Indian Journal of Natural Products and Resources
Vol. 7(3), September 2016, pp. 234-239

Studies on potential breeding habitats of dengue and chickungunya vector mosquitoes in Ramanathapuram district, Tamil Nadu, India

P Senthamarai Selvan* and A Jebanesan

Division of Vector Biology, Department of Zoology, Annamalai University, Annamalainagar – 608 002, Tamil Nadu, India

Received 08 April 2016; Revised 28 July 2016

In recent past, entomological survey has not been carried out in the rural villages of Ramanathapuram district, Tamil Nadu, India. Keeping this in view, larvae and pupae of *Aedes* mosquitoes from different artificial containers were collected from higher altitudes of the Ramanathapuram, viz. Paramakudi, Rameshwaram, Tiruvadanai, Kadaladi, and Ramanathapuram during pre (May-June) and post (November-December) monsoon seasons in 2015 to understand the seasonal distribution, so as to forecast the risk of dengue transmission. Collected immature were transported to laboratory and allowed to emerge as adult. The adults were identified up to the species level. *Aedes aegypti* mosquitoes were highest in water tanks followed by in discarded tyres and the maximum number of collection was made from Ramanathapuram followed by Rameshwaram. The Shannon-Weiner diversity index value of *Aedes* mosquitoes in the study areas recorded for *Ae. aegypti*, *Ae. albopictus*, and *Ae. vittatus* were 1.1571, 0.1105, and 0.0674, respectively. Presence of *Ae. aegypti* and *Ae. albopictus* in both seasons reveals re-emergence of vector borne diseases at higher altitudes.

Keywords: *Aedes*, Diversity, Ramanathapuram, Tamil Nadu, India.
IPC code; Int. cl. (2015.01)– A01M 1/00

Introduction

Dengue fever (DF) and its severe forms, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are mosquito transmitted arboviral diseases belonging to genus *Flavivirus*, family Flaviviridae. It affects the tropical and the subtropical regions of the world[1]. The incidences of the disease have increased over the last 50 years[2] with 2.5 billion people living in areas where dengue is endemic and it affects up to 100 million people each year, with 500,000 cases of DHF and DSS, and around 30,000 deaths, mostly children[3]. In recent years, dengue fever and its more serious forms, DHF and DSS, have emerged as a major public health problem with expanded geographic distribution and increased epidemic activity[4].

Mosquitoes breed in various habitats such as ponds, marshes, ditches, pools, drains, water containers, tree holes, etc. Different genera of mosquitoes may have a specific breeding preference[5]. The abundance of *Aedes* mosquitoes is strongly influenced by availability of water sources and changes in climate. Human ecology is responsible for the creation of mosquitogenic environment; humans directly or indirectly create such a situation. Containers are probably the most important factor for the breeding of *Aedes* mosquitoes[6].

In India, DF and DHF have spread to many different parts of the country[7] including Southern India[8,9]. Among the 32 districts of Tamil Nadu, 29 districts were found to be affected with dengue infections, which include DHF outbreaks in Chennai[8], Dharmapuri[10], Tiruchirappalli[11], and Virudhunagar district[12].

Materials and Methods

Study area

Investigations on *Aedes* mosquitoes were carried out in Ramanathapuram district, Tamil Nadu, India at Paramakudi, Rameshwaram, Tiruvadanai, Kadaladi, and Ramanathapuram in two different seasons, pre-monsoon (May-June, 2015) and post-monsoon (November-December, 2015). The study areas were situated in 9° 23’ 0” N latitude and 78° 50’ 0” E longitude with the elevation of 7 feet. During the survey, *Aedes* mosquitoes breeding was observed in a variety of domestic and rain water containers. The most important breeding habitats observed were water

*Correspondent author
Email: psselvan@ymail.com
Mob: +91-9786694626.

tanks, discarded tyres, tree holes, mud pots, coconut shells, etc. People, especially in slums and coast store water in drums and tanks due to scarcity. They do not empty the drum completely even once in a month, which results in heavy breeding of dengue vectors in these habitats.

Mosquito collection and identification

Habitat evaluation method as described by Service[13] was adopted in collecting the larvae from different habitats. The adult mosquitoes were collected with the help of suction tube[14] and sweep net. Mosquito collection was carried out at dawn during 06:00-09:30 and dusk during 18:00-21:30 h, twice per month in May, June, November, and December 2015 at indoor resting and outer areas. The collected mosquitoes were identified to species level by identification keys and nomenclature[15,16]. The collected *Aedes* immatures were transported to the laboratory and allowed them to emerge as adult and identified up to species level. Other species of mosquitoes were discarded safely by heating the water. The voucher specimens (No. ZAe 1, ZAe 2, and ZAe 3) were deposited in the Department of Zoology, Annamalai University, Tamilnadu.

