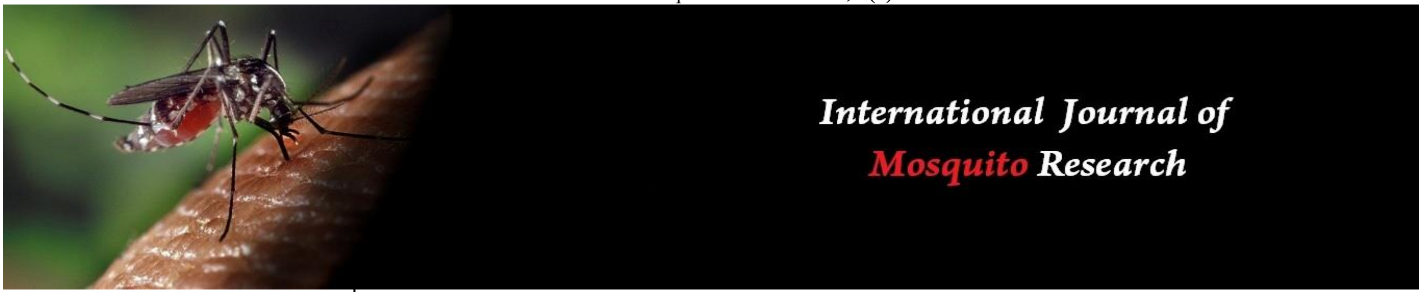

ISSN: 2348-5906
CODEN: IJMRK2
IJMR 2021; 8(2): 134-144

www.dipterajournal.com
Received: 17-01-2021
Accepted: 04-03-2021

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Seasonal dynamics and relative abundance of seven Japanese encephalitis vectors of *Culex sitiens* group and their association with meteorological factors in various ecological habitats of Chandigarh and its surrounding areas

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DOI: https://doi.org/10.22271/23487941.2021.v8.i2b.527

Abstract
Japanese encephalitis (JE) is one of the most common cause of acute viral encephalitis which poses serious infection of the brain. Species under *Culex sitiens* group are major vectors responsible for the transmission of JE. The knowledge about the occurrence of *Culex sitiens* group in Chandigarh and its adjoining areas is very scanty. Hence, present investigations have been carried out to study seasonal dynamics and relative abundance of JE vectors in these areas. Detailed surveys have been carried out in various ecological habitats of Chandigarh from June 2017–November 2019. The monthly meteorological parameters of the study–area like temperature, rainfall, and relative humidity were compared and statistically correlated with JE vector density. As many as 34 mosquito species belonging to 8 genera have been recorded. Genus *Culex* contributed maximum (69.40%) of the total catch which included 7 incriminated JE species from the studied habitats. These vectors showed highest density in monsoon and post–monsoon periods. The abundance of *Culex sitiens* vectors indicates their importance for evaluation of the seasonal dynamics which ultimately demarcates the risk of JE outbreak. The observation over a period of three years is highly significant to assess the seasonal dynamicity of JE vector population in different seasons and its abundance in different ecological habitats.

Keywords: Japanese encephalitis, *Culex*, *sitiens* group, vector, Chandigarh

1. Introduction

Japanese encephalitis (JE) is one of the most dreadful and emerging mosquito borne viral disease prevalent in India. About a century ago, the disease was endemic in East Asia, especially in Japan, China, Korea and Taiwan and spread widely in Southeast Asia subsequently causing outbreaks of varying magnitudes in India, Indonesia, Burma, Sri Lanka and Thailand [1]. It was less recognized in India untill 1973, confined only to southern parts of India with low prevalence. Since then, various outbreaks have been observed in other states like Bihar, Uttar Pradesh, Assam, Manipur, Maharashtra, Haryana, West Bengal, Orissa and union territories of Goa and Pondicherry [2]. The incidence of JE in recent times is showing an increasing trend in the north-western states of India. Roop *et al*. (2013), reported the first indigenous transmission of JE in four urban areas of Delhi [3]. The Directorate of National Vector Borne Disease Control Programme (NVBDCP) reported 1214 cases and 181 deaths due to JE from 15 states/UTs in 2011. However, since 2014 to till date, NVBDCP reported 2560 cases and 297 deaths from Uttar Pradesh, 499 cases, 90 deaths from Bihar whereas, 13 cases with 2 deaths in Haryana, 3 cases from Punjab and 19 other states [4]. In 2018, two children have been found positive with JE virus in Solan district of Himachal Pradesh and one death of a child due to JE was also reported from Dera Bassi near Chandigarh in 2018 (Unpublished data). In India, so far Japanese encephalitis virus has been isolated from 10 species of genus *Culex* [4].

These species prefer to breed in variety of ecological habitats such as stagnant water in paddy fields or drainage ditches, temporary freshwater habitat, polluted waters, small pools, water with filamentous algal growth [4] and water body with high abundance of aquatic weeds like

Eichhornia, *Phragmites*, *Typha* etc. (Mosquito Research and Management 2013). Change in the environment has a great impact on the breeding habitats of different species. The meteorological factors affect adult mosquito species by altering the quality and quantity of breeding habitats. The relationship between weather parameters and mosquito population can provide important information to determine vector abundance and risk associated with their increasing density.

