



Original Research Article

Entomological investigation and distribution of *Aedes* mosquitoes in Tirunelveli, Tamil Nadu, India

Mohd. Ayoub Bhat* and K. Krishnamoorthy

Division of Epidemiology and Operational Research, Vector Control Research Centre, Indian Council of Medical Research, Indira Nagar, Pondicherry, India-605006

*Corresponding author

ABSTRACT

Keywords

Entomological study;
Aedes mosquitoes;
Breeding sources;
Larval indices;
Adult density based indices;
Tirunelveli.

Entomological survey was carried out extensively (covering the large areas) following the major dengue outbreak (2012) in Tirunelveli district of Tamil Nadu. The study was performed to investigate the various breeding sources and distribution dynamics of dengue vectors. Different technique indices were integrated in this study to understand the significance of entomological surveillance in maintaining the populations of dengue vectors. The most common storage containers used by the residents of Tirunelveli were plastic drums, cement tanks, plastic containers and aluminum utensils which were found the major breeding sources in Tirunelveli. The larval indices like house index, container index, breteau index, and pupal index varied from 5.00 – 30.00, 0.87-6.43, 5.00 – 30.00 and 00 – 86.67 respectively. The pupae/container and pupae per positive container varied from 0.00 – 0.13 and 0.00 – 3.50. Adult female *Aedes* mosquitoes were collected from the same selected premises as those studied during the larval survey and various adult density based indices were calculated. The adult premise index varied from 5.00 – 23.33 whereas females per house inspected and females per positive house (for *Aedes* mosquitoes) varied from 0.08 – 0.30 and 1.00 – 2.50 respectively. The study showed that the three species of *Aedes* mosquitoes (both immatures and adults) were abundantly distributed and expanded their range in all the study areas of Tirunelveli District.

Introduction

The vector borne diseases (VBDs.) mostly the mosquito borne diseases (MBDs.) have become important world health problems because there is no vaccine or drug available against these diseases so far. These diseases are standing as the potential risks to the major population of the world. Nowadays dengue fever, one of the mosquito borne diseases, has created havoc to all over world.

It is an acute febrile illness and crucial public health problem. It has been reported that 2.5 billion people are at risk globally and this disease is found all over the world (Fradin *et al.*, 2002). Every year newer areas of the world are invaded by this dreadful dengue infection. The disease may cause hemorrhages and plasma leakage giving birth to fatal diseases; the dengue hemorrhagic fever (DHF)

and dengue shock syndrome (DSS) respectively (WHO, 1997) which are the main causes of infant mortality (Monath *et al.*, 1994).

In India, DF and DHF has knocked the doors of many different parts of the country (Agarwal *et al.*, 1996) including southern India (Kabilan *et al.*, 2003; Victor *et al.* 2007). Among 30 districts of Tamil Nadu, 29 districts were found infected with dengue infections which includes DHF outbreaks in Chennai (Kabilan *et al.*, 2003), Dharmapuri (Victor *et al.*, 2002), Tiruchirappalli (Rajesh *et al.*, 2013) and Virudhunagar district (Wilson *et al.*, 2014). It was reported in daily newspaper, The Hindu (dated; 15 July 2012) that a total 9,000 cases and 50 deaths were reported in districts of Madurai, Tirunelveli and Kanyakumari. Although *Aedes aegypti*, the principal vector (WHO, 2003) is extensively distributed in many towns and cities of India (Sharma *et al.*, 2000; Tandon *et al.*, 2000), the dengue virus has also been detected in *Ae. Albopictus* (Das *et al.*, 2004). The various meteorological factors like temperature, rainfall and humidity affect the distribution of *Aedes* mosquitoes and the dengue infection generally become apparent during or after rainfall, as an outcome of rise in vector population (Pandya, 2014).

Entomological survey was carried out extensively following the major dengue outbreak (2012) in Tirunelveli district of Tamil Nadu. The study was performed to investigate the various breeding sources and distribution dynamics of dengue vectors. Different technique indices were integrated in this study to understand the significance of entomological surveillance in maintaining the populations of dengue vectors.

Materials and methods

The entomological survey was carried out (from September 2013 – December 2013)

extensively (covering large areas) following the major dengue outbreak (2012) in Tirunelveli district of Tamil Nadu.

