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**“STUDIES ON THE BIODIVERSITY AND DISTRIBUTION
OF TREE HOLE MOSQUITOES OF SOUTHERN INDIA”**

Submitted to



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INTRODUCTION

Mosquitoes are dipteran insects and blood sucking flies pest of man. They have always given tough time to men as important carriers of various diseases. People fight globally against mosquitoes and mosquito borne diseases. Mosquito vectors transmit parasites responsible for diseases such as Malaria, Dengue Fever (DF), Chikungunya (CG), Filariasis, Yellow Fever and various forms of Encephalitis such as Japanese Encephalitis (JE), Eastern Equine Encephalitis, St. Louis Encephalitis, Western Equine Encephalitis, Venezuelan Equine Encephalitis, etc. In January 2016, the World Health Organization (WHO) said that the Zika virus was likely to spread throughout the majority of the America by the end of the year.

Mosquitoes breed in permanent, semi-permanent and temporary water bodies viz., running water, human dwelling, cattle shed, cess pits, tree holes, rock holes, cess pools, containers and discarded materials. The larval stage is aquatic and mosquito larval habitats are varied, which starts from tree holes to ponds and lakes.

Tree holes provide a unique specialized type of ecological habitat which is different from the usual breeding places of the other species of mosquitoes. Tree holes habitats (phytotelmata) are small natural containers formed by living or dead plant parts when rainwater is collected in bark depressions. They are the most commonly found when main branches fork or brake, behind scar tissues (McCafferty, 1998).

Tree holes are among the most abundant standing water in many tropical and temperate forests, they are the primary breeding sites for many disease vectors especially mosquitoes. Tree holes and bamboo stumps with standing water for long periods are used more likely to have mosquito population than those containing water for shorter periods (Sota *et al.*, 1994). Mosquito species groups, sub genus and genus have their own preferred habitat based on location and condition of the water body (Hopkins, 1952). The mosquito family *Culicidae* is regarded as ancestrally part-time dwellers, with two of its small families, *Sabethini* and *Toxorhynchitinae* are exclusively phytotelm dwellers (Kitching, 2001).

The physicochemical compositions of water bodies are complicated and determine their condition and fauna composition. They include salts, dissolved inorganic and organic matter, turbidity and presence of suspended mud. Other hydrologic factors that affect pre-imaginal mosquito populations in water are the presence or absence of

plants, temperature, light and shade, hydrogen ion concentration, presence of food substances (living or dead), presence of predacious mosquito larvae and other insects.

OBJECTIVES OF THE PROJECT

- To survey, collect and identify tree hole mosquitoes from various locations (plains, forest and coastal areas) in the districts of Tamilnadu and selected places of Andhra Pradesh, Kerala and Karnataka.
- To investigate the relationship between climatic factors and physico-chemical parameters of tree hole mosquitoes.
- To study the morphology of the tree holes (height, diameter, depth,) and location at sampling site with tree hole mosquito distribution.
- To identify the species of mosquito breeding in the tree holes of Southern states of India.
- To update and prepare exclusive key to tree hole mosquitoes of Southern India.
- Generation of a repository of tree hole mosquitoes of Southern India.

MATERIALS AND METHODS

Study locations

Altogether survey tours were carried out between April, 2013 to March, 2017 viz., Monsoon (Jun-Oct), Winter (Nov-Feb) and Summer (Mar-May) around 32 districts of Tamilnadu and some places of Andhra Pradesh, Karnataka, Kerala and Puducherry (Fig.3). Study was mainly focused hill ranges viz., Anaimalai hills (Coimbatore), Kodaikanal hills (Palani), Yercaud hills (Salem), Kolli hills (Namakkal), Sitheri hills (Dharmapuri), Megamalai (Theni), plains, and in rural and urban areas. Since the studies were confined to Tamilnadu and selected places of Andhra Pradesh, Karnataka, Kerala and Puducherry, it is mainly the Western and Eastern slopes of the Western and Eastern Ghats which have been surveyed. During the survey for tree hole mosquitoes, we mostly concentrated forest fringe villages and tourist places. Due to the climatic variability recorded in the different hill areas, it is possible to observe large differences in the average temperatures and precipitations registered between the different categories of the natural tree holes studied.