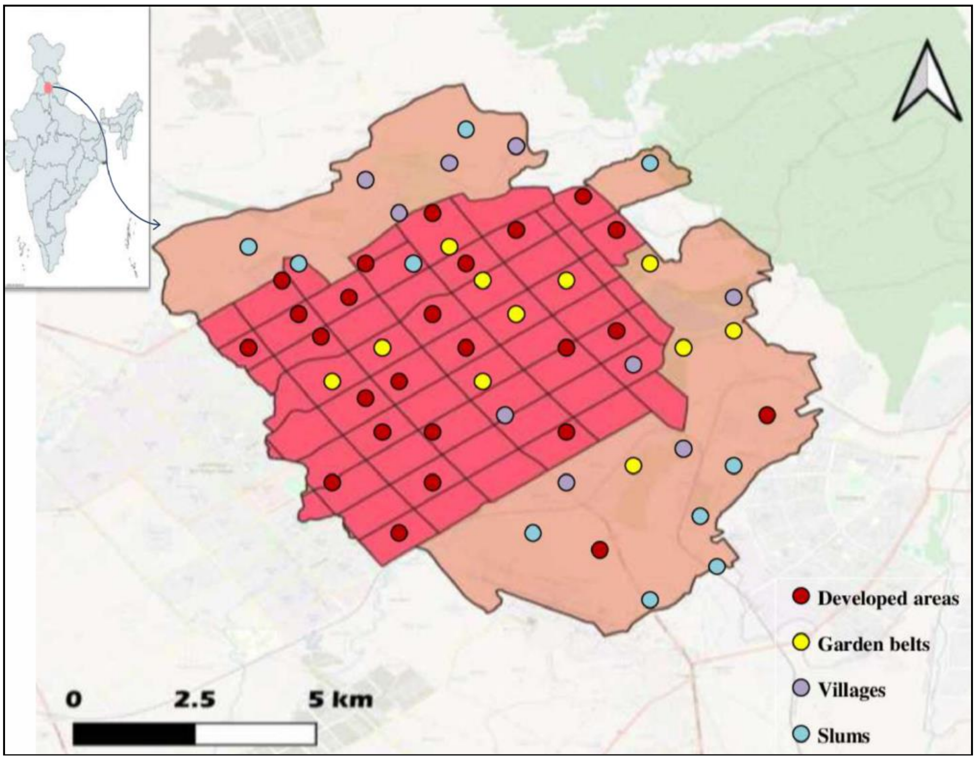
Hence, it is worthwhile to mention here, that the disease is spreading at an alarming rate in almost every part of the country, including northwest India but, except for few references, the data on mosquito species, particularly of JE vectors prevailing in Chandigarh and its adjoining areas is not available. In the present communication, entomological surveys were conducted for the period of three years to find out the seasonal prevalence and relative abundance of mosquito species (known to be the vectors of Japanese encephalitis virus) from different ecological habitats of Chandigarh. Out of the collected nineteen species of genus *Culex*, seven species associated with JE have been recorded in this area. Therefore, keeping in view, the importance of mosquito species in carrying JE virus, the present investigations have been carried out to explore the association with weather parameters with JE vectors in various rural and urban ecological habitats in and around Chandigarh from pre–monsoon to post–monsoon periods.

2. Material and Methods

2.1 Study area: The study was conducted in different ecological habitats of Chandigarh. Chandigarh is located near foothills of the Shivalik range of Himalayas in northwest India (30.74º N, 76.79º E) bordering Punjab and Haryana states. The study areas have variety of favourable habitats for mosquito breeding like thick vegetation which cover around 8.77% of total geographical areas, 3245.30 hectares forest area, green belts of gardens, lakes full of flora and fauna (also attracts various species of migratory birds from parts of Siberia and Japan in the early winter season), paddy fields and slum areas on its outskirts. This area also receive occasional rainfalls of 1103.7mm annually, maximum in monsoons (80%) followed by post–monsoons. Different seasons like (i) Summer occur in March–May, (ii) Pre–monsoon (June–July), (iii) Monsoon (August–September), (iv) Post–monsoon (October–November) and (v) Winter (December – Mid March).

2.2 Mosquito collection and Identification: Collection sites covering both urban and rural habitats were selected and more than 109 rounds of surveys were conducted for three years June 2017– November 2019 to catch mosquito samples. These sites were further categorized into four different ecological habitats viz; developed urban areas, garden belts, surrounding villages and slum areas (Fig. 1). The collection of adult mosquitoes was made with oral aspirator and hand–nets from various resting sites i.e. in and around cattle sheds, human dwellings, mixed dwellings and other sites in and around Chandigarh from 6am to 9am in the morning and 4pmto 8pm in the evening hours.

These samples were then brought to the laboratory, killed at 30–37°C for 8–10 minutes and preserved in insect storage boxes after pinning. The specimens were individually counted, examined and further identification was done under a Stereo Zoom Trinocular microscope for generic and species separation by following the standard identification keys [5, 6, 7, 8, 9]. The species were further confirmed by studying the details of their male and female genitalia. The slides of male and female genitalia were prepared by following the method of Siverly and Shroyer (1974) [10] and examined at 20x and 40x magnification using compound microscope and photographed by using digital Gentx camera and Gryphax software



Garden Belts	Villages	Slums	Developed Urban areas
1.Bougainvillea Garden I	1.Maloya	1.Khuda Lahora	1.Sector 4
2.Leisure Valley	2.Nayagaon I	2.Mauli Jagran	2.Sector 8
3. Zakir Hussain Rose Garden	3.Khuda Ali sher	3.BapuDham Colony I	3.Sector14
4.Fragnance Garden	4.Dhanas	4.BapuDhamColony II	4.Sector 15
5.Terraced Garden	5.Kishan Garh	5.Burail	5.Sector 38
6.Topiary Garden	6.Hallomajara	6.Dadu Majra	6.Sector 39
7. Pointsettian & Ixora Garden	7.Shangariwala	7.Sector 25	7.Sector 46
8. Botanical Garden (Sec-14)	8.Mastgarh	8.Madanpur	8.Sector 48
9. Hibiscus Garden	9.Sarangpur	9.Raipur khurd	9.Sector 2
10.Dahlia Garden	10.Kajheri	10.Makkanmajra	10.Sector 9
11.Garden of Silence	11.Mataur	11.Railway Colony	11.Sector 16
12.Botanical Garden (Dhanas)	12.Kumbra	12.Palsora	12.Sector 24
13.Valley of Animal	13.Kaimbra		13.Sector 34
14. Garden of Springs	14.Kaimbali		14.Sector 36
15. Garden of Palms (Sec-42)	15.Jagatpura		15.Sector 45
16. Bougainvillea Garden II	16.Bairmajra		16.Baltana
17. Japanese Garden	17.Behlana		17.Sector 31
18.Shanti Kunj	18.Kishangarh		18.Sector 40
19. Botanical Garden(Sukhna)	19.Saketri		19.Bal Bhawan
20.Butterfly Garden	20.Kaimbwala		20.Mohali
21 Rose Garden (Sec-14)	21.Karoran		21.Nada sahib
	22.Karor Upali		22.Panchkula
	23.Kansal		23.Zirakpur
	24.Mullanpur		24.Manimajra
	25.Tagon		25.Sukhna Lake
	26.Khuda Lohora		26.New Lake Sec-42
	27. Khuda Jassu		27. Mohali 6 Phase
	28. Daria		28. Mohali 3B2
	29.Raipur Kalan		29. Sector-19
	30. Nayagoan II		30. Sector 22
	31. Nizampur Burail		(& other sectors of Chandigarh)

Fig 1: Map showing Study areas of Chandigarh and its surroundings (via QGIS mapping)

2.3 Meteorological Data Collection: For meteorological parameters like maximum and minimum temperature, relative humidity and rainfall data during the period June 2017–November 2019 was collected on monthly basis from India Meteorology Department, Sector–39, Chandigarh. Average maximum temperature of the last three years was 34.55°C while minimum 24.40°C. Maximum temperature was recorded in the pre–monsoons and early months of monsoons while minimum temperature was recorded during the late post–monsoon period. The average relative humidity and average rainfall for survey period was 65.11% and 3.86mm respectively (Table 1).