The study was performed in randomly selected houses in 12 different areas of Tirunelveli Tamil Nadu, India (namely Puliankudi, Sankarankiol, Tenkasi, Ramaiyanpatti, Marathamputhur, Chettiyur, Shenkottai, Sivagiri, Alangulam, Panaagudi, Surandai and Vellanguli) (Fig. 1). Various breeding sources and distribution dynamics of *Aedes* mosquitoes was operated in our study. Tirunelveli district is located 700 km to the southwest of the state capital, Chennai and covers an area of 108.65 km². It is situated at 8.73⁰ N and 77.7⁰E with an average elevation of 47 meters. The climate of Tirunelveli is generally hot and humid with an average annual rainfall of 680 millimeters.

Larval Collection

All kinds of indoor and outdoor breeding habitats were examined to collect the *Aedes* immatures by following the dipper method (Reuben, 1978). 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 in Vector Control Research Centre (VCRC), Pondicherry. The immatures (larvae and pupae) were counted and reared in enamel 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 analyzed in terms of different larval survey techniques like House Index (HI), Container Index (CI), Breteau Index (BI), Pupal Index (PI), Pupae Per Container Index (PCI) and Pupae Per Positive Container Index (PPCI) according to various methods (WHO, 2003; Service, 1976). 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.

Breteau 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.

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 (Sundeep *et al.*, 2009 and Chan *et al.*, 1971). 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 plastic vials 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).

Adult identification

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 (Tyagi *et al.*, 2012; Barraud & Philip James, 1934). Adult *Aedes* mosquitoes were identified by the exposed patterns of the thorax formed by black, white or silvery scales and the legs were often black with white rings. The *Ae. aegypti* is identified by the presence of mesonotum marked with a pair of lateral curved white lines usually with a pair of sub median yellowish line. The tibia is without rings and clypeus consists of two dots of white scales. Another dengue vector; *Aedes albopictus* is identified by the presence of narrow median silvery white line in mesonotum. The pleurae were arranged in irregular patches with white lines and tibia is without white line.

The *Aedes vittatus*, another species collected from the study areas was identified due to the presence of 4-6 small white spots on the mesonotum and having tibiae with white rings.

Results and Discussion

Total of 550 houses were surveyed from 18 residential areas (Table 1) and 107 houses were had positive breeding sources for *Aedes* mosquitoes. Both artificial and natural breeding sources were examined. Out of 3778 containers screened 150 containers were found to support the *Aedes* mosquito breeding (Table 1). On the basis of positive houses and positive containers observed, the various larval indices were calculated to determine the distribution dynamics of *Aedes* species and to detect the dengue prone areas. In our study the

HI, CI, BI, and PI varied from 5.00 – 30.00, 0.87-6.43, 5.00 – 30.00 and 00 – 86.67 respectively (Table 1). The indices related to the pupal production other than pupal index (PI) like the pupae/container and pupae per positive container varied from 0.00 – 0.13 and 0.00 – 3.50 (Table 2) and thus depending on the size and material of breeding source, the pupal productivity fluctuates which was also observed from American studies (Burkot *et al.*, 2007). The major breeding sources (Table 2) observed were Cement tanks 17 (4.79%) followed by Plastic drums 13 (4.36%), Tyres 2 (2.56%), Grinding stones 3 (2.29%), Plastic containers 10 (2.18%), Aluminium utensils 10 (1.76%), Flower pots 1 (1.10%). Plastic

buckets and Iron pots were not found positive for breeding. Similar studies have also been conducted in Tiruchirappalli (Rajesh *et al.*, 2013) and Virudhunagar (Wilson *et al.*, 2014) districts of Tamil Nadu, India. As all the collected immatures were reared and allowed to emerge into the adults, the composition of emerged species consisted of three species of *Aedes* mosquitoes; *Aedes aegypti* 281 (49.13%), *Aedes vittatus* 224 (39.16%) and *Aedes albopictus* 67 (11.71%) (Fig 2 and Table 3). Similar results were obtained in other studies conducted in Tiruchirappalli (Rajesh *et al.*, 2013) and Virudhunagar (Wilson *et al.*, 2014) districts of Tamil Nadu, India.