Entomological indices

All the possible water containers were sampled for mosquito larvae and immature, both indoors and outdoors. In this study, the first and second instars larvae and pupae were separated, because immature mosquitoes at these stages could not be identified. There were a total of 531 container categories surveyed in this study. Three indices like House index (HI), Container index (CI), and Breteau index (BI) were worked out as per WHO guidelines[17]. The results were calculated by,

$$\text{House index} = \frac{\text{House positive}}{\text{Total no. of house searched}} \text{ X } 100$$

$$\text{Container index} = \frac{\text{Container positive}}{\text{Total no. of container searched}} \text{ X } 100$$

$$\text{Breteau index} = \frac{\text{Container positive}}{\text{Total no. of house searched}} \text{ X } 100$$

Shannon-Weiner diversity index[18] is commonly used to characterize species diversity in a community, according to both abundance and evenness of the species present.

$$\text{Shannon-Weiner diversity Index } (H') = -\sum pi \text{ In (Pi)}$$

Results

A total of 194 houses from 27 villages of 5 taluks of Ramanathapuram district were surveyed for various types of containers (Table 1). Among them 78 houses were positive breeding sources of *Aedes* mosquitoes. Both, artificial and natural breeding sources were examined in and around the houses (indoor and outdoor). Out of 531 containers screened, 208 containers were found to support the *Aedes* mosquito breeding (Table 2). On the basis of positive houses and positive containers observed, various larval indices were calculated to determine the distribution dynamics of *Ae. aegypti, Ae. albopictus*, and *Ae. vittatus* and to detect the dengue prone areas. In the present study, the HI, CI, and BI varied in between 21.95–53.33, 25.53–41.26, and 48.00–148.78, respectively (Table 2).

Table 1—*Aedes* larval occurrence in both indoor and outdoor containers during the study period in Ramanathapuram district, Tamil Nadu, India

Container type	*Aedes* larval occurrence				
	Ae. aegypti	*Ae. albopictus*	*Ae. vittatus*	Male	Female
Indoor					
Used cans	X	-	X	3	1
Vessels	X	-	-	11	23
Cattle sheds	X	X	-	0	3
Water plant pots	X	-	X	2	6
Outdoor					
Water tanks	X	X	X	17	49
Large earthen jars	-	X	X	1	6
Cement tanks	X	-	-	2	13
Coconut shells	-	-	X	1	3
Discarded tyres	X	X	-	6	16
Tree holes	X	X	-	9	16
Mud pots	-	-	X	2	7
Total	172	25	11	54	154

(X) represents present, (-) represents absent

Table 2—Larval indices and distribution of *Aedes* mosquitoes breeding habitats at different locations in Ramanathapuram district

Location	No. of villages surveyed	Total houses	Positive houses	Total containers	Positive containers	House index (HI)	Container index (CI)	Breteau index (BI)
Paramakudi	4	38	12	63	24	31.57	38.09	63.15
Rameshwaram	7	41	9	150	61	21.95	40.66	148.78
Tiruvadanai	3	30	16	82	33	53.33	40.24	110.00
Kadaladi	4	25	13	47	12	52.00	25.53	48.00
Ramanathapuram	9	60	28	189	78	46.66	41.26	130.00
Total	27	194	78	531	208	40.20	39.17	107.21

Table 3—Shannon-Weiner diversity index of *Aedes* mosquitoes in the study areas of Ramanathapuram district during the study period May-June (Pre-monsoon) and November-December (Post-monsoon) 2015

Mosquito	fi	%	fi log fi	Pi	$(Pi)^2$ or $(ni/Ni)^2$	Ni (ni-1)/ N(N-1)	Pi log Pi	Pi In Pi	Pi $(In Pi)^2$	Shannon-Weiner diversity index H=(N log N - ∑ fi log fi / N
Ae. aegypti	172	82.69	384.5	0.8269	0.6837	0.6831	-1.1571	-0.1571	0.0298	1.1571
Ae. albopictus	25	12.01	34.94	0.1201	0.0144	0.0139	-0.1105	-0.2545	0.5394	0.1105
Ae. vittatus	11	5.28	11.45	0.0528	0.0027	0.0025	-0.0674	-0.1552	0.4567	0.0674
Total	208	100	430.89	0.9998	0.7008	0.6995	-1.335	-0.5668	1.0259	1.335

fi: Number of individual; *N*: Total number of individuals; Pi: Proportion of individuals found in the species; In*:* The natural (Naperian) logarithms (log); $(ni/N)^2 = (Pi)^2$