2.4 Statistical analysis: The statistical analysis was performed to determine the correlation between climatic factors and JE vector density by using Pearson correlation coefficient. Further, regression analysis including p–value and correlation coefficient (r) was computed by using SPSS® 16.0 to test the significant statistical difference between mosquito density and associated climatic factors.

Table 1: Average annual climatic data of Chandigarh during June 2017 to November 2019

S.no.	Year	Months	Temperature (°C)		Relative Humidity	Average Rainfall
			Min Temperature	Max Temperature	(%)	(mm)
1.	2017	Pre-monsoon (June)	26.36 °C	36.38 °C	55.08%	4.52mm
		Monsoon (July–September)	25.58 °C	33.83 °C	74.54%	6.95mm
		Post–monsoon (October–November)	14.81 °C	29.70 °C	66.74%	0.0mm
2.	2018	Pre-monsoon (June)	26.96 °C	37.15 °C	57.59%	5.66mm
		Monsoon (July–September)	25.34 °C	33.12 °C	79.05%	8.94mm
		Post–monsoon (October–November)	14.53 °C	33.5 °C	63.43%	0.23mm
3.	2019	Pre-monsoon (June)	27.07 °C	45.07 °C	45.00%	0.88mm
		Monsoon (July-September)	25.25 °C	33.77 °C	76.43%	6.77mm
		Post–monsoon (October–November)	15.76 °C	28.51 °C	68.21%	0.83mm

Source: Meteorology Department Chandigarh

3. Results and Discussion

3.1 Species composition: During present investigations, a total of **21,970** mosquitoes belonging to 8 genera viz; *Culex*, *Aedes*, *Armigeres*, *Verrallina*, *Mansonia*, *Anopheles*, *Coquillettidia* and *Mimomyia* were collected. Out of these, genus *Culex* was the most dominant with 69.40% of total catch followed by *Aedes* (23.18%), *Armigeres* (5.62%), *Verrallina* (1.16%), *Mansonia* (0.26%), *Anopheles* (0.11%), *Coquillettidia* (0.11%) and *Mimomyia* (0.13%). The genus *Culex* was represented by 19 species *viz*; *Culex (Culex) quinquefasciatus*, *Culex (Culex) fuscocephala*, *Culex (Culex) vagans*, *Culex (Culex) univittatus*, *Culex (Culex) theileri*, *Culex (Culex) hutchinsoni*, *Culex (Culex) tritaeniorhynchus*, *Culex (Culex) pseudovishnui*, *Culex (Culex) vishnui*, *Culex (Culex) summorosus*, *Culex (Culex) whitei*, *Culex (Oculeomyia) bitaeniorhynchus*, *Culex (Culex) sitiens*, *Culex (Oculeomyia) sinensis*, *Culex (Culex) gelidus*, *Culex (Culex) whitmorei*, *Culex (Oculeomyia) luzonensis*, *Culex (Oculeomyia) infula* and *Culex (Culex) mimulus*. Out of these, 13 species belonging to *sitiens* group have been found, of which seven are important vectors of Japanese encephalitis in India (Table 2). Hence, keeping in view, the importance of these species in carrying Japanese encephalitis virus, the present studies have been carried out to know the distribution, relative abundance and seasonal prevalence of seven vector species from Chandigarh and its surrounding areas.

Table 2: Composition of species belonging to *Culex sitiens* group in Chandigarh and its adjoining areas

S. No.	Species Name	Total captured			Total percentage
		2017	2018	2019	
1.	*Culex (Culex) tritaeniorhynchus**	3282	3564	2473	76.52
2.	*Culex (Culex) pseudovishnui**	398	512	36	7.76
3.	*Culex (Culex) vishnui**	198	276	54	4.33
4.	*Culex (Culex) summurosus*	28	441	361	6.81
5.	*Culex (Culex) whitei*	37	1	–	0.31
6.	*Culex (Culex) sitiens*	87	277	112	3.90
7.	*Culex (Culex) whitmorei**	25	25	5	0.45
8.	*Culex (Culex) gelidus**	36	130	111	2.27
9.	*Culex(Oculeomyia)bitaeniorhynchus**	92	82	25	1.63
10.	*Culex (Oculeomyia) sinensis*	47	38	13	0.80
11.	*Culex (Oculeomyia) luzonensis*	6	–	11	0.13
12.	*Culex (Oculeomyia) infula**	4	2	4	0.08
13.	*Culex (Culex) mimulus*	1	–	1	0.01
Total		4241	5352	2585	12,178

(*) Marked species are vectors of Japanese encephalitis virus in India [4].

3.2 Seasonal prevalence and relative abundance of JE vectors: The relationship of weather parameters like maximum temperature, minimum temperature, relative humidity and rainfall with JE vector population for the three years in transient seasons like pre–monsoon, monsoon and post–monsoon from June 2017 to November 2019 (Table 3) was observed. Highest peaks of most vectors were observed during monsoon to initial post–monsoon while smallest peaks during pre–monsoon (June) and late post–monsoon in almost all ecological habitats (Fig. 3). Out of the three weather variables, the statistically significant values ($p<0.05$) of JE vectors have been observed with relative humidity (55%–75%) and rainfall (4mm–6.9mm) as compared to temperature during three years (Table 4).

The seasonal distribution of JE vectors reveals the fluctuations in different seasons from June 2017–November 2019. These species appeared in pre–monsoon season (2.67%) and reach the major peaks during monsoon period (80.74%) and decline during post–monsoon season (16.57%) (Fig. 2). However, monthly distribution of JE vectors has shown different trends throughout survey periods in different ecological habitats of the study area.