Table.1 Larval indices and distribution of *Aedes* mosquito breeding habitats at different locations in Tirunelveli district

Location	Total houses	Positive houses	Total containers	Positive containers	Pupae	HI	CI	BI	PI
Puliankudi	30	9	140	9	26	30.00	6.43	30.00	86.67
Sankarankoil	25	5	132	5	8	20.00	3.79	20.00	32.00
Tenkasi	35	3	210	4	10	8.57	1.90	11.43	28.57
Ramaiyanpatti	40	5	200	7	15	12.50	3.50	17.50	37.50
Maruthamputhur	25	2	100	2	0	8.00	2.00	8.00	0.00
Chettiyur	20	1	115	1	0	5.00	0.87	5.00	0.00
Shenkottai	30	3	210	5	20	10.00	2.38	16.67	66.67
Sivagiri	45	5	260	6	25	11.11	2.31	13.33	55.56
Alangulam	25	2	130	2	5	8.00	1.54	8.00	20.00
Panaagudi	38	4	300	6	10	10.53	2.00	15.79	26.32
Surandai	30	3	260	3	5	10.00	1.15	10.00	16.67
Vellanguli	27	3	243	6	15	11.11	2.47	22.22	55.56
Total	370	45	2300	56	139	12.16	2.43	15.14	37.57

HI-House Index, CI-Container Index, BI-Breteau Index, PI- Pupal Index.

Table.2 Container positivity and pupal indices

Container type	No. of containers examined	No. of Positive containers	Container positivity	No. of pupae collected	Pupae/container	Pupae/positive container
Coconut shells	22	0	0.00	0	0.00	0.00
Cement tanks	355	17	4.79	45	0.13	2.65
Plastic drums	298	13	4.36	25	0.08	1.92
Plastic containers	458	10	2.18	29	0.06	2.90
Plastic buckets	182	0	0.00	0	0.00	0.00
Aluminium utensils	569	10	1.76	28	0.05	2.80
Grinding stones	131	3	2.29	5	0.04	1.67
Tyres	78	2	2.56	7	0.09	3.50
Flower pots	91	1	1.10	0	0.00	0.00
Iron pots	111	0	0.00	0	0.00	0.00
Total	2300	56	2.43	139	0.06	2.48

Container positivity = Number of positive containers divided by total containers searched multiplied by 100.

Table.3 Composition of *Aedes* mosquitoes emerged from different breeding habitats

Container type	<i>Ae. aegypti</i>				<i>Ae.vittatus</i>				<i>Ae.albopictus</i>			
	M	%	F	%	M	%	F	%	M	%	F	%
Coconut shells	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Cement tanks	55	9.62	31	5.42	36	6.29	24	4.20	6	1.05	3	0.52
Plastic drums	44	7.69	32	5.59	38	6.64	17	2.97	5	0.87	3	0.52
Plastic containers	25	4.73	12	2.10	27	4.72	22	3.85	3	0.52	1	0.17
Plastic buckets	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Aluminium utensils	41	7.17	24	4.20	24	4.20	22	3.85	7	1.22	4	0.70
Grinding stones	6	1.05	5	0.87	5	2.87	7	1.22	2	0.35	3	0.52
Tires	1	0.17	1	0.17	0	0.00	0	0.00	17	2.97	11	1.92
Flower pots	1	0.17	3	0.52	1	0.17	1	0.17	1	0.17	1	0.17
Iron pots	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
Total	173	30.24	108	18.88	131	22.90	93	16.26	41	7.17	26	4.55

M = Male mosquitoes, F=Female mosquitoes, % = percentage of mosquitoes

Table.4 Distribution of collected adult *Aedes* mosquitoes from different locations in Tirunelveli district.

Village name	Houses inspected	Positive houses			Females/positive	
		(for <i>Ae. sp.</i>)	Females collected	API	house	house (for <i>Ae. sp.</i>)
Puliankudi	30	7	9	23.33	0.30	1.29
Sankarankoil	25	5	7	16.00	0.28	1.75
Tenkasi	35	4	5	8.57	0.14	1.67
Ramaiyanpatti	40	3	4	7.50	0.10	1.33
Maruthamputhur	25	3	3	8.00	0.12	1.50
Chettiyur	20	2	2	5.00	0.10	2.00
Shenkottai	30	1	5	6.67	0.17	2.50
Sivagiri	45	2	7	13.33	0.16	1.17
Alangulam	25	6	2	8.00	0.08	1.00
Panaagudi	38	2	4	10.53	0.11	1.00
Surandai	30	4	4	10.00	0.13	1.33
Vellanguli	27	3	4	7.41	0.15	2.00
Total	370	2	56	10.54	0.15	1.44

API = Number of positive houses for adult female *Aedes* mosquitoes divided by the number of inspected houses multiplied by 100.