Table 4—Composition of *Aedes* mosquitoes emerged from different breeding habitats in Ramanathapuram district, Tamil Nadu, India

Container type	*Ae. aegypti*				*Ae. albopictus*				*Ae. vittatus*			
	M	%	F	%	M	%	F	%	M	%	F	%
Used cans	2	4.34	0	0.00	1	16.66	1	5.26	0	0.00	0	0.00
Vessels	11	23.91	22	17.46	0	0.00	0	0.00	0	0.00	1	11.11
Cattle sheds	0	0.00	3	2.38	0	0.00	0	0.00	0	0.00	0	0.00
Water plant pots	2	4.34	6	4.76	0	0.00	0	0.00	0	0.00	0	0.00
Water tanks	13	28.26	39	30.95	3	50.00	7	36.84	1	50.00	3	33.33
Large earthen jars	1	2.17	4	3.17	0	0.00	1	5.26	0	0.00	0	0.00
Cement tanks	3	6.52	11	8.73	0	0.00	1	5.26	0	0.00	1	11.11
Coconut shells	1	2.17	3	2.38	0	0.00	0	0.00	0	0.00	0	0.00
Discarded tyres	4	8.69	21	16.66	1	16.66	5	26.31	1	50.00	2	22.22
Tree holes	8	17.39	11	8.73	1	16.66	3	15.78	0	0.00	2	22.22
Mud pots	1	2.17	6	4.76	0	0.00	1	5.26	0	0.00	0	0.00
Total	46	100	126	100	6	100	19	100	2	100	9	100

M-Male, F-Female

Overall, the indices related to the species diversity i.e. Shannon-Weiner diversity index values were recorded as 1.1571 for *Ae. aegypti*, 0.1105 for *Aedes albopictus*, and 0.0674 for *Ae. vittatus* (Table 3), which depended on the size and the material of breeding sources. The major breeding sources (Table 4) observed were water tanks, vessels, discarded tyres, and tree holes having more *Ae. aegypti* mosquitoes followed by *Ae. albopictus*. Overall, indoor and outdoor water tanks had *Ae. aegypti* (28.26 % male and 30.95 % female), *Ae. albopictus* (50.00 % male and 36.84 % female) and *Ae. vittatus* (50.00 % male and 33.33 % female). In discarded tyres also, *Ae. aegypti* (8.69 % male, 16.66 % female), *Ae. albopictus* (16.66 % male and 26.31 % female), and *Ae. Vittatus* (50.00 % male and 22.22 % female) mosquitoes were observed (Table 4). Similar studies have also been conducted in Tiruchirappalli, Coimbatore[1], Virudhunagar[12], and Tirunelveli[19] districts of Tamil Nadu, India.

The collected immatures were reared and allowed to emerge into the adults. The emerged adults consisted of three species of *Aedes* mosquitoes: 82.69 % (172) *Ae. aegypti*, 12.01 % (25) *Ae. albopictus*, and 5.28 % (11) *Ae. vittatus* (Table 3). Rajesh *et al.* conducted similar study[11]. Regarding the sex ratio of emerged

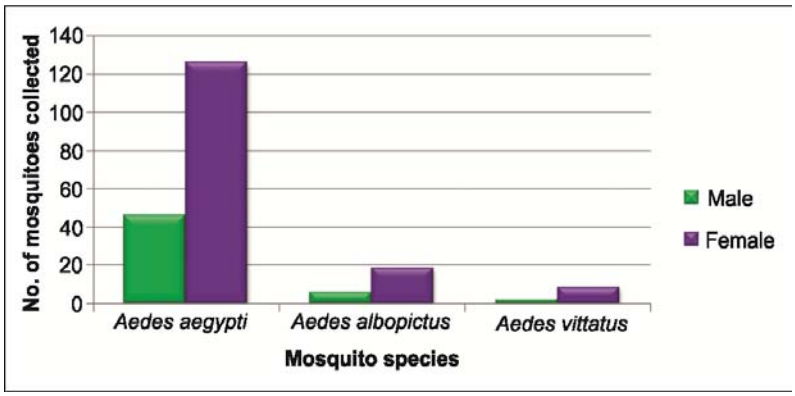

Fig. 1—Sex ratio of collected *Aedes* mosquitoes in study areas during the study period of Pre-monsoon (May-June) and Post-Monsoon (November-December), 2015

mosquitoes, 54 (25.96 %) were male and 154 (74.04 %) were female (Fig 1 and Table 1). A higher dengue vector population was recorded in post monsoon season when compared with pre monsoon due to rain water collections in various types of containers in post monsoon seasons (Fig 1).

