



A STUDY ON BREEDING PREFERENCE OF DENGUE VECTORS IN ANNAMALAI NAGAR, CHIDAMBARAM, TAMIL NADU, INDIA

Vineet J Jemuel*, Elangovan A and Vinita Vinjoy Jerusha

Department of Zoology, Annamalai University, Annamalai Nagar-608 002, Chidambaram, Tamil Nadu, India

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ABSTRACT

Aedes mosquitoes are the most dangerous species of mosquito which causes the deadly dengue fever in India and all over the world. The aim of this study was to find out the breeding preference ratio and identification of *Aedes aegypti* and *Aedes albopictus* in Annamalai Nagar (Town panchayat), Chidambaram (Taluk), Cuddalore (District), Tamil Nadu, India. The number of positive containers found in two of our study area Tiruvetkalam, Kothankudi kuppam are 524 and 481 respectively. The breeding preference ratio was higher in the mud pot followed by cement tank in Tiruvetkalam, whereas Kothankudi kuppam shows cement tank followed by mud pot. In Tiruvetkalam, overall 1010 larvae were collected during the four seasons (January to December 2016) from 524 containers. Among them, 918 immatures were found to be *Ae. aegypti* and remaining 92 were *Ae. albopictus*. In Kothankudi kuppam, out of 1024 mosquito larvae collected the population of *Ae. aegypti* and *Ae. albopictus* were 885 and 139, respectively. Moreover, the *Ae. aegypti* population was found to be higher in both study sites compared to *Ae. albopictus*. Additionally, the population of *Aedes* mosquitoes were higher in pre-monsoon season compared to other seasons. Thus, this study clearly showed that *Ae. albopictus* was mostly located in tyres, plastic materials and vessels, whereas *Ae. aegypti* were located in all the containers. From these results, it can be concluded that the study areas Kothankudi kuppam and Tiruvetkalam are prone to dengue outbreaks.

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INTRODUCTION

The transmission rate of any vector born disease depends on the prevalence of that vector species. Thus the prevalence of vector species denotes the transmission risk and also gives the basic information for developing methods of personal protection and government control programmes. According to Directorate of National Vector Borne Disease Control Programme (NVBDCP) in India, the major vector-borne diseases include Malaria, Dengue, Lymphatic Filariasis, Japanese Encephalitis and Chikungunya. Dengue is a mosquito-borne viral disease that in recent years has rapidly spread in all regions of the World [1]. Dengue virus is transmitted by female mosquitoes mainly of the species *Aedes aegypti* and, to a lesser extent, *Aedes albopictus*, which also transmits chikungunya, yellow fever and Zika virus infections [2]. Dengue is widespread throughout the tropics, with local variations in risk influenced by rainfall, temperature and unplanned rapid urbanization. Dengue, with its two clinical severe manifestations, i.e., dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), poses a serious health

concern in India [3]. Changes in the season also influence in the prevalence of mosquitoes [4][5].

Studies on the population dynamics shows a phenomenon of annual pulsation, i.e., the population of mosquitoes remain almost confined in central humid cooler part of the locality, thus spreading around only during the rainy season [7]. Distribution of mosquitoes are found in various habitats that include opened vessels indoor and outdoor along with ponds, marshes, ditches, pools, drains, tree holes, etc. Different genera of mosquitoes breed in different habitats and may also have a specific breeding preference [8]. The population dynamics and distribution pattern knowledge is essential for the spatial location of the vectors in the epidemic and endemic area so that the management strategy can be implemented accordingly. *Ae. aegypti* being a primary vector for dengue, chiefly feeds on humans and rests in undisturbed locations inside and outside the house, whereas *Aedes albopictus* act as the secondary vector for dengue virus [9].

Previously, Senthamarai Selvan and Jebanesan (2014) reported that, a high number of *Aedes* mosquitos were observed in Chidambaram and their surrounding places. Moreover, based on our previous study, we observed a high range of larval indices (House index, Container index and Breteau index) in

*Corresponding author: Vineet J Jemuel

Department of Zoology, Annamalai University, Annamalai Nagar-608 002, Chidambaram, Tamil Nadu, India

Kothankudi kuppam and Tiruvetkalam, Annamalai Nagar, Chidambaram and Tamil Nadu [5]. In the light of the above literature, the present study was designed to find out breeding preference ratio (BRP) and the population of *Aedes* in Annamalai Nagar, Chidambaram, Tamil Nadu and India.