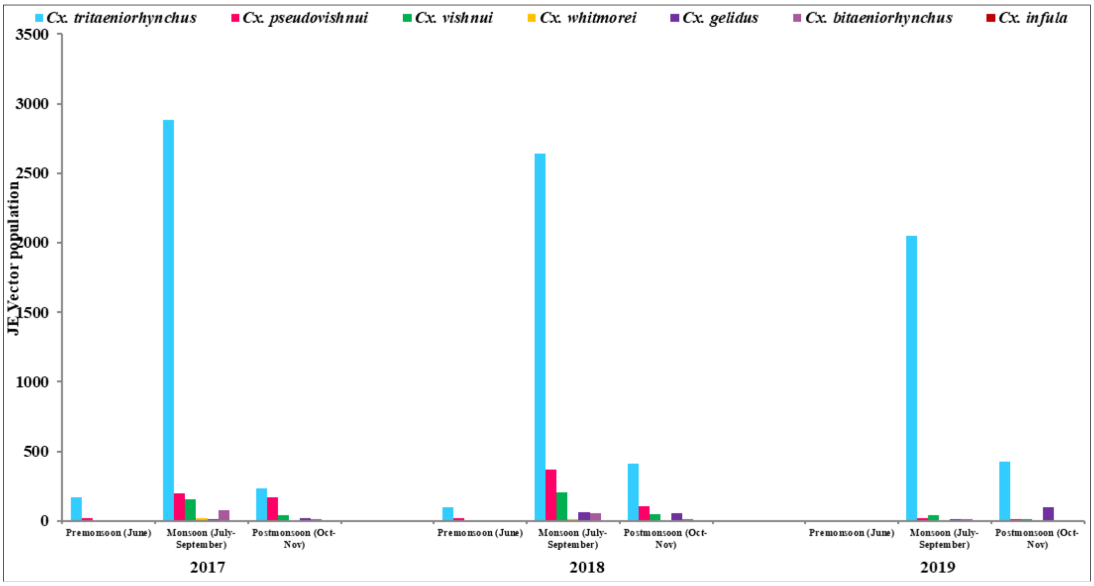
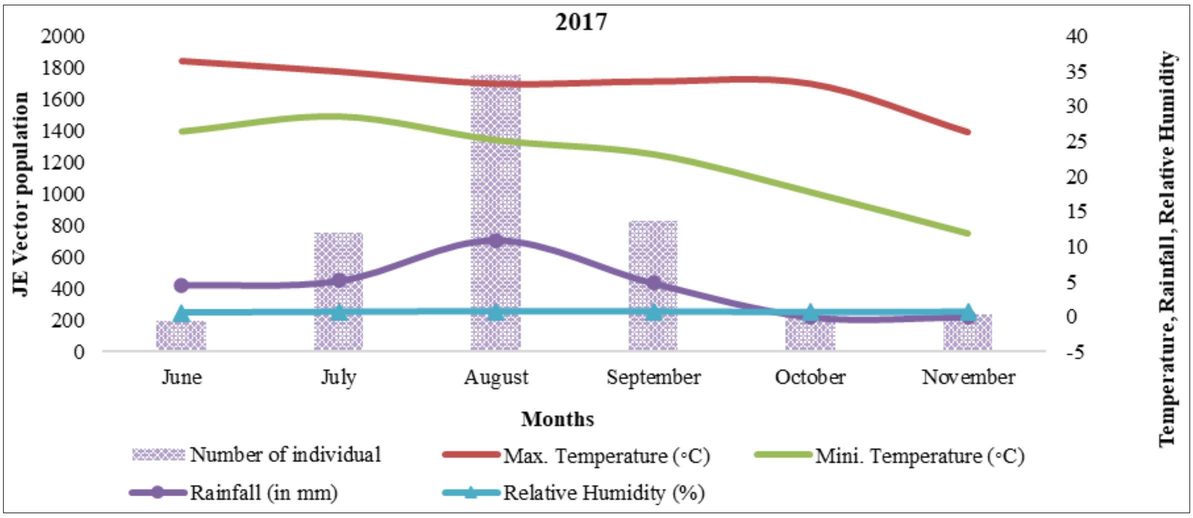


Fig 2: Seasonal abundance of JE vectors in Chandigarh and its surrounding areas

Table 3: Monthly Relationship between JE Vector Populations and Weather Parameters in Chandigarh and its surroundings

Study Years	Survey periods	Total number of JE vector captured							Total (n)	Weather Parameters (Mean±SD)			
		Cx. tritaeniorhynchus	*Cx. pseudovishnui*	*Cx. vishnui*	*Cx. whitmorei*	*Cx. gelidus*	*Cx. bitaeniorhynchus*	*Cx. infula*		Max temp. (˚C)	Min temp. (˚C)	RH	Rain fall (mm)
2017	Pre–monsoon	168	24	01	01	01	04	–	199	36.39±0.01	26.33±0.04	55±0.007	4.47±0.07
	Monsoon	2880	201	155	18	14	75	01	3344	33.83±0.92	25.58±2.71	75±0.04	6.95±3.43
	Post–monsoon	234	173	42	06	21	13	03	492	33.31±4.87	20.43±4.15	67±0.04	00±00
2018	Pre–monsoon	99	18	–	–	–	02	–	119	37±0.07	26.96±0.66	58±0.004	5.66±0.11
	Monsoon	2644	366	203	15	60	55	01	3344	33.12±1.22	25.34±2.06	79±0.01	8.94±1.80
	Post–monsoon	415	103	48	08	57	15	01	647	33.5±2.55	14.53±2.55	63±0.04	0.23±0.06
2019	Pre–monsoon	–	–	–	–	01	01	–	02	45.07±0.007	27.07±0.01	0.45±0.007	0.81±0.014
	Monsoon	2047	22	40	03	12	17	–	2141	33.77±0.45	25.25±1.51	76±0.007	6.78±3.19
	Post–monsoon	426	14	14	01	98	05	–	558	28.51±3.32	15.76±3.16	68±0.07	0.81±1.12

n- total number of JE vectors in different survey periods and SD-Standard Deviation