Discussion

Ae. aegypti, the principal mosquito vector of dengue viruses is an insect closely associated with humans and their dwellings. People not only provide the mosquitoes with blood meals, but also water-holding containers in and around the home needed to complete their development. The mosquito lays her eggs on the sides of containers with water and eggs hatch into larvae after a rain or flooding. A larva changes into a pupa in about a week and thereafter into a mosquito in two days. People also furnish shelter as *Ae. aegypti* preferentially rests in darker cool areas, such as closets leading to their ability to bite indoors.

This study demonstrated that the number of *Aedes* larvae was higher after the rainy season (post-monsoon) than in the May–June (pre-monsoon). Many studies have reported similar findings in other parts of India and Tamil Nadu. The seasonality of *Aedes* larvae in Coimbatore and Tiruchirappalli showed a similar pattern to that observed by Selvan *et al*., that *Aedes* larvae remained low in summer and winter seasons, but increased in the rainy season[1]. However, *Aedes* larvae in Tamil Nadu showed a non-seasonal fluctuation pattern due to water-filled containers being present round the year.

Ae. albopictus is capable of breeding in a wide range of container types and water holding containers. General breeding sites, such as coconut shells, fruit peels, water jars, unused and discarded tyres, and old boats or cars holding water have been found to contain *Ae. albopictus* larvae[20]. Key breeding sites (i.e. the most abundant larval habitats) of *Ae. aegypti* are cement tanks and earthen jars inside and outside the dwellings[21,22]. Previous studies reported that *Ae. albopictus* is capable of breeding in small aquatic sites such as tree holes in forested habitats as well as in a variety of other habitats in rural and suburban areas[23-27].

In addition to the domestic water storage containers, *Ae. aegypti* breeds in a plethora of waste containers such as discarded tyres, empty metal cans, plastic containers, bottles, jars, and old automobile bodies. Household water storage is likely to change with intensive educational programs. Indoor waste jars produce more pupae per house than all the other containers combined. The infestation rate of covered containers was significantly higher than that of uncovered containers, because loose-fitting lids allowed entrance of gravid females to the attractive, darkened interior of the container[28]. Some measures like filling up of all the defective ground surfaces with sand, mud, or cement, wrapping up disused tyres properly or puncturing them to prevent water being trapped, changing or removing water in flower vases or saucers underneath potted plants at least once a week will definitely prevent breeding in these areas.

The present results support previous findings that the number of *Ae. aegypti* larvae is higher than that of *Ae. albopictus* larvae in the winter and rainy seasons[14,27,29-31]. Although *Ae. aegypti* was introduced in India a long time ago, it is now the primary dengue vector and has greater prevalence than *Ae. albopictus*. This could be due to four possible reasons. Firstly, *Ae. aegypti* larvae are competitively superior to *Ae. albopictus* larvae. Secondly, *Ae. aegypti* has a higher

net reproductive rate than *Ae. albopictus*. Thirdly, eggs of *Ae. aegypti* are more desiccation-resistant than those of other *Aedes* species. Lastly, the females of these two species select different ovipositing habitats. *Ae. aegypti* females prefer to oviposite in all types of artificial and natural indoor water containers, whereas *Ae. albopictus* females prefer to oviposit in outdoor habitats, especially trash containers.

Wongkoon *et al*.[22] and Dieng[25] studied *Aedes* larval occurrence in Nakhon Si Thammarat, Thailand and found *Ae. aegypti* and *Ae. albopictus* larvae in six water storage containers including pot plants, animal pans, tires, small water jars, bathroom tanks, and concrete tanks. They found that from these six containers, there was a higher number of *Ae. aegypti* larvae in water containers in bathrooms and water tanks than *Ae. albopictus*[22]. The present results support the previous findings and show that key breeding sites of *Ae. aegypti* were the ceramic or earthen jars both inside and outside the dwellings, and water storage tanks served as the main breeding places of *Ae. aegypti* whereas preserved jars, metal boxes, and coconut shells found outdoors were the major breeding site of *Ae. albopictus*.

