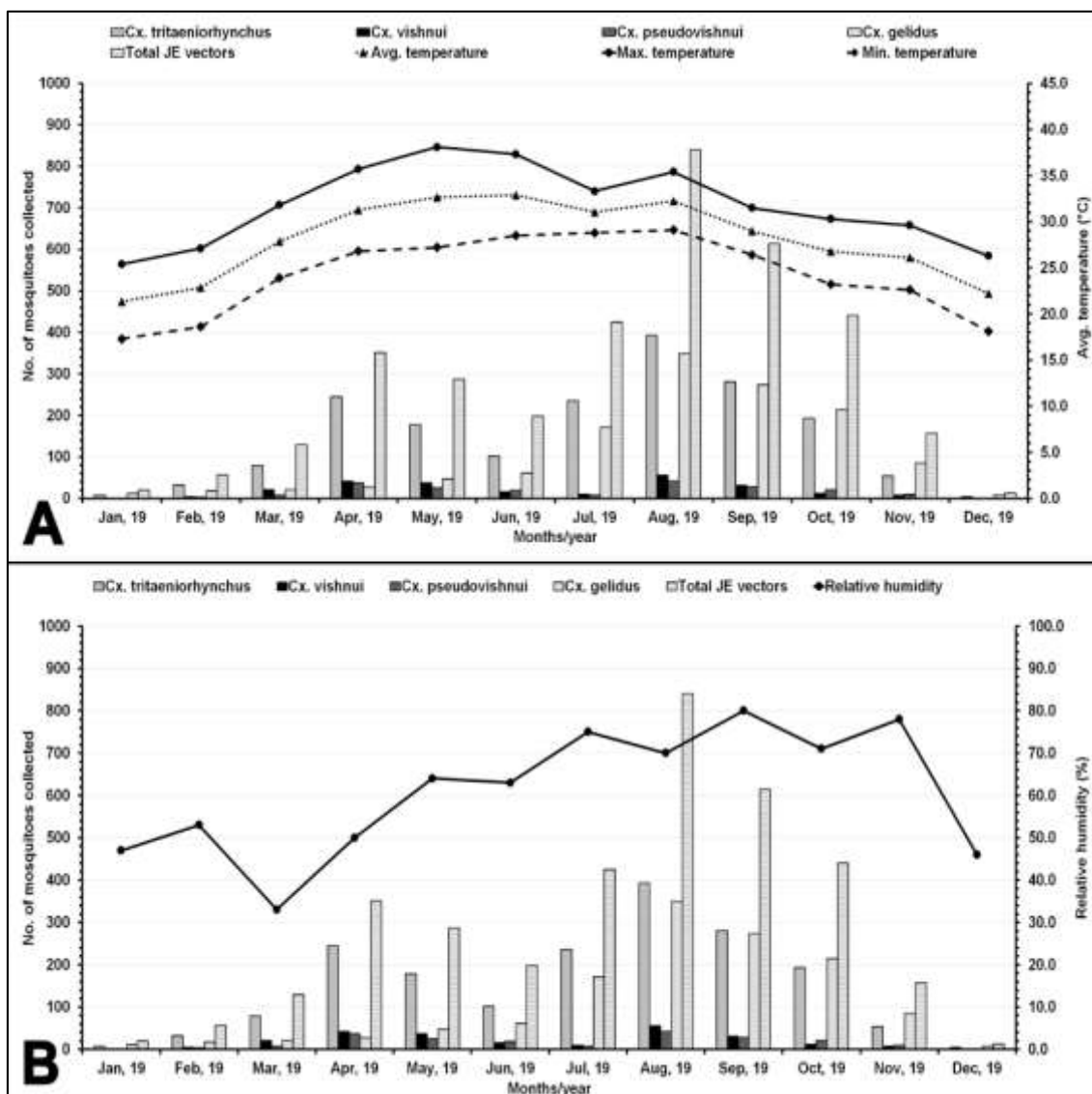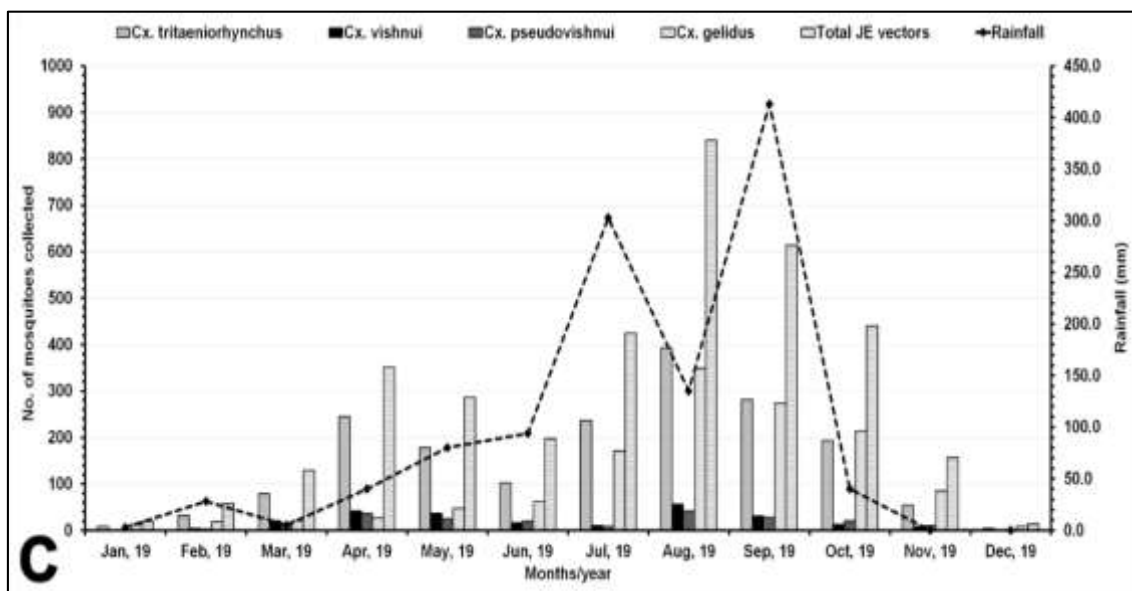
Fig 5: Influence of environmental factors on population density of JE vector species in Darjeeling district, West Bengal. A: Maximum, minimum and average temperature, B: Relative humidity and C: Average rainfall

Fig 6: Influence of environmental factors on population density of JE vector species in Jalpaiguri district, West Bengal. A: Maximum, minimum and average temperature, B: Relative humidity and C: Average rainfall

Fig 7: Influence of environmental factors on population density of JE vector species in Uttar Dinajpur district, West Bengal. A: Maximum, minimum and average temperature, B: Relative humidity and C: Average rainfall

5. Conclusion
The results of this study may serve as baseline data on population dynamics of JE vector and impact of environmental factors on JE vector population which may be helpful for formulation of effective vector control strategies. Abundance of several species of JE vectors was determined through this prospective entomological study in the three northern districts of West Bengal, which indicates the vulnerability and possible threat of future JE outbreaks in the study area. The vectors were found almost throughout the year with highest peak in monsoon and post-monsoon months. So, implementation of vector control and surveillance programme during monsoon and post monsoon months in northern districts of West Bengal is the need of the hour.

Authors' contribution
AKM and PS designed the study; SB, MC, AB, TP performed the field survey, , collection and identification of mosquitoes; PS, MC, SB, AB, TP, AKM perform the data analysis and interpretation; AKM, PS, MC, SB prepare the manuscript.

Conflicts of interest
We have no conflicts of interest concerning the work reported in this article.

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