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Entomological surveillance of dengue vectors in Tamil Nadu, India

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Abstract
Dengue infection is most important rapidly growing arboviral disease of public health concern. On the basis of repeatedly occurrence of dengue outbreaks in India, wide and extensive (covering large areas) entomological surveillance was conducted in different districts of Tamil Nadu to detect the dengue prevalence and transmission by analysing the major breeding sources and abundance of *Aedes* mosquitoes. The entomological survey data was analysed in terms of various larval indices. The survey of adult female mosquitoes has also been carried out to assess its entomological Importance. The larval indices analysed included HI, CI, BI, and PI which varied from 2.50 – 18.26, 0.83 – 9.03, 5.00– 31.71 and 0.00 – 46.15 respectively. The Pupae Per Container and Pupae Per Positive Container varied from 0.00 – 0.33 and 0.00 – 5.50 respectively. The Container Positivity varied from 25.93 (Earthen Pots) –0.46 (Plastic Buckets). The Adult Premise Index showed variation from 1.82 – 18.26. Whereas Females Per House inspected varied from 0.07-0.33 and Females Per Positive House (for *Aedes* mosquitoes) showed variation from 1.00 – 13.00. The *Aedes aegypti* followed by *Aedes vittatus* and *Aedes albopictus* were abundantly reported. It can be concluded that due to the availability of various breeding sources, *Aedes* larval and adult stages were detected throughout study areas of Tamil Nadu and thus, there are the chances of occurrence of dengue fever infections. The various organisations of vector control should take the necessary steps to educate the people of study areas. The people should be informed about the practices of source reduction in order to reduce the chances of dengue outbreaks.

Keywords: Extensive Study, *Aedes* Species Composition, Breeding Sources, Larval Indices, Pupal Indices, Adult Density Based Indices, Tamil Nadu, India.

1. Introduction
Dengue infection is most important rapidly growing arboviral disease of public health concern. Dengue epidemics occurred regularly in tropical, subtropical and temperate areas of the world. It has been reported that 2.5 billion people are living in areas of risk, with millions of cases occurring each year [1-3]. The severe forms of dengue infection; dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) causes massive mortality especially among infants [4-7]. Dengue infections are reported throughout the world including India, where the first dengue outbreak was reported in Delhi in 1988 [8]. Now the dengue infection has been reported from all over the country [5-6, 9-12], with major outbreaks reported from Tamil Nadu [13-16]. The dengue cases and deaths are increasing and spreading their range to the new areas of Tamil Nadu and all the four serotypes of dengue virus were reported. The number of dengue cases and deaths in Tamil Nadu since 2008 is shown in Table 6 (NVBDCP website http://nvbdcp.gov.in/den-cd.html). As no vaccine/drug is available yet, the Vector Control Programme has proven, valuable tool to control the dengue fever. The Vector Control Programme is based on the various techniques of entomological surveillance which play an important role in determining the population density and distribution, to detect and monitor the larval habitats of *Aedes* mosquitoes in a particular area and thus helps in early alarming about the dengue outbreak [17-19]. On the basis of repeatedly occurrence of dengue outbreaks in India, wide and extensive (covering large areas) entomological surveillance was conducted in different districts of Tamil Nadu to detect the dengue prevalence and transmission by analysing the major breeding sources and abundance of *Aedes* mosquitoes. The entomological survey data was analysed in terms of various larval indices. The survey of adult female mosquitoes has been also carried out to assess its entomological Importance.

2. Materials and methods
The entomological survey was carried out (December 2013 – April 2014) extensively (covering large areas) in 21 villages of 10 districts of Tamil Nadu India. (Table 1, Fig 1).

Table 1: Location of study areas.

District name	Latitude	longitude	Villages inspected
Villupuram	11° 57' N	79° 32' E	Kallidayalam
			Sendiakuppam
			Senithal
Kanyakumari	8° 3' N	77° 15' E	Nagerkoil
			Kalkulam
Madurai	9° 58' N	78° 10' E	Melur
			Anna nagar
Tiruchirappalli	10° 50' N	78° 46' E	Lalgudi
			Pullambedi
Thanjuvur	10° 47' N	79° 10' E	Thanjuvur
			Pattukottai
Thoothukudi	8° 77' N	78° 19' E	Tuticorin
			Kovilpatti
Nammakal	11° 13 ' N	78° 13' E	Pudupatti
			Valayapatty
Perambalur	11° 14' N	78° 56 ' E	Perambalur
			Kunan
Dharmapuri	12° 08' N	78° 13' E	Dharmapuri
			Sithri hills
Virudhunagar	9° 35' N	77° 57' E	Vendurayapuram
			Naranapuram