Results revealed that storage jars and water storage tanks (indoor and outdoor) were the main breeding sites of *Aedes* larvae indoor and outdoor, in both wet and dry seasons. On the other hand, indoor small earthen jars, plant plates, plant pots, drinking water storage jars, ant guards and natural sites served as minor breeding sites during both seasons. The present results also support the previous study[21], which revealed that *Ae. aegypti* breed in both domestic and peridomestic sites. However, peridomestic breeding sites such as discarded containers, flower vessels, tire dumps, and water meter chambers supported the maximum breeding in both wet and dry seasons.

It is very difficult to control or eliminate *Ae. aegypti* mosquitoes because they have adaptations to the environment that make them highly resilient. They have the ability to rapidly bounce back to initial numbers after disturbances resulting from natural phenomena like droughts or human interventions like control measures. One such adaptation is the ability of the eggs to withstand desiccation (drying) and to survive without water for several months on the inner walls of containers. For example, if we were to eliminate all larvae, pupae, and adult *Ae. aegypti* at once from a site, its population could recover two weeks later as a result of egg hatching following rainfall or the addition of water to containers harboring eggs.

Conclusion

The prevalence of dengue vector and silent circulation of dengue viruses have been detected in rural and urban Tamil Nadu, which is ever increasing. Identification of *Ae. aegypti* during the study was an important indication of re-emergence of dengue vectors in the study area. The house indexes of all the study areas were higher than the WHO standard for high DHF risk areas i.e. 10 %. Water holding containers produced by humans are the main source of *Aedes* mosquito. Source reduction is an effective way for the community to manage the populations of many kinds of mosquitoes. People should practice integrated vector management in and around their living places as recommended by WHO.

Acknowledgement

The authors are grateful to the University Grants Commission for providing financial assistance under UGC– Major Research Project (Letter no: 42-558/2013 (SR), dt. 22.03.2013) and also thank Professor and Head, Department of Zoology, Annamalai University for the facilities provided. The authors acknowledge the local people for their timely help in mosquito survey.

References

1 Selvan P S, Jebanesan A and Divya G, Breeding of *Aedes aegypti* (L.) and its Impact on Dengue / DHF in Rural Areas of Tamil Nadu, India, *In*: Proc of Brain Storming Conference on Dengue Scenario in India, B K Tyagi, Ed, 2013, **1**, 132-138.

2 Gubler D J, Dengue and dengue haemorrhagic fever: its history and resurgence as a global public health problem, 1997, 1-22.

3 Guzman M G and Kouri G, Dengue: An update, *Lancet Infect Dis*, 2002, **2**, 33-42.

4 World Health Organization, Strengthening implementation of the global strategy for dengue fever and dengue haemorrhagic fever, prevention and control, Report of the informal consultation, Geneva, Switzerland, 18-20 October, 1999.

5 Jebanesan A, Biodiversity of mosquitoes and their diseases in India, *In*: Biodiversity; Issues, impacts remediation and significance, VL media solutions, New Delhi, 2013, **5**(4), 321-330.

6 Selvan P S and Jebanesan A, Survey and Epidemiology of tree hole breeding mosquitoes in Annamalai University campus, Tamilnadu, India, *Int J Curr Res*, 2014, **6**(5), 6462-6465.

7 Agarwal R, Kapoor S, Nagar R A, Misra A, Tandon R, Mathur A, *et al*., A clinical study of the patients with dengue

hemorrhagic fever during the epidemic of 1996 at Lucknow, India, *J Trop Med Public*, 1999, **30**, 735-740.

8 Kabilan L, Balasubramanian S, Keshava S M, Thenmozhi V, Sekar G, Tewari S C, *et al*., Dengue disease spectrum among infants in the 2001 dengue epidemic in Chennai, Tamil Nadu, India, 2003, **41**, 3919-3921.

9 Victor T J, Malathi M, Asokan R and Padmanaban P, Laboratory-based dengue fever surveillance in Tamil Nadu, India, *Indian J Med Res*, 2007, **126**, 112-115.

10 Victor T J, Malathi M, Gurusamy D, Desai A, Ravi V, Narayanasamy G, *et al*., Dengue fever outbreaks in two villages of Dharmapuri district in Tamilnadu, *Indian J Med Res*, 2002, **116**, 133-139.

11 Rajesh K, Dhanasekaran D and Tyagi B K, Survey of container breeding mosquito larvae (Dengue vector) in Tiruchirappalli district, Tamil Nadu, India, *J Entomol Zool Stud*, 2013, **1**, 88-91.