Regarding the sex ratio of emerged mosquitoes 345 (60.31%) were male and 227 (39.69%) were females (Fig. 3). A total of 56 adult female *Aedes* mosquitoes were collected from 39 positive houses (Table 4). The composition of adult female collection includes *Aedes aegypti* 32 (57.14%), *Aedes vittatus* 20 (35.71%) and *Aedes albopictus* 4 (7.14%) (Fig 4). As long as larval indices plays vital role in determining the dengue risks, adult density is also related to know the chances of dengue transmission. The API varied from 5.00 – 23.33 whereas females per

house inspected varied from 0.08 – 0.30 and females per positive house (for *Aedes* mosquitoes) showed variation from 1.00 – 2.50 (Table 4).

The study shows that the three species of *Aedes* mosquitoes (both immatures and adults) were abundantly distributed and had expanded their range in all the study areas of Tirunelveli District. Dengue is caused by the *Aedes aegypti* (principle dengue vector) and *Aedes albopictus* (secondary vector).

Fig.1 Map showing locations of study areas in Tirunelveli district.

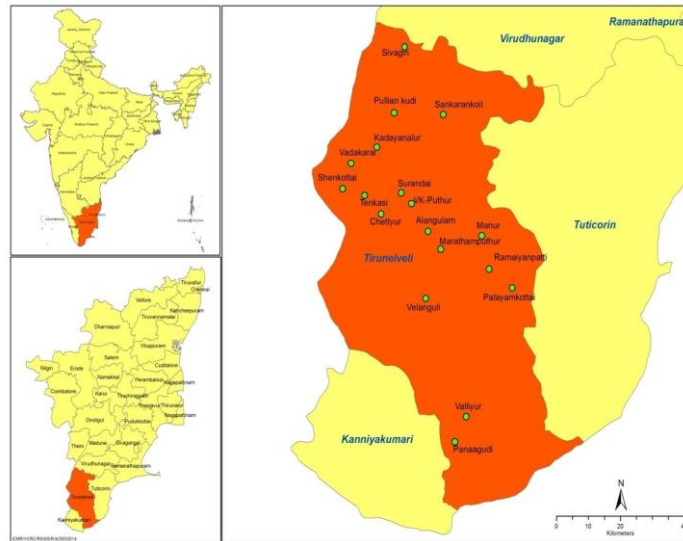
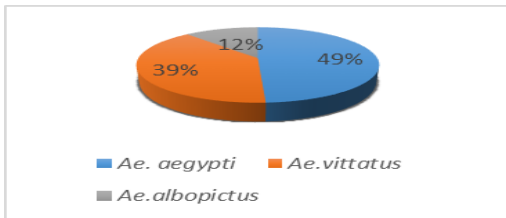


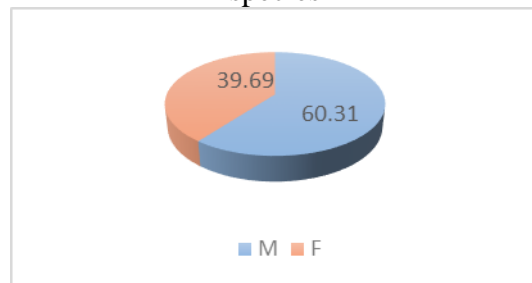
Fig. 2 Composition of emerged mosquito species.



These vectors lay their eggs in various breeding sources present in and around the human dwellings such as water storage containers (Maciel, 2007). The water storage containers constituted the main breeding habitats for *Aedes* mosquito in Tirunelveli district. Due to poor rainfall and shortage of water supply, the residents of Tirunelveli district stored the water in various containers and the most commonly used water storage containers included plastic drums, cement tanks, plastic containers and aluminium utensils and these containers constituted the major breeding sources in Tirunelveli district (Table 2). The water storage containers remained filled with water and undisturbed for long durations to meet the daily water requirements for diverse purposes of humans

and other domestic animals. All these water storage practices are favorable to give rise to breeding of *Aedes* mosquitoes (Swaddiwudhipong *et al.*, 1992) and are closely associated to the spread of dengue infection.