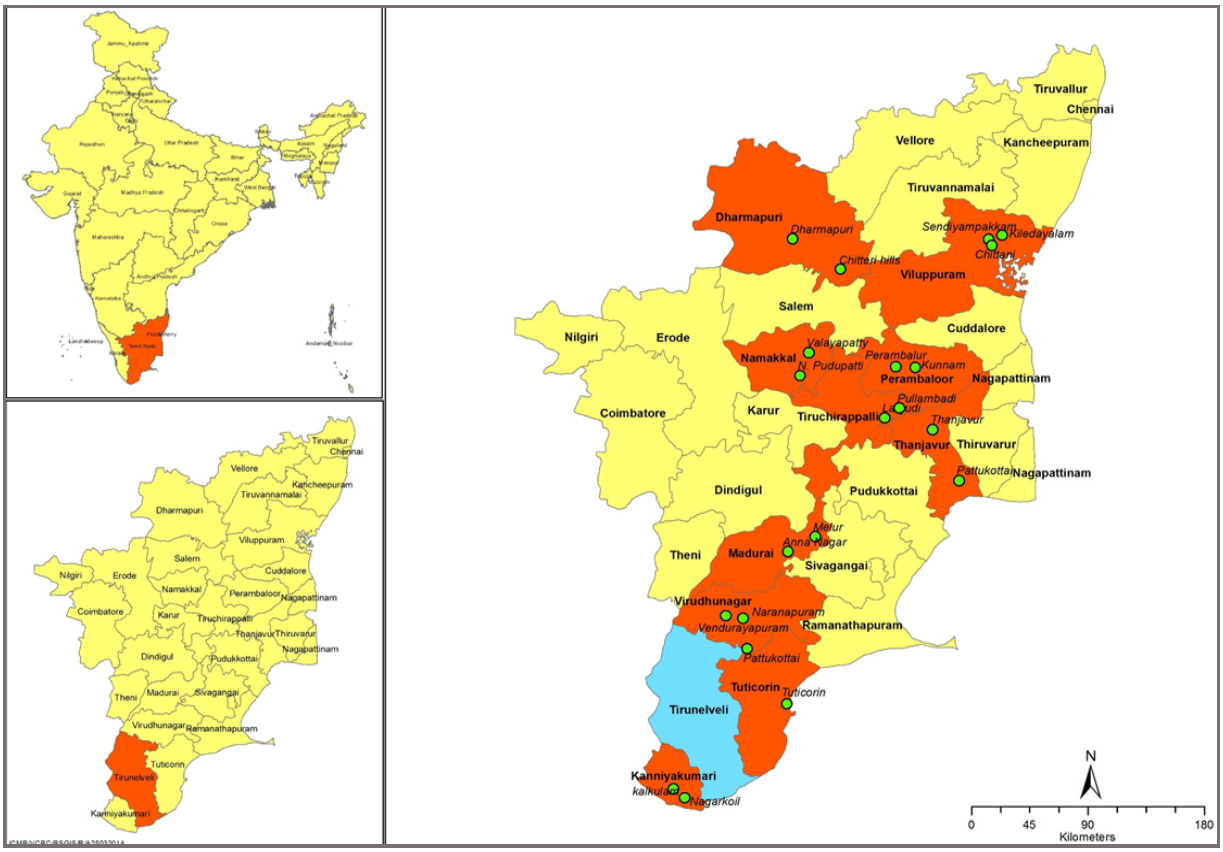

Fig 1: Map showing study areas.

The study was performed in randomly selected houses to determine the preferred breeding sources and distribution dynamics of dengue vectors across the Tamil Nadu which is sixth most populous and the eleventh largest state in India, covering an area of 130,058 square kilometres. The bordering states are Kerala to the west, Karnataka to the northwest, Andhra Pradesh to the north, and the Bay of Bengal to the east. The southern most tip of the Indian Peninsula is located in Tamil Nadu. At this point is the town of Kanyakumari which is the meeting point of the Arabian Sea, the Bay of Bengal, and the Indian Ocean. Tamil Nadu is heavily dependent on monsoon rains, and thereby is prone to droughts when the monsoons fail. The climate of the state ranges from dry sub-humid to semi-arid. The normal annual rainfall of the state is about 945 mm (37.2 in). Since the state is entirely dependent on rains for recharging its water resources, monsoon failures lead to acute water scarcity and severe drought.