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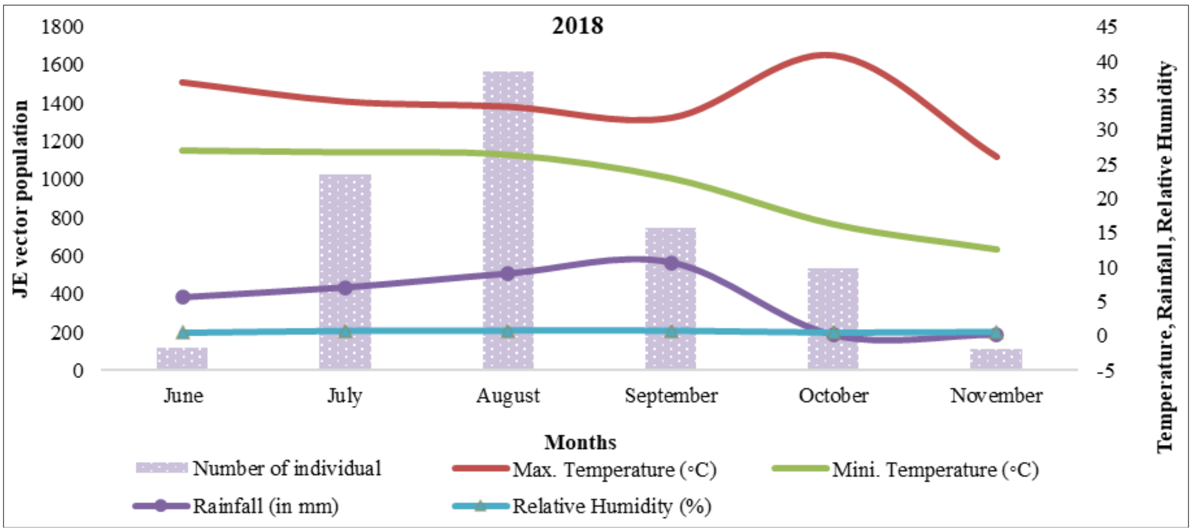

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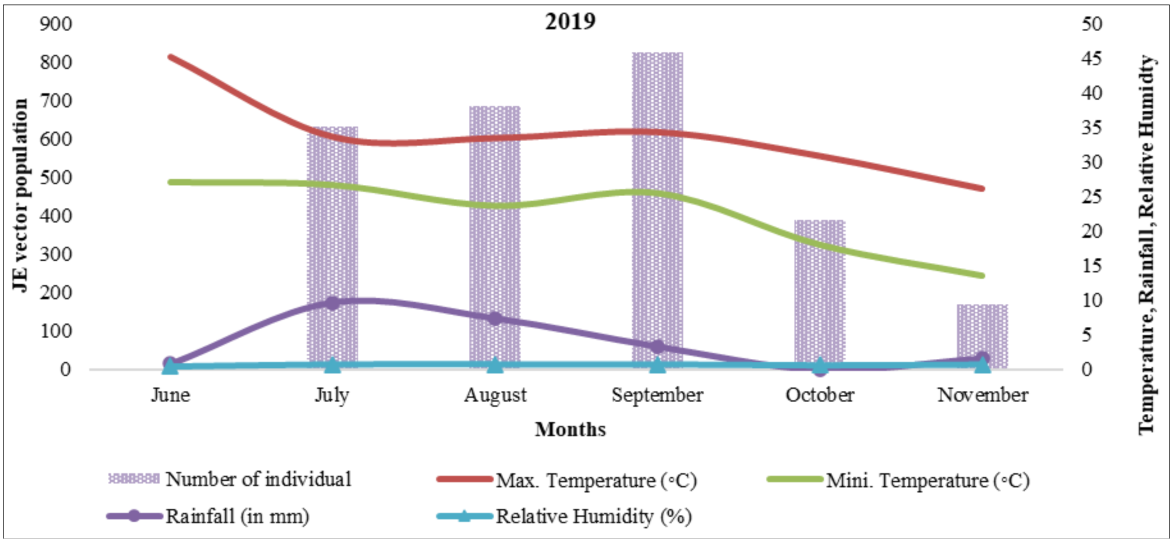

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Fig 3: Seasonal occurrence of JE vectors with meteorological parameters like temperature, rainfall and relative humidity in study areas during (A) 2017, (B) 2018, and (C) 2019.

3.3 Seasonal fluctuation of seven JE vector populationsand its relationship with different meteorological parameters in study habitats

3.3.1 *Culex (Culex) tritaeniorhynchus* Giles

The maximum density of *Culex tritaeniorhynchus* has been recorded from urban areas (36.66%), followed by garden belts (28.07%), villages (24.75%) and slum areas (10.50%). In urban areas, the population of *Cx. tritaeniorhynchus* started appearing in June (1.17%), and increased immediately with the onset of monsoon. The maximum adult density of *Culex tritaeniorhynchus* (89.69%) was observed during July–September which declined gradually from October–November (9.1%). Murty *et al*., (2010) also observed a high density of *Culex tritaeniorhynchus* in urban areas as compared to rural areas irrespective of the season [11]. Although rice fields are the most preferable breeding sites in villages, its high population dynamics in urban areas than rural areas is pointing towards the most congenial ecological conditions in the vicinity of residential areas.

In gardens, the adult density was (89.14%) in July–September, which declined sharply to 9.9% in October–November. Their presence in gardens in July–September may be due to the availability of temporary water bodies which developed due to monsoon rains and soon got dried after the rainy season. In slums areas too, the maximum number of individuals were recorded from July–September (86.10%) as compared to 12.56% in October–November. In surrounding villages, the individuals of *Culex tritaeniorhynchus* were recorded throughout the period of June–November with maximum density seen in July–September (73%) and 18.16% in October–November. Its availability in pre–monsoon, monsoon and post–monsoon season in villages may be due to its preferences to breed in rural settings in rice fields, ground pools, ponds with aquatic vegetation and also due to the presence of a variety of hosts like cattle, birds, horses, pigs and other domestic animals.

*Cx. tritaeniorhynchus*has showed strong positive correlation between abundance and relative humidity ($p<0.05$) in 2017 and with lag rainfall ($p<0.05$) both in 2018–2019 (Table 5). Out of all ecological habitats, a strong positive correlation has been observed between abundance and relative humidity in villages ($p<0.05$) and slums ($p<0.05$) (Table 6).

Cx. tritaeniorhynchus is considered an important vector of Japanese encephalitis (JE) in India and also in many countries of Southeast Asia. *Cx. tritaeniorhynchus* has yielded total 79 JE isolations viz; 9 virus isolations from Vellore (Tamil Nadu) and Kolar (Karnataka), 2 from Mandya (Karnataka), 58 from Cuddalore (Tamil Nadu), 7 from Kuttanadu (Kerala), 1 from Gorakpur of Uttar Pradesh, North India and 2 from Karnataka [12-16]. So far, incidence of JE has been reported from 15 states of India [17].