MATERIALS AND METHODS

Study area

The study was carried out for one year, from January 2016 to December 2016, in Annamalai Nagar (Town panchayat) Chidambaram (Taluka), Tamil Nadu, India. Chidambaram is an important tourist spot in the area, with a great number of tourists coming from abroad and from other states of India. The main attractions include some establishments of religious meaning, as well as natural attractions like mangrove forest and cultural traditions.

The Annamalai Nagar is one of the fast-growing towns, and also it is also an educational, important Town Panchayat in Tamil Nadu. The study places, Tiruvetkalam and Kothankudi kuppam, are located at 11°23'41.7"N 79°43'08.4"E and 11°23'38.0"N 79°42'26.6"E, respectively. In these study areas, four distinct seasons are observed, and the field survey was made during these seasons. The seasons are (i) Post-monsoon season (January-March), (ii) Summer season (April-June), (iii) Pre-monsoon season (July-September) and (iv) Monsoon season (October December).

A door-to-door survey was carried out in houses and peridomestic areas to detect habitats preferred by *Aedes aegypti* and *Aedes albopictus* with a view to study their prevalence, distribution of *Aedes* mosquitoes and also to identify high risk areas prone to dengue. Periodical larval collections were made effectively in each locality following the single larval technique of WHO [10]. All kinds of indoor and outdoor breeding habitats were examined to collect the *Aedes* immatures by following the dipper method [11]. A container containing any amount of water was considered as a wet container, and the wet container containing any number of immatures (larvae, pupae or both) was considered as a positive container. The immatures were collected by using different immature collecting materials like pipettes, dipper, strainer depending upon the type and size of the breeding source. The collected immatures were kept in plastic containers labelled with the code of breeding source, locality code, house identification code and date of collection. 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 [10]. The container preferences of *Ae. aegypti* and *Ae. albopictus* breeding were assessed by calculation of Breeding preferences ratio (BPR) as suggested by [12]. The breeding preference ratio was calculated by using following formula:

$$\text{Breeding preference ratio} = \frac{\text{X\% (Percentage of Breeding places of Aedes)}}{\text{Y\% (Percentage of Aedes in each container)}}$$

Statistics

Differences between mosquito abundance and productivity between different seasons and areas were analyzed using Chi-square test. Mosquito larval indices, i.e., CI, HI, BI and PI were calculated as per standard WHO guidelines.

RESULTS

In our previous study, the two areas Kothankudi kuppam and Tiruvetkalam had shown a high number of positive houses, house index, container index and breteau index. In the present study, overall 9390 containers were observed in the two study sites in the four seasons. Out of these, a total of 979 containers were found to be as positive containers for breeding of *Aedes* species. Table 1 shows the comparison between study areas and productivity (breeding activity based on positive containers) of *Aedes* mosquitoes based on overall seasonal distribution (January to December 2016). Moreover, the number of positive containers was found to be increased in pre-monsoon compared to other seasons and the results were found to be significant at $p < 0.001$ and $p < 0.05$.

Table 1 Comparison between study areas and productivity (breeding activity based on positive containers) of *Aedes* mosquitoes based on overall seasonal distribution (January to December 2016)

Study areas	Total No. of observed containers in overall seasons	Total No. of positive containers	χ^2
Tiruvetkalam	4562	524	40.764*#
Kothankudi kuppam	4828	481	27.715*#

*: Significant at $p < 0.001$; #: Significant at $p < 0.05$

In the study sites, all the containers were divided into ten container categories such as cement tanks, coconut shells, discarded tea/water cups, discarded tyres, flower pots, grinding stones, mud pots, plastic materials, tree holes and vessels. In this investigation, overall 4,562 containers in Tiruvetkalam site were searched in the four seasons. Out of these, 524 containers were found to be positive for larvae and pupae. The BPR (Table 2) of *Aedes* were found to be high in mud pots (1.99) followed by cement tank (1.68), vessels (1.19), coconut shells (1.10), discarded tyres (1.02), plastic materials (0.96) grinding stone (0.95), discarded tea/water cups (0.70), flower Pot (0.51) and tree holes (0.44).