2.1 Larval Collection

All kinds of indoor and outdoor breeding habitats were examined to collect the *Aedes* immatures by following the dipper method (Reuben, 2014) [20]. A container containing any amount of water was considered as wet container and the wet container containing any number of immatures (larvae, pupae or both) was considered as positive container. The immatures were collected by using different immature collecting materials like pipettes, dipper, strainer depending upon the type and size of breeding source. The collected immatures were kept in plastic containers labeled with the code of breeding source, locality code, house identification code and date of collection. The samples were carried to the laboratory at Vector Control Research Centre (VCRC), Pondicherry. The immatures (larvae and pupae) were counted and reared in enamel (rearing) trays for their emergence into adults. Every day the emerged mosquitoes were collected and identified according to species and sex. In this way species composition and sex ratio of emerged mosquitoes was calculated. The larval survey data was calculated and analysed in terms of different larval survey techniques like House Index (HI), Container Index (CI), Breateau Index (BI), Pupal Index (PI), Pupae Per Container (PPC) and Pupae Per Positive Container (PPPC) according to standard methods [21]. The calculation of larval indices is based on the following mathematical formulae:

House Index (HI) = Number of houses infested/ Total number of houses inspected multiplied by 100.

Container Index (CI) = Number of positive containers infested/ Total number of containers inspected multiplied by 100.

Breateau Index (BI) = Number of positive containers/ Total number of houses inspected multiplied by 100.

Pupal Index (PI) = Number of pupae collected/Total number of houses inspected multiplied by 100.

Container Positivity = Number of positive containers (infested)/Total number of containers inspected multiplied by 100.

2.2 Adult collection

Adult female *Aedes* mosquitoes were collected from the same selected premises as those studied during the larval survey. The collection of female *Aedes* mosquitoes was performed by following the standard protocol [19, 22]. Both indoor and outdoor resting places were explored to collect the female adult *Aedes* mosquitoes using mechanical/oral aspirators and flash torch was used to locate the resting places of mosquitoes from dark areas. The collected adult mosquitoes were stored in test tubes labelled with locality code, house identification code and date of collection. The adult density was calculated by means of Adult Premise Index (API, number of positive houses for adult female *Aedes* mosquitoes divided by the number of inspected houses multiplied by 100); Adults Per House (number of female adult *Aedes* mosquitoes collected divided by houses inspected); Adults Per Positive House (number of collected adult female *Aedes* mosquitoes per positive house for *Aedes* mosquitoes). The collected and emerged adults were pinned and identified under microscope to separate them according to species and sex by using the standard taxonomic keys [23, 27].

3. Results

Out of 606 houses surveyed, 70 houses were found to support the breeding of *Aedes* mosquitoes and 111 containers were reported positive for the presence of *Aedes* immatures (Table 2). The distribution of *Aedes* mosquitoes were calculated in terms of various Larval and Adult Density Based Indices (ADBI). The larval indices were analysed in terms of HI, CI, BI, and PI which varied from 2.50 (Tiruchirappalli) – 18.26 (Villupuram), 0.83 (Tiruchirappalli) – 9.03 (Villupuram), 5.00(Tiruchirappalli) – 37.71 (Nammakal) and 0.00 (Dharmapuri) – 46.15 (Kanyakumari) respectively (Table 2). Based on the pupal collection, the indices other than PI related to the positive containers has also been calculated. The Pupae Per Container (PPC) and Pupae Per Positive Container (PPPC) varied from 0.00 – 0.33 and 0.00 – 5.50 respectively (Table 3). The Container Positivity varied from 25.93 (earthen pots) – 0.46 (plastic buckets) (Table 3). The Container Positivity is important to determine the major and preferred breeding sources of *Aedes* mosquitoes.

Table 2: Entomological indices used to assess the level of *Aedes* infestations.

District name	Houses		Containers			Larval indices			
	Inspected	Positive	Examined	Positive	pupae	HI	CI	BI	PI
Villupuram	115	21	321	29	35	18.26	9.03	25.22	30.43
Kanyakumari	65	7	650	12	30	10.77	1.85	18.46	46.15
Madurai	65	6	560	9	15	9.23	1.61	13.85	23.08
Tiruchirappalli	40	1	241	2	3	2.50	0.83	5.00	7.50
Thanjuvur	55	3	344	5	7	5.45	1.45	9.09	12.73
Thoothukudi	85	8	650	11	26	9.41	1.69	12.94	30.59
Nammakal	41	6	245	13	8	14.63	5.31	31.71	19.51
Perambalur	50	9	276	12	12	18.00	4.35	24.00	24.00
Dharmapuri	36	2	237	4	0	5.56	1.69	11.11	0.00
Virudhunagar	54	7	548	14	15	12.96	2.55	25.93	27.78

HI – House Index, CI- Container Index, BI – Breateau Index

Table 3: Container Positivity and Pupal Indices.