3.3.2 *Culex (Culex) vishnui* Theobald

The adult density of *Cx. vishnui* was also observed maximum in urban areas (42.61%), gardens (24.43%), villages (24.24%) and in slums (8.71%). In urban areas, the maximum percentage of *Cx. vishnui* (88%) was recorded during July–September as compared to 12% in October–November. Similarly in gardens, this species was recorded as 79.06% in July–September, which decreased to 20.93% in October–November. In villages too, it was 72.65% in July–September and decreased to 26.56% in October–November. In slums, during July–September, the individuals obtained were 58.69% which decreased to 41.30% during October–November. During present studies, a strong correlation has been observed between abundance of *Cx. vishnui* and maximum temperature in developed areas ($p<0.05$), minimum temperature in slums ($p<0.05$) while, relative humidity in villages ($p<0.05$). However, no positive correlation has been observed between abundance and weather parameters in garden belts ($p>0.05$) of Chandigarh (Table 6).

In 2013, the Department of Medical Parasitology, PGI conducted a survey on medically important mosquitoes and reported the presence of *Cx. vishnui* in large numbers in the vicinity of houses of Dhanas villages and Sector 25 (unpublished data). The breeding places of *Cx. vishnui* are very similar to *Cx. tritaeniorhynchus* [18] and both the species can be found in association with one another. *Culex vishnui* is another important vector of Japanese encephalitis in India. So far, 30 virus isolations have been made from *Culex vishnui* in Tamil Nadu, Karnataka and West Bengal [4].

3.3.3 *Culex (Culex) pseudovishnui* Colless

The adult density of *Culex pseudovishnui* also followed a similar pattern to *Culex vishnui* and *Culex tritaenorhynchus*. The maximum number of individuals of *Cx. pseudovishnui* were observed in urban areas (43.96%) followed by surrounding villages (22.45%), slums (18.96%) and gardens (14.61%). In urban areas, 72.53% adult density of *Cx. pseudovishnui* was obtained during July–September which reduced to 27.22% in October–November. In gardens, 76.81% of *Cx. pseudovishnui* was obtained during July–September. In villages, 45.75% of density was found in July–September followed by 39.15% in October–November. In slum areas, 55.7% of density was noticed in July–September while, it was 40.22% in October–November. A similar pattern for the prevalence of *Cx. pseudovishnui* which showed peak in August during the period of an epidemic of JE has been made in Goa [19]. During present investigations, a strong positive correlation of *Cx. pseudovishnui* with minimum temperature has been observed in developed urban areas ($p<0.05$), maximum temperature in slums ($p<0.05$) and with relative humdity in villages ($p<0.05$) around Chandigarh. However, no significant correlation of weather parameters with abundnace of *Cx. pseudovishnui* in garden belts has been seen (Table 6).

Culex pseudovishnui is also a potential vector of JE in India as total eight JE virus from Kolar (Karnataka) and Goa have been isolated so far [4, 19, 20]. The occurence of three vector species of *Cx. vishnui* subgroup viz; *Cx. vishnui*, *Cx. pseudovishnui* and *Cx. triateniorhynchus* has been reported along with various intraspecific variations from Chandigarh province [21, 22]. All the three species belonging to *Cx. vishnui* subgroup prefer to breed in ground pools, ponds, wells, ditches, puddles containing fresh or polluted water with grasses and aquatic vegetation [7]. The breeding habits of *Cx. pseudovishnui* are very similar to *Cx. tritaeniorhynchus* and *Cx. vishnui*. It breeds in freshwater ground pools, rice fields and stream pools [23]. Reuben (1971) reported the presence of larvae in rice fields when plant reached 0.3 m in height [20].

3.3.4 *Culex (Culex) whitmorei* Giles

Cx. whitmorei was obtained 32.72% in villages and 30.90% in urban areas, 29.09% in gardens while in slums it was 7.27%. In villages, the maximum percentage was found in July–September (83.33%) which reduced to 11.1% in October–November. In urban areas too, the high density was observed in July–September (70.58%), which declined to 29.41% in October–November. However, in slums areas a different trend has been seen. These were highest i.e., 75% during post monsoon period (October–November). In addition to this, in almost all habitats, maximum female specimens of *Cx. whitmorei* were found, i.e., about 0.5% of total catch. Earlier workers have also reported only 5% of adults of total catch [20, 25, 26]. During present work, strong correlation with maximum temperature ($p<0.05$) has been found in urban areas. However, other meteorological parameters like minimum temperature, rainfall and relative humidity have shown no significant relation with abundance in villages, garden belts and slums (Table 6).

The breeding places of *Cx. whitmorei* are freshwater ground pools, puddles, ditches in rice fields with aquatic vegetation [18]. It is a chief vector of JE in India, a total of four JE virus isolations have been made in India so far out of which, two were from Vellore (Tamil Nadu) and Krishna (AP) and two from Kolar (Kar) and West Bengal [4, 18].

3.3.5 *Culex (Culex) gelidus* Theobald

The abundance of *Cx. gelidus* was 36.23% in villages followed 32.97% in gardens, 27.89% in urban areas and slum areas it was 2.87%. In urban areas, the percentage of *Cx. gelidus* was 15.58% during July–September which increased to 84.41% during post-monsoon season i.e., October–November. However, in villages, these were abundant during July–September (60%) and reduced to 39% in October–November. In slums, these were found only during post–monsoon season (87.5%). In gardens too, highest peak density of adults was obtained in post–monsoon season (85.71%). Hence, except in villages, this species was available in post–monsoon periods in most areas of Chandigarh. During these studies, a strong correlation between minimum temperature and abundance of *Cx. gelidus* ($p<0.05$) was observed in 2019 whereas, other parameters like relative humidity and rainfall have not been found to be correlated with its abundance (Table 5). Out of four ecological habitats, *Cx. gelidus* has shown no significant correlation between abundance and all meteorological parameters in developed urban areas, villages, gardens and slums in and around Chandigarh (Table 6).