Table 2 *Aedes* breeding preference ratio in different artificial habitats in Tiruvetkalam, from January to December 2016

Containers	Number of containers	X%	Number of positive containers	Y%	BPR (Y/X)
Cement Tanks	343	7.52	66	12.60	1.68
Coconut Shells	632	13.85	80	15.27	1.10
Discarded tea/water cups	894	19.60	72	13.74	0.70
Discarded Tyrese	180	3.95	21	4.01	1.02
Flower Pot	430	9.43	25	4.77	0.51
Grinding stone	210	4.60	23	4.39	0.95
Mud pot	302	6.62	69	13.17	1.99
Plastic Material	497	10.89	55	10.50	0.96
Tree holes	394	8.64	20	3.82	0.44
Vessels	680	14.91	93	17.75	1.19
Total	4562	100	524	100	

The Table 3 shows the identified mosquitoes from larvae collected from artificial containers in Tiruvetkalam (January to December 2016). In Tiruvetkalam, overall 1010 larva were collected during four seasons from 524 containers. Among them, 918 immatures were *Ae. aegypti* and remaining 92 were *Ae. albopictus*. The highest number of *Ae. aegypti* were observed in pre-monsoon (355) followed by monsoon (257) summer (160) and post-monsoon (146).

Table 3 *Ae. aegypti* and *Ae. albopictus* mosquitoes identified from larvae collected from artificial containers in Thiruvetkalalam, from January to December 2016

Containers	Post monsoon						Summer						Pre monsoon						Monsoon					
	Containers Positive		<i>Aedes aegypti</i>		<i>Aedes albopictus</i>		Containers Positive		<i>Aedes aegypti</i>		<i>Aedes albopictus</i>		Containers Positive		<i>Aedes aegypti</i>		<i>Aedes albopictus</i>		Containers Positive		<i>Aedes aegypti</i>		<i>Aedes albopictus</i>	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Cement Tanks	4	4.49	14	9.59	0	0	8	7.143	21	13.13	0	0	29	15.51	48	13.52	0	0	25	18.4	51	19.84	2	8
Coconut Shells	11	12.36	21	14.38	0	0	21	18.75	27	16.88	0	0	27	14.44	53	14.93	2	5	21	15.4	41	15.95	0	0
Discarded tea/water cups	16	17.98	23	15.75	0	0	19	16.96	30	18.75	0	0	24	12.83	54	15.21	3	7.5	13	9.56	24	9.34	0	0
Discarded Tyrese	4	4.49	4	2.74	7	47	6	5.357	3	1.88	6	50	6	3.209	13	3.66	17	42.5	5	3.68	10	3.89	13	52
Flower Pot	9	10.11	15	10.27	2	13	2	1.786	3	1.88	1	8.33	10	5.348	22	6.20	3	7.5	4	2.94	12	4.67	4	16
Grinding stone	6	6.74	11	7.53	0	0	3	2.679	5	3.13	0	0	8	4.278	17	4.79	0	0	6	4.41	14	5.45	0	0
Mud pot	12	13.48	15	10.27	0	0	16	14.29	21	13.13	0	0	23	12.3	47	13.24	0	0	18	13.2	32	12.45	0	0
Plastic Material	10	11.24	14	9.59	3	20	13	11.61	15	9.38	1	8.33	17	9.091	30	8.45	5	12.5	15	11	27	10.51	2	8
Tree holes	1	1.12	3	2.05	0	0	6	5.357	8	5.00	0	0	7	3.743	18	5.07	2	5	6	4.41	12	4.67	1	4
Vessels	16	17.98	26	17.81	3	20	18	16.07	27	16.88	4	33.3	36	19.25	53	14.93	8	20	23	16.9	34	13.23	3	12
Total	89	100	146	100	15	100	112	100	160	100	12	100	187	100	355	100	40	100	136	100	257	100	25	100

The maximum number of *Ae. albopictus* were found to be in pre-monsoon (40) followed by monsoon (25), post-monsoon (15) and summer (12). From the collected mosquitoes high number of *Ae. aegypti* was observed in coconut shells (142) followed by vessels (140), cement tanks (134), discarded tea / water cups (131) mud pots (115), plastic materials (86), flower pots (52), grinding stone (47), tree hole (41) and discarded tyres (30). *Ae. albopictus* was profoundly found in discarded tyres (43) followed by vessels (18), plastic materials (11), flower pots (10), discarded tea/water cups (3), tree holes (3) cement tanks (2) and coconut shells (2). The number of *Ae. aegypti* observed was high in Tiruvetkalam when compare to *Ae. albopictus*, whereas Coconut Shells showed more number of *Ae. aegypti* and discarded tyres showed more number of *Ae. albopictus*.