Container type	Containers examined	Positive containers	Pupae collected	Container positivity	Pupae per container	Pupae per positive container
Coconut Shells	126	6	13	4.76	0.10	2.17
Cement Tanks	623	21	29	3.37	0.05	1.38
Plastic Drums	663	22	34	3.32	0.05	1.55
Plastic Containers	687	23	30	3.35	0.04	1.30
Plastic Buckets	439	2	11	0.46	0.03	5.50
Aluminum Utensils	874	20	21	2.29	0.02	1.05
Grinding Stones	104	2	2	1.92	0.02	1.00
Tires	75	1	0	1.33	0.00	0.00
Flower Pots	135	3	2	2.22	0.01	0.67
Iron Pots	319	4	0	1.25	0.00	0.00
Earthen Pots	27	7	9	25.93	0.33	1.29

The emerged mosquitoes consisted of three species of *Aedes* mosquitoes; *Aedes aegypti* 523 (62.71%), *Aedes vittatus* 290 (34.77%) and *Aedes albopictus* 21 (2.52%) (Fig. 2, Table 4). The emerged species consisted of 508 (60.91%) male and 326 (39.09%) females *Aedes* mosquitoes (Fig. 3)

Total of 106 adult *Aedes* female mosquitoes were collected and 51 houses were found positive for the adult females *Aedes* mosquito collection (Table 5). The composition of adult collection consisted of same three species of mosquitoes as that of emerged mosquitoes composition i.e. *Aedes aegypti* 58 (54.72%), *Aedes vittatus* 32 (30.19%) and *Aedes albopictus* 16 (15.09%) (Fig 4, Table 5). The Adult Premise Index showed variation from 1.82 – 18.26. Whereas Females Per House inspected varied from 0.07-0.33 and Females Per Positive House (for *Aedes* mosquitoes) showed variation from 1.00 – 13.00 (Table 5). The study showed that all the study areas supported various kinds of breeding sources due to which the dengue vectors were abundantly reported.

Table 4: Species composition of emerged mosquitoes.

Container type	*Ae. aegypti*				*Ae.vittatus*				*Ae.albopictus*			
	M	%	F	%	M	%	F	%	M	%	F	%
Coconut Shells	8	0.96	6	0.72	18	2.16	4	0.48	0	0.00	0	0.00
Cement Tanks	51	6.12	40	4.80	30	3.60	25	3.00	0	0.00	0	0.00
Plastic Drums	67	8.03	40	4.80	21	2.52	30	3.60	2	0.24	2	0.24
Plastic Containers	53	6.35	41	4.92	23	2.76	22	2.64	0	0.00	0	0.00
Plastic Buckets	10	1.20	10	1.20	22	2.64	7	0.84	0	0.00	0	0.00
Aluminum Utensils	71	8.51	45	5.40	42	5.04	19	2.28	0	0.00	0	0.00
Grinding Stones	4	0.48	6	0.72	3	0.36	0	0.00	0	0.00	0	0.00
Tires	0	0.00	0	0.00	0	0.00	0	0.00	3	0.36	2	0.24
Flower Pots	7	0.84	0	0.00	0	0.00	2	0.24	8	0.96	4	0.48
Iron Pots	11	1.32	0	0.00	8	0.96	1	0.12	0	0.00	0	0.00
Earthen pots	33	3.96	20	2.40	13	1.56	0	0.00	0	0.00	0	0.00