The corresponding facts regarding abundance of *Cx. gelidus* in the post–monsoon period was also observed in Bareilly, Uttar Pradesh [27]. It might be due to the presence of swampy water bodies as this species prefers to breed in marshy water containing abundant aquatic vegetation or in muddy pools with a high concentration of organic matter [7, 28]. They also observed that accumulated manures and stagnant water left over in the paddy fields facilitates *Cx. gelidus* breeding. Murty *et al*., (2010) observed predominantly high percentage (68.05%) in urban areas and least percentage in rural areas of Kurnool district of Andhra Pradesh [11]. Philip *et al*., (2016) mentioned the gradual replacement of *Cx. tritaeniorhynchus* with *Cx. gelidus* in urban and semi–urban areas [29].

Cx. gelidus has been reported from various states of India viz; Maharashtra, Goa, Rajasthan, Karnataka, Kerala, Tamil Nadu, Andhra Pradesh, West Bengal and Assam. But, from Chandigarh and its adjoining areas,it has been observedwith various variations in morphology. In India, earlier studies carried out on *Cx. gelidus* showed the negligible percentage of the total population of mosquitoes while, in recent years, it has increased to more than 50% of the total mosquito population in the same area (NIV Annual report 2009–2010, 2012–2013).

Cx. gelidus has shown wide susceptibility to arboviruses associated with family *Togaviridae*, *Flaviviridae*, *Rhabdoviridae* and *Bunyaviridae*. The natural isolations of Getah (GETV), Japanese encephalitis virus (JEV), Ross River (RRV) and Tembusu virus have already been documented suggesting their susceptibility to these viruses [30]. Recent experimental studies have also shown that the mosquitoes are susceptible to Chikungunya virus (CHIKV), Chandipura virus (CHPV) and Chittoor (CHITV, Batai group) virus in Pune (Sudeep, unpublished data). Many Isolations of JEV have also been made from *Cx. gelidus* in India. A total number of 8 JE isolations have been made of which 5 isolations are from Cuddalore district of Tamil Nadu and 3 from Mandya in Karnataka [4, 15].

3.3.6 *Culex* (*Oculeomyia*) *bitaeniorhynchus* Giles

Culex bitaeniorhynchus was found in almost all the ecological habitats with maximum density in urban areas (40.7%) followed by gardens (38.3%), villages (14.4%), slums (6.46%). In urban areas, the adults were abundant during July–September (82.92%) while, got decreased to 15.86% during October– November. In gardens too, almost 79.22% of adults were reported during July–September which reduced drastically to 19.48% in October–November. In villages, these were 62.06% during July–September (monsoon) and decreased to 24% in October–November. In slums, 53.84% in monsoon period (July–September) while, reduced to 38% in the post-monsoon period (October–November). As far as its realtion with weather parameter is concerned, it showed a strong positive correlation with relative humidity and lag rainfall but no significance in relation with temperature in 2017 (Table 5). Out of 4 habitats, strong positive correlation (p<0.05) was observed in garden belts but no significant correlation in urban areas, villages and slums around Chandigarh (Table 6).

During present investigations, two forms of *Culex bitaeniorhynchus* i.e., ‘*typical’* and ‘*tenax’* form has also been observed along with various intraspecific variants. *Culex bitaeniorhynchus* breeds in swamps, ponds, irrigation ditches filled with a dense mass of filamentous green algae [7, 18]. *Culex bitaeniorhynchus* is important from viewpoint of spreading various diseases like Japanese encephalitis and filariasis in tropical and subtropical regions of Asia. First JE virus isolation from *Culex bitaeniorhynchus* was reported from Malaysia [31]. In Korea, it has also been incriminated as a possible vector of Japanese encephalitis [32]. Further, 3 isolations of JE virus have been made in Karnataka and West Bengal of India [4]. Iyengar (1938) reported *bitaeniorhynchus* naturally infected with *Wuchereria bancrofti* in the oriental region, Travancore, India [33].

3.3.7 *Culex* (*Oculeomyia*) *infula* Theobald

Cx. infula was reported at its highest density in villages (83.33%), followed by urban areas (1.66%) whereas not even a single adult was found in gardens and slum areas, During the present investigations, 80% of catch was recorded in villages during post–monsoon seasons while 20% in late monsoon period. In urban areas, only one adult was recorded during late monsoon period. *Cx. infula* has showed strong positive correlation (p<0.05) with rainfall whereas no significant correlation with other meteorological parameters in all of the ecological habitats in and around Chandigarh (Table 5, 6).

Cx. infula mainly feeds on cattle but a few fraction on birds and humans as larvae are available in water courses with high content of algae [34]. *Cx. infula* is also considered as an important vector of JE, as total of 6 JE virus isolations were made from *Cx. infula* viz; 1 from Madurai (TN) and 5 from Cuddalore (TN) [35, 36].

Table 4: Analysis of Pearson’s correlation coefficient (r–value) between the meteorological parameters and JE vectors population density

Meteorological parameters	2017		2018		2019	
	r–value	p–value	r–value	p–value	r–value	p–value
Maximum Temperature (°C)	0.15	0.76	0.08	0.87	-0.26	0.60
Minimum Temperature (°C)	0.44	0.37	0.52	0.28	0.33	0.52
Relative Humidity (%)	0.90	0.01*	0.79	0.05*	0.63	0.17
Rainfall (mm)	0.78	0.05*	0.62	0.18	0.76	0.05*

(Pearson’s correlation coefficient (r); Significant p value* (confidence interval of 95%) p <0.05 considered to be statistically significant)

Table 5: Pearson’s correlation coefficient (r) of most JE vectors with meteorological parameters and their significant (p) value