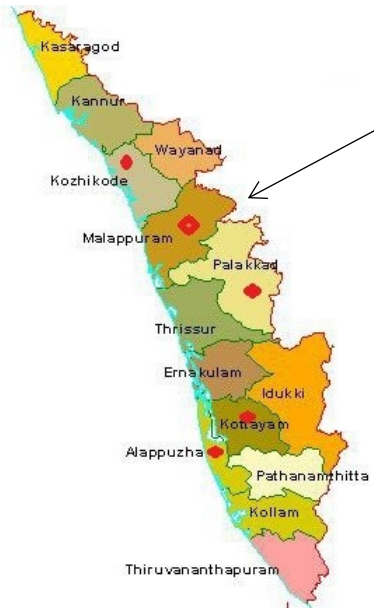
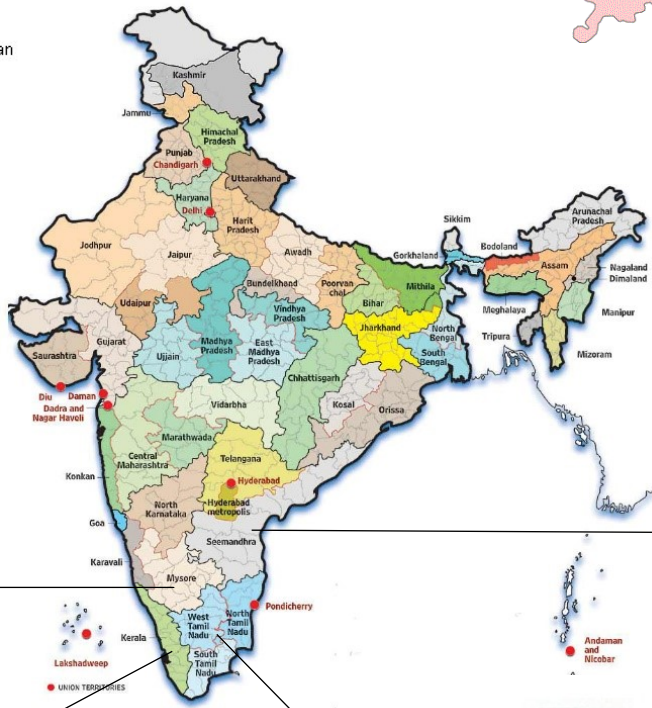
Fig.1 Map of Study Areas



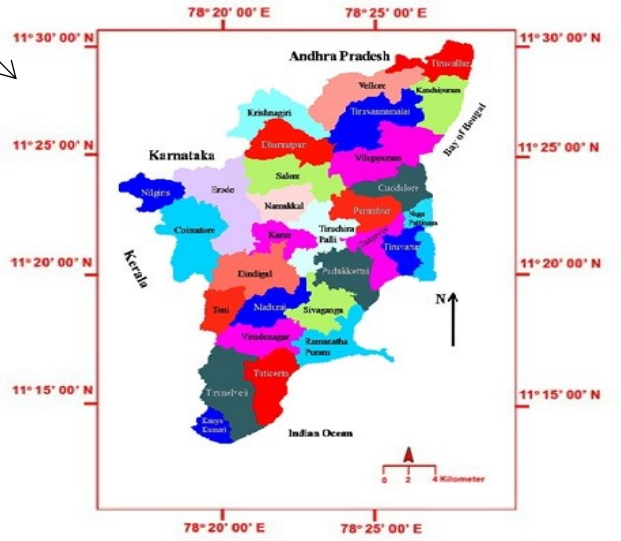
Karnataka



Andhra Pradesh



Kerala



Tamilnadu

Mosquito collection techniques and equipment's used

The basic equipments were used for field collection of mosquitoes viz., suction tube, needle, forceps, hand magnifier, insect killing jar, rectified sprit (formalin), aspirator, cotton, test tubes, marker pen, kerosene pump, torch light, etc. A collection bag (roughly 14" × 18") made of canvas, muslin or other materials are helpful to carry equipments.

A random sampling method was carried out across the study locations by selecting all the suitable trees (having tree holes) to accommodate immature forms of mosquitoes. Mosquito collection was carried out in atleast 25 tree holes in each site at dawn (06:00 – 09:30) and dusk (18:00 – 21:30) hours at frequently twice per month. Immatures (larvae and pupae) were sampled by sucking 20 - 40 mL (500 mL) samples followed by a standardized larval sampling protocol at each tree holes (WHO, 2013; Silver, 2008).