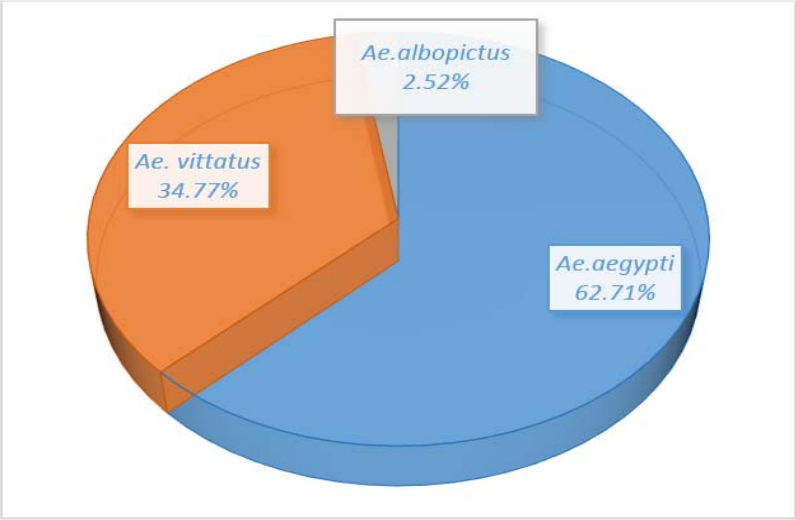

Fig 2: Species composition of emerged mosquitoes.

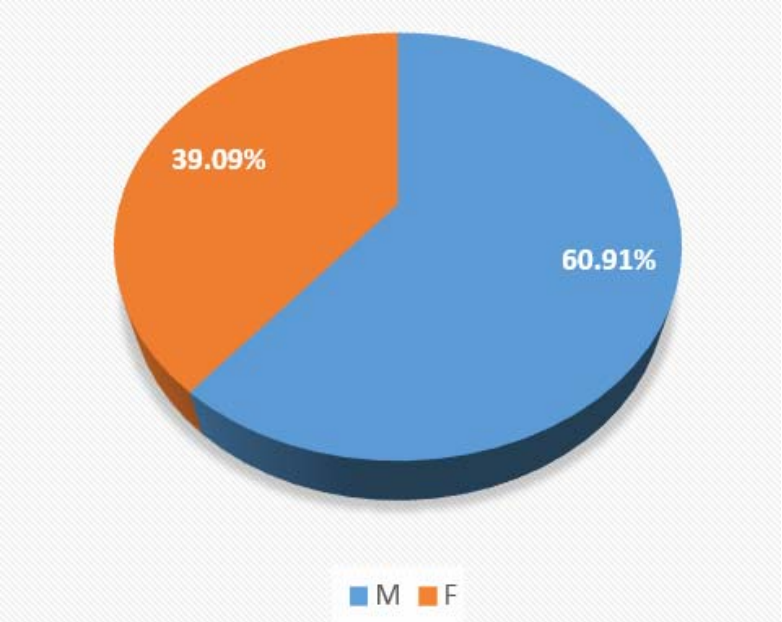

Fig 3: Sex ratio of emerged mosquitoes.

Table 5: Adults *Aedes* female mosquito collection and Adult Density Based Indices.

District name	Total houses	Positive houses for female adults	Total number of females collected	Adults Premise Index (API)	Females Per House	Females Per Positive House
Villupuram	115	21	30	18.26	0.26	1.43
Kanyakumari	65	5	14	7.69	0.22	2.80
Madurai	65	4	8	6.15	0.12	2.00
Tiruchirappalli (Tiruchi)	40	1	13	2.50	0.33	13.00
Thanjuvur	55	1	9	1.82	0.16	9.00
Thoothukudi	85	3	6	3.53	0.07	2.00
Nammakal	41	5	11	12.20	0.27	2.20
Perambalur	50	3	5	6.00	0.10	1.67
Dharmapuri	36	2	4	5.56	0.11	2.00
Virudhunagar	54	6	6	11.11	0.11	1.00

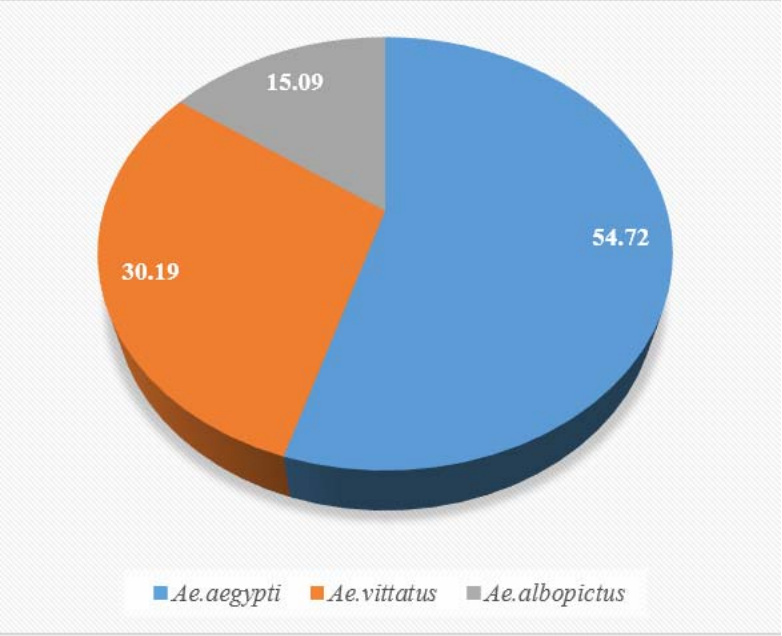

Fig 4: Species composition of adult collected female mosquitoes.