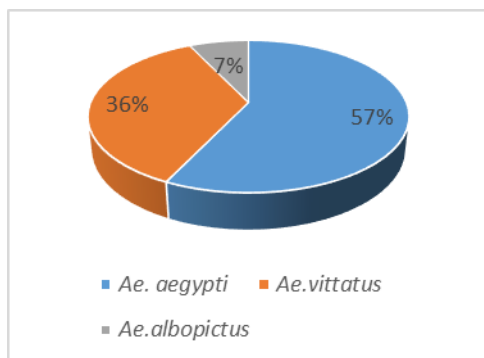
Fig. 3 Sex ratio of emerged mosquito species



M = Male mosquitoes, F = Female mosquitoes.

Among various larval indices, house index and container index provides information on the extent of breeding and intensity of breeding respectively. The breteau index combines the information of both the houses and containers, and thus it is an excellent risk indicator of dengue outbreaks (Tun-Lin *et al.*, 1996). Pupal indices are important to know the intensity of transmission and were considered the better and alternate indicator for adult mosquito abundance (Wai *et al.*, 2012).

Fig. 4 Composition of collected adult *Aedes* female mosquito population in Tirunelveli district.



The adult based density indices are more proper and applicable measures for entomological studies to detect the dengue risk and dengue prone areas because the female mosquitoes are the lone stages which can transmit the dengue virus. Both immatures and adult females of the *Aedes* mosquitoes were recorded in abundance from the study areas. It is clear that there are many chances of spreading of mild viral infection of dengue among the residents. Thus there is a need of establishment of mosquito control program at Tirunelveli district. Such a program will educate and encourage the community that the effective way to deal with the increasing level of mosquito populations is the source reduction (Dame & Fasulo, 2003) and the *Aedes* mosquitoes can be controlled by eliminating the water-filled containers to destroy the oviposition sites and all the aquatic stages of *Aedes* mosquitoes which in turn reduces the chances of spreading of the dengue risks in Tirunelveli district.

Acknowledgement

The author Mr. Mohd Ayoub Bhat is very thankful to Pondicherry University for providing the financial support. The Director, Vector Control Research Centre (ICMR) also provided great support by making the availability of various institutional facilities.

The author is also very gratified to the respected Dr. Ambrose, Entomology division, Xavier college of Pallayamkottai, Tirunelveli, Tamil Nadu for his kind support to complete the study.

References

- Agarwal R., Kapoor S., Nagar R., A. Misra A., Tandon R., Mathur A., Misra A.K., Srivastava K.L., and U. C. Chaturvedi U.C. 1999. A clinical study of the patients with dengue hemorrhagic fever during the epidemic of 1996 at Lucknow, India. *J Trop Med Public.* 30: 735-740.
- Barraud and Philip James. 1934. The Fauna of British India, including Ceylon and Burma. Diptera. Vol. 5. Family Culicidae. Tribes Megarhinini and Culicini. Vol. 5.
- Burkot T. R., Handzel T., Schmaedick M.A., Tufa J., Roberts J.M., and Graves P.M. 2007. Productivity of natural and artificial containers for *Aedes polynesiensis* and *Aedes aegypti* in four American Samoan villages. *Med Vet Entomol.* 21: 22-29.
- Chan, Y. C., Ho B.C., and Chan K.L. 1971. *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in Singapore City: 5. Observations in relation to dengue haemorrhagic fever. *Bulletin of the World Health Organ.* 44: 651.
- Dame D., Fasulo T.R. 2003. Mosquitoes. In: Public health pesticide applicator training manual for USA and its territories. Gainesville: University of Florida.
- Das B. P., L. Kabilan K., Sharma S.N., Lal S., Regu K., V. K. Saxena V.K.. 2004. Detection of dengue virus in wild caught *Aedes albopictus* (Skuse) around Kozhikode airport, Malappuram district, Kerala, India. *Dengue Bull.* 128: 210-212.
- Fradin, Mark S., and John F. D. 2002. Comparative efficacy of insect repellents against mosquito bites. *New England Journal of Medicine.* 347: 13-18.