Data Analysis

Community analysis was carried out during rainy season when majority of the mosquitoes were at the peak of their growth. In every study sites, 15 tree species of 25 trees were randomly selected in all the districts of the quadrats of 10 m X 10 m (100 sq. m). The important quantitative analysis such as Relative abundance and Relative frequency of tree hole mosquitoes were determined as per Curtis and McIntosh (1950). The species richness, diversity and dominance were calculated by following the procedure of Shannon-Weiner diversity index (1949), Simpson's dominance index (1949). The faunal structure with environmental variables among sampling sites and effect of seasonality on mosquitoes were subjected to multivariate analysis of Principal Component Analysis (PCA) by using PAST version 3.06 (Hummer *et al.*, 2001).

Fig.2 Field collections of mosquitoes from southern India



RESULTS

The mosquito species found in southern India (Tamilnadu, Kerala, Karnataka, Andra Pradesh and Puducherry Union Territory) were identified and presented in Table 1 and Plate.1. Totally 15 genera of 88 species were collected in Southern, India.

Table.1 Identification of mosquitoes

S. No	Mosquitoes	S. No	Mosquitoes
Subfamily - Anophelinae		26	<i>Culex bailyi</i>
1	<i>Anopheles stephensi</i>	27	<i>Culex fragilis</i>
2	<i>Anopheles aitkenii</i>	28	<i>Culex pallidothorax</i>
3	<i>Anopheles culiciformis</i>	29	<i>Culex brevipalpis</i>
4	<i>Anopheles insulaeflorum</i>	30	<i>Culex khazani</i>
5	<i>Anopheles interruptus</i>	31	<i>Culex flavicomis</i>
6	<i>Anopheles sintoni</i>	32	<i>Culex lasiopalpis</i>
7	<i>Anopheles elegans</i>	33	<i>Culex mammilifier</i>
8	<i>Anopheles jeyporiensis</i>	34	<i>Culex minor</i>
9	<i>Anopheles maculatus</i>	35	<i>Culex minutissimus</i>
10	<i>Anopheles mirans</i>	36	<i>Culex uniformis</i>
11	<i>Anopheles annularis</i>	37	<i>Culex tritaeniorhynchus</i>
12	<i>Anopheles culicifacies</i>	38	<i>Culex pipiens</i>
13	<i>Anopheles tessellatus</i>	39	<i>Culex tarsalis</i>
14	<i>Anopheles subpictus</i>	40	<i>Culex decens</i>
Subfamily - Culicinae		41	<i>Culex nebuloses</i>
Genus - Armigeres		Genus - Downsiomyia	
15	<i>Armigeres subalbatus</i>	42	<i>Downsiomyia albolateralis</i>
16	<i>Armigeres joloensis</i>	43	<i>Downsiomyia nivea</i>
17	<i>Armigeres inchoatus</i>	Genus - Fredwardsius	
18	<i>Armigeres flavus</i>	44	<i>Fredwardsius vittatus</i>
Genus - Christophersiomyia		Genus - Heizmannia	
19	<i>Christophersiomyia annularis</i>	45	<i>Heizmannia chandi</i>
20	<i>Christophersiomyia gombakensis</i>	46	<i>Heizmannia grenii</i>
21	<i>Christophersiomyia thomsoni</i>	47	<i>Heizmannia indica</i>
Genus - Culex		48	<i>Heizmannia discrepans</i>
22	<i>Culex mimulus</i>	Genus - Lutzia	
23	<i>Culex pseudovishnui</i>	49	<i>Lutzia fuscana</i>
24	<i>Culex quinquefasciatus</i>	50	<i>Lutzia halifaxii</i>
25	<i>Culex vishnui</i>		