Table 6: Dengue Cases and Deaths in the Tamil Nadu, India since 2008.

2008		2009		2010		2011		2012		2013*		2014**	
Cases	**Deaths**	**Cases**	**Deaths**	**Cases**	**Deaths**	**Cases**	**Deaths**	**Cases**	**Deaths**	**Cases**	**Deaths**	**Cases**	**Deaths**
530	3	1072	7	2051	8	2501	9	12826	66	6122	0	732	0

* Provisional till 31st December 2013 | ** Provisional till 31st July 2014

4. Discussion

The study has been carried out to cover the large areas in Tamil Nadu, to detect the dengue prone areas on the basis entomological surveillance of dengue vectors. The knowledge on distribution and ecology of mosquitoes are important parts of vector control program and should be continued to provide the updated information to control the vectors [24]. The *Aedes aegypti* and *Aedes albopictus* are the main dengue vectors, which showed close association with humans and are anthropophilic mosquitoes [25]. *Aedes vittatus* was also abundantly recorded from the study areas. The entomological indices are important indicators to know the dengue fever transmission. House Index and Container Index are important determinants of extent of breeding and intensity of mosquito breeding respectively. The House Index has been widely used to monitor the infestation levels. The Container Index provides the valuable information on the proportion of water holding containers that are infested. However, the Breateau Index is more applicable, as it correlates the Positive Containers and Houses inspected and thus it is regarded as an excellent risk indicator of dengue outbreaks [26, 27]. Other than these larval indices, Pupal Indices are important to know the intensity of transmission and are considered the better and alternate indicators for adult mosquito abundance [28]. The Pupal index has been frequently used for Operational Research purposes. The larval indices varied among different districts of Tamil Nadu and all the areas had positive breeding places. Similar results were reported from previous studies [29]. The study areas were densely populated and having shortage of regular water supply, due to which the people used to store the water in various containers for different purposes of daily life and all these practices are favourable to increase the dengue vector populations [27, 30]. The major breeding sources recorded from various places included Earthen Pots (25.93%) followed by Coconut Shells (4.76%), Cement Tanks (3.37%), Plastic Containers (3.35%), Plastic Drums (3.35%), Aluminium Utensils (2.26%), Flower Pots (2.22%), Grinding Stones (1.92%), Tires (1.33 %), Iron Pots (1.25%) and Plastic Buckets (0.46%). Similar studies have also been conducted in other districts in India [27, 31-33].

All the above mentioned breeding sources were found inhabited by *Aedes aegypti* and *Aedes vittatus* where as the main breeding sources inhabited by *Aedes albopictus* recorded in this study included Tires, Flower Pots and Plastic Drums. Thus the *Aedes* species were observed to breed in all artificial available containers filled with stagnant water, retained for long duration without disturbing the breeding source. Similar results were also obtained in previous studies [27].

The species composition of adults emerged and adults collected mosquitoes consisted of three species of *Aedes* mosquitoes. The *Aedes aegypti* followed by *Aedes vittatus* and *Aedes albopictus* were abundantly reported and thus the study areas are dengue prone and susceptible to outbreaks. Similar results were obtained in other studies conducted in other areas in Tamil Nadu [27, 31-32]. The adult female collection plays vital role in determining the dengue prone areas because the females are lone stages of mosquitoes which can transmit the dengue virus [27, 34]. The adult vector surveillance plays important role in determining the seasonal population trends, transmission dynamics and transmission risks. It also provides the valuable information for the evaluation of adulticide interventions.

It can be concluded that that due to the availability of various breeding sources, *Aedes* larval and adult stages were detected throughout all the areas of Tamil Nadu and thus there are the chances of occurrence of dengue fever infections. The various organisations of vector control should take the necessary steps to educate the people of study areas. The people should be informed about the practices of source reduction, to destroy all the breeding sources including all the aquatic stages of mosquitoes due to which the chances of dengue outbreaks can be reduced.

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