Species	Maximum temp. (T_{max})		Minimum temp. (T_{min})		Rainfall		Relative Humidity	
	r	p–value	r	p–value	r	p–value	r	p–value
2017								
Culex tritaeniorhynchus	0.21	0.68	0.51	0.29	0.71	0.08	0.92	0.008*
Cx. pseudovishnui	-0.45	0.35	-0.48	0.32	0.15	0.77	0.54	0.26
Cx. vishnui	-0.02	0.96	0.09	0.86	0.73	0.09	0.67	0.13
Cx. whitmorei	-0.39	0.43	-0.66	0.14	0.98	0.85	-0.62	0.18
Cx. gelidus	0.00	0.99	-0.27	0.95	0.57	0.23	0.24	0.63
Cx. bitaeniorhynchus	0.12	0.81	0.45	0.36	0.81	0.04*	0.87	0.02*
Cx. infula	-0.28	0.58	-0.49	0.31	-0.24	0.64	-0.71	0.11
2018								
Culex tritaeniorhynchus	0.03	0.95	0.50	0.30	0.77	0.007*	0.64	0.16
Cx. pseudovishnui	0.22	0.66	0.35	0.49	0.30	0.55	0.10	0.84
Cx. vishnui	0.21	0.68	0.45	0.36	0.69	0.12	0.45	0.36
Cx. whitmorei	-0.18	0.71	-0.49	0.32	0.01	0.97	0.13	0.79
Cx. gelidus	0.47	0.34	-0.02	0.96	0.31	0.54	0.19	0.71
Cx. bitaeniorhynchus	0.28	0.58	0.36	0.47	0.70	0.11	0.61	0.19
Cx. infula	0.37	0.46	-0.30	0.56	-0.02	0.96	-0.01	0.98
2019								
Culex tritaeniorhynchus	-0.20	0.69	0.39	0.43	0.75	0.018*	0.66	0.14
Cx. pseudovishnui	-0.60	0.19	-0.28	0.58	-0.007	0.98	0.68	0.13
Cx. vishnui	-0.37	0.46	0.94	0.86	0.61	0.19	0.75	0.08
Cx. whitmorei	-0.62	0.18	-0.86	0.02*	-0.02	0.95	-0.62	0.18
Cx. gelidus	-0.10	0.84	0.27	0.60	0.33	0.52	0.06	0.89
Cx. bitaeniorhynchus	-0.15	0.76	0.19	0.71	0.58	0.22	0.24	0.63
Cx. infula	0.03	0.95	0.27	0.59	0.29	0.05*	-0.06	0.90

Table 6: Statistical analysis of Pearson's correlation coefficient (r) between weather parameters and Population density (PD) of JE vectors from four ecological

Species	Developed urban area		Villages		Garden belt		Slums	
	r	p–value	r	p–value	r	p–value	r	p–value
Culex tritaeniorhynchus								
Mean T_{max} *vs* PD	-0.67	0.53	-0.62	0.56	0.22	0.98	-0.67	0.52
Mean T_{min} *vs* PD	-0.27	0.82	-0.90	0.27	-0.42	0.72	-0.93	0.23
Mean RH% *vs* PD	-0.13	0.91	1.00	0.01*	0.75	0.45	0.99	0.02*
Mean Rainfall *vs* PD	-0.32	0.07	0.98	0.10	0.86	0.33	0.97	0.14
Cx. pseudovishnui								
Mean T_{max} *vs* PD	-0.87	0.32	-0.61	0.57	0.06	0.96	0.99	0.002*
Mean T_{min} *vs* PD	-0.99	0.03*	-0.90	0.28	-0.38	0.74	-0.89	0.289
Mean RH% *vs* PD	0.93	0.22	1.00	0.02*	0.72	0.48	0.64	0.55
Mean Rainfall *vs* PD	0.85	0.34	0.98	0.10	0.84	0.36	0.49	0.67
Cx. vishnui								
Mean T_{max} *vs* PD	-0.99	0.02*	-0.64	0.55	0.43	0.97	-0.94	0.21
Mean T_{min} *vs* PD	-0.91	0.26	-0.91	0.26	-0.40	0.73	-0.99	0.05*
Mean RH% *vs* PD	0.61	0.53	0.99	0.001*	0.73	0.47	0.86	0.33
Mean Rainfall *vs* PD	0.51	0.65	0.98	0.12	0.85	0.35	0.75	0.45
Cx. whitmorei								
Mean T_{max} *vs* PD	-0.99	0.03*	-0.85	0.34	0.04	0.97	-0.98	0.12
Mean T_{min} *vs* PD	-0.91	0.25	-0.54	0.63	-0.40	0.73	-0.78	0.42
Mean RH% *vs* PD	0.68	0.52	0.16	0.89	0.73	0.47	0.47	0.68
Mean Rainfall *vs* PD	0.53	0.64	-0.02	0.98	0.84	0.35	0.30	0.80
Cx. gelidus								
Mean T_{max} *vs* PD	0.99	0.08	-0.16	0.89	0.97	0.15	-0.64	0.55
Mean T_{min} *vs* PD	0.82	0.37	-0.58	0.60	0.76	0.44	-0.24	0.84
Mean RH% *vs* PD	-0.53	0.64	0.85	0.34	-0.43	0.71	-0.17	0.89
Mean Rainfall *vs* PD	-0.36	0.76	0.94	0.22	-0.26	0.83	-0.35	0.77
Cx. bitaeniorhynchus								
Mean T_{max} *vs* PD	-0.99	0.08	-0.86	0.34	-0.67	0.53	-0.72	0.47
Mean T_{min} *vs* PD	-0.94	0.20	0.54	0.63	-0.93	0.23	-0.95	0.18
Mean RH% *vs* PD	0.74	0.46	0.16	0.89	0.99	0.02*	0.99	0.07
Mean Rainfall *vs* PD	0.60	0.59	-0.02	0.98	0.97	0.14	0.95	0.19
Cx. infula								
Mean T_{max} *vs* PD	-0.01	0.99	-0.95	0.18	–	–	–	–
Mean T_{min} *vs* PD	-0.45	0.69	-0.73	0.47	–	–	–	–
Mean RH% *vs* PD	0.77	0.43	0.39	0.74	–	–	–	–
Mean Rainfall *vs* PD	0.87	0.31	0.21	0.86	–	–	–	–

Pearson's correlation coefficient (r); Significant p value* (confidence interval of 95%) $p < 0.05$ considered to be statistically significant, PD-Population Density

4. Conclusions

The present study has been done keeping in view the importance of JE vector species and their relation with meteorological factors in the divided ecological habitats of the study area. The present analysis has shown that suitable ecological conditions are available for the establishment of these seven JE vectors in almost all ecological habitats i.e., developed urban areas, villages, garden belts and slums of study areas. These vector populations have shown highest density in July–September (monsoon period) except *Cx. gelidus* and *Cx. infula* which were recorded maximum in post–monsoon months. As far as weather parameters are concerned, statistically significant values ($p<0.05$) have been observed with relative humidity (55%–75%) and rainfall (4mm–6.9mm). Since the information about distribution, seasonal prevalence and relative abundance of JE vectors in and around Chandigarh was not available, the present investigations will be immensely beneficial to know the dynamics of Japanese encephalitis in this area and to forecast the outbreak of the disease and its control well in time.

5. Acknowledgments

The authors are highly thankful to DST–SERB for providing financial grant in the form of Core Research project (EMR/2016/003185) under which the present work has been carried out.

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