Overall 4,828 containers in Kothankudi kuppam study site were searched in four seasons, from January to December 2016. Out of these, 481 positive containers were found with larvae and pupae. In Kothankudi kuppam, the BPR (Table 4) was found to be high in cement tanks (1.42), followed by mud pots (1.40), vessels (1.27), plastic materials (1.21), discarded tyres (0.97), coconut shells (1.10), grinding stone (0.97), discarded tea/water cups (0.72), flower pots (0.57) and tree holes (0.39).

Table 4 *Aedes* Breeding Preference Ratio in different artificial habitats in Kothankudi kuppam, from January to December 2016

Containers	Number of containers	X%	Number of positive containers	Y%	BPR (Y/X)
Cement Tanks	367	7.60	52	10.81	1.42
Coconut Shells	678	14.04	74	15.38	1.10
Discarded tea/water cups	859	17.79	62	12.89	0.72
Discarded Tyrese	187	3.87	18	3.74	0.97
Flower Pot	460	9.53	26	5.41	0.57
Grinding stone	249	5.16	24	4.99	0.97
Mud pot	460	9.53	64	13.31	1.40
Plastic Material	481	9.96	58	12.06	1.21
Tree holes	391	8.10	15	3.12	0.39
Vessels	696	14.42	88	18.30	1.27
Total	4828	100	481	100	

The Table 5 shows the *Ae. aegypti* and *Ae. albopictus* mosquitoes identified from larvae collected from artificial containers in Kothankudi kuppam

(January to December 2016). In Kothankudi kuppam, overall 1024 mosquito larva were collected from 481 positive containers. From these, *Ae. aegypti* and *Ae. albopictus* were 885 and 139, respectively. The pre-monsoon showed the highest number of *Ae. Aegypti* (334) followed by monsoon (234), summer (159) and post monsoon (158). The highest number of *Ae. albopictus* were collected in pre-monsoon (55) followed by the monsoon (38), summer (25) and post-monsoon (21). *Ae. aegypti* were found in containers like coconut shells (127), vessels (115), plastic materials (115), cement tanks (114), mud pots (108), discarded tea / water cups (104), flower pots (73), grinding stones (71), tree holes (33) and discarded tyres (25). *Ae. albopictus* was found in containers like discarded tyres (44), mud pots (22), flower pots (20), plastic materials (18), vessels (12), tree holes (8), coconut shells (3) and discarded tea/water cups (1). The number of *Ae. aegypti* observed was high in Kothankudi kuppam when compare to *Ae. albopictus*, whereas coconut shells showed more number of *Ae. aegypti* and discarded tyres showed more number of *Ae. aegypti* (Table 3 and Table 5).

As per the obtained results, more number of *Ae. aegypti* were collected in Tiruvetkalam than Kothankudi kuppam, whereas more number of *Ae. albopictus* were collected in Kothankudi kuppam than Tiruvetkalam (Table 3 and Table 5). Moreover, the number of *Aedes* mosquitoes was found to be increased in pre-monsoon season compared to other seasons (Table 3 and Table 5).

DISCUSSION

Dengue is caused by several closely related viruses, called den virus types 1, 2, 3 and 4. The disease is transmitted from person to person mainly by *Aedes aegypti* but *Aedes albopictus* can also act as a secondary vector. *Ae. aegypti* and *Ae. albopictus* females lay eggs in human-made breeding sites such as used/discarded tires, metal drums, recycling containers and domestic water storage containers [7]. In the present study, the results support previous reports that *Ae. aegypti* and *Ae. albopictus* may have different key breeding sites from one area to another [8]. In the present study most of the *Ae. Aegypti* were found in cement tanks, coconut shells, vessels, discarded tea / water cups, mud pots, plastic materials, while *Ae. albopictus* was abundant in tyres, plastic materials, mud pots and vessels.

Table 5 *Ae. aegypti* and *Ae. albopictus* mosquitoes identified from larvae collected from artificial containers in Kothankudi kuppam, from January to December 2016