12 Wilson J J and Sevarkodiyone S P, Breeding preference ratio of dengue and chikungunya vectors in certain rural villages of Virudhunagar District, Tamil Nadu, South India, *World Appl Sci J*, 2014, **30**, 787-791.

13 Mosquito ecology: Field sampling methods, 2nd Edn, Service M W, Ed, Elsevier Applied Science, London, New York, 1993, 23-58.

14 Selvan P S, Jebanesan A, Divya G, and Ramesh V, Diversity of mosquitoes and larval breeding preference based on physico-chemical parameters in Western Ghats, Tamilnadu, India, *Asian Pacific J Trop Dis*, 2015, **5**(1), S59-S66.

15 Barraud P J, The fauna of British India, including Ceylon and Burma (Diptera: Culicidae), Taylor and Francis, London, 1934, **5**, 1-59.

16 Christophers S R, The Fauna of British India, Including Ceylon and Burma, Diptera, Family *Culicidae*, Tribe *Anophelini,* Taylor & Francis*,* London, 1933, **4**, 371.

17 World Health Organization, Global Strategic frame work for Integrated Vector Management, Geneva, Document No. WHO/CDC/CPE/PVC/2004, 1-15.

18 Shannon C E and Weiner W, The Mathematical Theory of Communication, University of Illinois press, Urbana-III, 1949.

19 Ayoub B and Krishnamoorthy K, Entomological investigation and distribution of *Aedes* mosquitoes in Tirunelveli, Tamilnadu, India, *Int J Curr Microbio App Sci*, 2014, **3**(10), 253-260.

20 Thavara U, Tawatsin A and Chompoosri J, Evaluation of attractants and egg-laying substrate preference for oviposition by *Aedes albopictus* (Diptera: Culicidae), *J Vector Ecol,* 2004, **29**(1), 66-72.

21 Ansari M A and Razdan R K, Seasonal prevalence of *Aedes aegypti* in five localities of Delhi, India, *Dengue Bull*, 1998, **22**, 28-32.

22 Wongkoon S, Jaroensutasinee M and Jaroensutasinee K, Larval infestation of *Aedes aegypti* and *Ae. albopictus* in Nakhon Si Thammarat, Thailand, *Dengue Bull*, 2005, **29**, 169-175.

23 Selvan P S, Jebanesan A, and Makesh Kumar C, Diversity and distribution of tree hole mosquitoes in Puducherry Union Territory, India, *J Coast Life Med,* 2015, **3**(7), 531-533.

24 Jebanesan A, Rajasekar P and Paul S A, Influence of climatic factors on the distribution of tree hole mosquitoes collected from Kashmir valley, India, *Int J Environ Bio*, 2012, **2**(2), 92-96.

25 Dieng H, Mwandawiro C, Boots M, Morales R, Satho T, Tuno N, *et al*., Leaf litter decay process and the growth performance of *Aedes albopictus* larvae (Diptera: Culicidae), *J Vector Ecol*, 2002, **27**(1), 31-8.

26 Hawley W A, The biology of *Aedes albopictus*, *J Am Mosq Control Assoc*, Suppl, 1988, **1**, 1-39.

27 Makesh Kumar C, Jebanesan A, Mary Nisha S and Selvan P S, *Aedes m*osquitoes in arboviral epidemic prone area of Chidambaram town, Cuddalore district, Tamil Nadu, India, *Indo –Asian J Multidis Res*, 2015, **1**(1), 70-72.

28 Kittayapong P and Strickman D, Distribution of container-inhabiting Aedes larvae (Diptera: Culicidae) at a dengue focus in Thailand, *J Med Entomol*, 1993, **30**, 601-606.

29 Chatterjee K K, Biswas D, Chandra G, Bhattacharyya A, Ghosh A and Hati A K, A longitudinal survey on mosquito larvae in relation to metro rail construction in Calcutta, *Indian Med Gaz* CXXII, 1988, **2**, 56-59.

30 Selvan P S and Jebanesan A, Preliminary study on Tree hole Breeding Mosquitoes diversity in Pachamalai hills, Eastern Ghats, Tamilnadu, India, *Annamalai University Sci J*, 2015, **48**, 73-78.

31 Selvan P S, Jebanesan A and Reetha D, Entomofaunal diversity of tree hole mosquitoes in Western and Eastern ghats hill ranges of Tamilnadu, India, *Acta Trop,* 2016, **159**, 69-82.