S. No	Mosquitoes	S. No	Mosquitoes
Genus - Ochlerotatus		71	<i>Aedes vittatus</i>
51	<i>Ochlerotatus anureostriatus</i>	72	<i>Aedes africanus</i>
52	<i>Ochlerotatus grenii</i>	73	<i>Aedes simpsoni</i>
53	<i>Ochlerotatus albocinctus</i>	74	<i>Aedes taylori</i>
54	<i>Ochlerotatus albotaeniatis</i>	75	<i>Aedes stokesi</i>
55	<i>Ochlerotatus chrysolineatus</i>	Genes - Tewarius	
56	<i>Ochlerotatus cogilli</i>	76	<i>Tewarius agastyai</i>
57	<i>Ochlerotatus deccanus</i>	77	<i>Tewarius reubenae</i>
58	<i>Ochlerotatus gubernatoris</i>	Genus - Tripteroides	
59	<i>Ochlerotatus khazani</i>	78	<i>Tripteroides aranoides</i>
60	<i>Ochlerotatus pseudotaeniatus</i>	79	<i>Tripteroides serratus</i>
Genus - Orthopodomyia		Genus - Uranotaenia	
61	<i>Orthopodomyia anopheloides</i>	80	<i>Uranotaenia bicolor</i>
62	<i>Orthopodomyia flavithorax</i>	81	<i>Uranotaenia novobscura</i>
Genus - Stegomyia		82	<i>Uranotaenia stricklandi</i>
63	<i>Aedes aegypti</i>	83	<i>Uranotaenia annandalei</i>
64	<i>Aedes albopictus</i>	84	<i>Uranotaenia campestris</i>
65	<i>Aedes annandalei</i>	85	<i>Uranotaenia hussaini</i>
66	<i>Aedes edwardsi</i>	Genus - Toxorhynchitinae	
67	<i>Aedes krombeini</i>	86	<i>Toxorhynchites minimus</i>
68	<i>Aedes novalbopicta</i>	87	<i>Toxorhynchites splendens</i>
69	<i>Aedes pseudalbopicta</i>	88	<i>Toxorhynchites viridibasis</i>
70	<i>Aedes subalbata</i>		

Among the collected 15 genera, *Aedes* was the predominant genus and was represented by 33.52 (3345 mosquitoes) per cent of the total mosquitoes collected, followed by *Culex* 29.34 % (2928 mosquitoes) and *Anopheles* 19.15 % (1911 mosquitoes) (Table.3.1 and Fig.3.1). These three genera together constituted 82.01 per cent of the total collection.

Plate 1. Adult mosquitoes



Ae. aegypti and *Ae. albopictus*



Cx. tritaeniorhynchus



Psoropora spp.



Cx. nigripalpus



Ae. triseriatus

Plate 1. Adult mosquitoes



An. mirans



An. culicifacies



Cx. quinquefasciatus



Oclerotatus spp.



Cx. pipiens



Culex spp.

Plate 1. Adult mosquitoes



Ar. subalbatus



Lutzia halifaxii



An. stephensi



Orthopodomyia anopheloides



Toxo. viridibasis



Toxo. splendens

Fig.3 Year wise collections of Tree hole Mosquitoes from April 2013 to March 2017