Containers	Post monsoon						Summer						Pre monsoon						Monsoon					
	Containers Positive		<i>Aedes aegypti</i>		<i>Aedes albopictus</i>		Containers Positive		<i>Aedes aegypti</i>		<i>Aedes albopictus</i>		Containers Positive		<i>Aedes aegypti</i>		<i>Aedes albopictus</i>		Containers Positive		<i>Aedes aegypti</i>		<i>Aedes albopictus</i>	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Cement Tanks	9	10.84	30	18.99	0	0	14	14.14	19	11.95	0	0	19	11.05	42	12.57	0	0	10	7.87	23	9.83	0	0
Coconut Shells	12	14.46	17	10.76	0	0	15	15.15	23	14.47	0	0	27	15.7	50	14.97	2	3.64	20	15.7	37	15.81	0	0
Discarded tea/water cups	10	12.05	19	12.03	0	0	7	7.071	15	9.43	0	0	26	15.12	37	11.08	1	1.82	19	15	33	14.10	0	0
Discarded Tyrese	2	2.41	5	3.16	7	33.33	2	2.02	4	2.52	9	36	8	4.651	7	2.10	15	27.3	6	4.72	9	3.85	13	34.2
Flower Pot	4	4.82	15	9.49	3	14.29	7	7.071	17	10.69	4	16	11	6.395	30	8.98	8	14.5	4	3.15	11	4.70	5	13.2
Grinding stone	3	3.61	8	5.06	0	0	4	4.04	11	6.92	2	8	11	6.395	33	9.88	7	12.7	6	4.72	19	8.12	3	7.89
Mud pot	12	14.46	21	13.29	2	9.52	13	13.13	12	7.55	3	12	20	11.63	43	12.87	10	18.2	19	15	32	13.68	7	18.4
Plastic Material	7	8.43	17	10.76	5	23.81	15	15.15	28	17.61	3	12	20	11.63	37	11.08	5	9.09	16	12.6	33	14.10	5	13.2
Tree holes	1	1.20	0	0.00	2	9.52	2	2.02	3	1.89	1	4	7	4.07	17	5.09	3	5.45	5	3.94	13	5.56	2	5.26
Vessels	23	27.71	26	16.46	2	9.52	20	20.2	27	16.98	3	12	23	13.37	38	11.38	4	7.27	22	17.3	24	10.26	3	7.89
Total	83	100	158	100	21	100	99	100	159	100	25	100	172	100	334	100	55	100	127	100	234	100	38	100

Kothankudi kuppam and Tiruvetkalam showed high BPR due to the usage of containers, improper drainage and high population, and more number of houses in these sites. Similarly, several previous studies had also reported the influence of similar factors that contributed to higher BPR of *Aedes* species in the study areas [8] [13].

Discarded containers and water storage containers were important breeding sources for *Ae. aegypti* and *Ae. albopictus*. *Aedes* are adapted to feed on humans and undergo larval and pupal development in natural (e.g. rock pools, tree holes, leaf axils) and artificial (e.g. water tanks, blocked drains, decorative pots and discarded tyres and food/beverage containers) freshwater collected container in the both urban and peri-urban environments. *Aedes* mosquitoes are common anthropophagic mosquitoes in urban areas [14]. Among the two *Aedes* species, *Ae. albopictus* is less domestic than *Ae. aegypti*, but tends to rest and feed outdoors and uses both artificial and natural breeding sites [15].

From the collected mosquitoes high number of *Ae. aegypti* was found in coconut shells followed by vessels, cement tanks, discarded tea/water cups, mud pots, plastic materials, flower pots, grinding stones, tree holes and discarded tyres. In this study, *Ae. albopictus* population was found to be high in discarded tyres followed by vessels, plastic materials, flower pots, discarded tea/water cups, tree holes, cement tanks and coconut shells. Our study indicates that *Ae. albopictus* prefers breeding in discarded tyres, plastic materials, flower pots and vessels, whereas shows less preference to coconut shells, discarded tea/water cups, cement tanks and grinding stones. Previously, Thavara *et al.* (2001), Preechaporn *et al.* (2006) and Thenmozhi *et al.* (2006) also reported that *Ae. albopictus* larvae were found in a wide range of artificial containers such as tyres, plastic containers, earthen jars, vessels, mud pots and natural containers (tree holes and coconut shells). Thus, from these result it can be concluded that *Ae. Aegypti* prefers breeding in all the above mentioned containers except in discarded tyres. Our results were in line with previously reported by Vijayakumar *et al.* (2014) that discarded tyres had a high positivity for breeding of *Ae. Albopictus*. Moreover, similar results were also observed in other studies conducted in different study areas in Tamil Nadu [20].

CONCLUSION

Based on the current study, we can conclude that the *Ae. aegypti* and *Ae. albopictus* larvae were detected in most of the

households of Tiruvetkalam and Kothankudi kuppam, Annamalai Nagar. Thus, the results of the study also indicate that these two study areas are prone to dengue outbreaks. Hence preventive measures should be taken to minimize the breeding potential of *Aedes* by various awareness programmes and also by implementing proper garbage disposal managements. Also government officials, local authorities and residents must take steps in introducing proper water management practices in these study areas.

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