- Kabilan L., Balasubramanian S., Keshava S.M., Thenmozhi V., Sekar G., Tewari S.C., Arunachalam N., Rajendran R., Satyanarayana K. 2003. Dengue disease spectrum among infants in the 2001 dengue epidemic in Chennai, Tamil Nadu, India. *J Clin Microbiol.* 41:3919-3921.
- Maciel D.F., Codeco R., Claudia T., Lourenco-De-Oliveira, Lourenco-De-Oliveira R. 2007. Daily survival rates and dispersal of *Aedes aegypti* females in Rio de Janeiro, Brazil. *Am J Med Hyg.* 76: 659-665.
- Monath, Thomas P. 1994. Dengue: The risk to developed and developing countries. *Proc Nat AcadSci* 91:2395-2400.
- Pandya G. 2014. Prevalence of dengue infection in India. *DefSci J* 32: 359-370.
- Rajesh K., Dhanasekaran D., Tyagi B.K. 2013. Survey of container breeding mosquito larvae (Dengue vector) in Tiruchirappalli district, Tamil Nadu, India. *J Entomol Zool Stud.* 1:88-91.
- Reuben R. 1978. A report on mosquitoes collected in the Krishna-Godavari delta, Andhra Pradesh. *Indian J Med Res* 68: 603-609.
- Service M.W. 1976. Mosquito ecology, field sampling methods. London: Applied Science Publishers.
- Sharma S.N., Raina V.K., Kumar A. 2000. Dengue/DHF: an emerging disease in India. *J Commun Dis.* 32:175-179.
- Sudeep A.B., Hundekar S.L., Jacob P.G., Balasubramanian R., Arankalle V.A., Mishra A.C. 2011. Investigation of a Chikungunya - like illness in Tirunelveli district, Tamil Nadu, India. *Trop Med Int Health.* 16: 585-588.
- Swaddiwudhipong W., Chaovakiratipong C., Nguntra P., Koonchote S., Khumklam P., Lerdlukanavong P., 1992. Effect of health education on community participation in control of dengue hemorrhagic fever in an urban area of Thailand. *J Trop Med Public Health* 23: 200-206.
- Tandon, Neelam, and Ray S. 2000. Breeding habitats and larval indices of *Aedes aegypti* and *Ae. albopictus* in the residential areas of Calcutta City. *J Commun Dis* 32: 180-184.
- Tun-Lin, W., B. H. Kay, Barne A., S. Forsyth, 1996. Critical examination of *Aedes aegypti* indices: correlations with abundance. *Am J Med Hyg.* 54(5): 543-547
- Tyagi B.K., Munirathinam A., Krishnamoorthy R., Venkatesh A. 2012. A field based handbook on Identification keys to mosquitoes of public health importance in India. CRME Madurai.
- Victor T.J., Malathi M., Gurusamy D., Desai A., Ravi V., Narayanasamy G., Anuradha L., Rani C., Krishnamurthy P. 2002. Dengue fever outbreaks in two villages of Dharmapuri district in Tamil Nadu. *Indian J Med Res* 116: 133-139.
- Victor T.J., Malathi M., Asokan R., Padmanaban P. 2007. Laboratory-based dengue fever surveillance in Tamil Nadu, India. *Indian J Med Res.* 126:112-115.
- Wai K.T., Arunachalam N., Tana S., Espino F., Kittayapong P., Abeyewickreme W., Dilini H., Petzold M. 2012. Estimating dengue vector abundance in the wet and dry season: implications for targeted vector control in urban and peri-urban Asia. *Pathog Glob Health* 106: 436-445.
- World Health Organization. 1997. Dengue hemorrhagic fever: diagnosis, treatment, prevention and control. Geneva. p. 84.
- World Health Organization .2003. Guidelines for Dengue surveillance and mosquito control (2 Ed.), Regional Office of the Western Pacific, Manila.
- Wilson J.J., Sevarkodiyone S.P., 2014. Breeding Preference Ratio of Dengue and Chikungunya Vectors in Certain Rural Villages of Virudhunagar District, Tamil Nadu, South India. *World Appl Sci.* J 30: 787-791