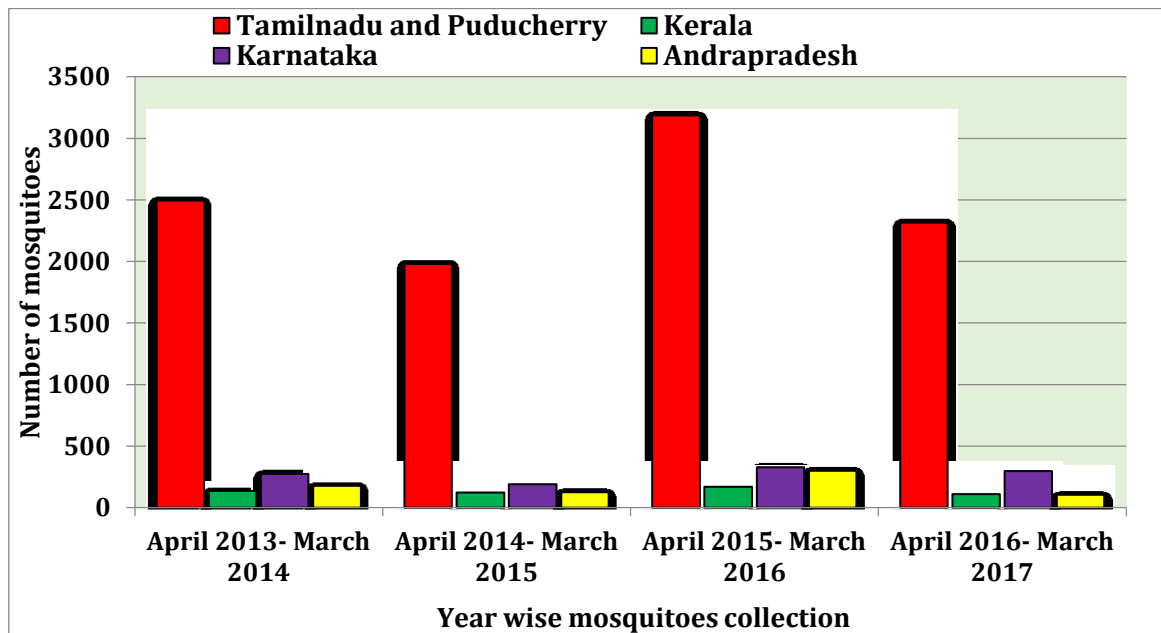


Table.2 Generic composition of collected mosquitoes in Tamilnadu during April 2013 – March 2017

Genus	No. of Species	Total no of specimens collected	Percentage (%)
<i>Aedes</i>	13	3345	33.52
<i>Anopheles</i>	14	1911	19.15
<i>Armigeres</i>	4	359	3.59
<i>Christophersiomyia</i>	3	139	1.39
<i>Culex</i>	20	2928	29.34
<i>Downsiomyia</i>	2	146	1.46
<i>Oclerotatus</i>	10	643	6.44
<i>Heizmannia</i>	4	96	0.96
<i>Orthopodomyia</i>	2	22	0.22
<i>Fredwardsius</i>	1	20	0.20
<i>Lutzia</i>	2	55	0.55
<i>Tripteroides</i>	2	42	0.42
<i>Uranotaenia</i>	6	97	0.97
<i>Tewarius</i>	2	21	0.21
<i>Toxorhynchites</i>	3	153	1.53
Total	88	9977	100

Table.3 Tree hole mosquitoes species diversity and dominance indices in Coimbatore, Dindugal, Salem, Namakkal, Theni and Dharmapuri districts of Tamilnadu, India (April 2013 – March 2017)

S. No	Name of the Species	f_i	$f_i \log f_i$	$f_i \log^2 f_i$	P_i	$\frac{N_i(n_i-1)}{n(N-1)}$	$P_i \log P_i$	$P_i \ln P_i$	$P_i (\ln P_i)^2$	Shannon-Weiner Index $H = (N \log N - \sum f_i \log f_i / N)$ (or) $-(P_i \log P_i)$	Simpson's Dominance Index $C = \sum (n_i/N)^2$
1	<i>Ae. aegypti</i>	184	416.7264	943.7912	0.0696	0.0048	-0.0805	-0.1854	0.4943	0.0805	0.0048
2	<i>Ae. albopictus</i>	174	389.8555	873.4452	0.0658	0.0043	-0.0777	-0.1790	0.4872	0.0777	0.0043
3	<i>Ae. pseudoalbopicta</i>	193	441.1125	1008.1355	0.0730	0.0053	-0.0829	-0.1910	0.5000	0.0829	0.0053
4	<i>Ae. subalbata</i>	130	274.8126	580.905	0.0492	0.0024	-0.0643	-0.1481	0.4463	0.0643	0.0024
5	<i>Ae. krombeini</i>	96	190.2980	377.1936	0.0363	0.0013	-0.0522	-0.1203	0.3991	0.0522	0.0013
6	<i>Ae. stokesi</i>	103	207.3222	417.2839	0.0389	0.0015	-0.0548	-0.1262	0.4100	0.0548	0.0015
7	<i>An. elegans</i>	37	58.0234	90.9904	0.0140	0.0001	-0.0259	-0.0597	0.4183	0.0259	0.0001
8	<i>An. stephensi</i>	96	190.2980	377.1936	0.0363	0.0013	-0.0522	-0.1203	0.3991	0.0522	0.0013
9	<i>An. aitekenii</i>	79	149.9125	284.4632	0.0299	0.0008	-0.0455	-0.1049	0.3683	0.0455	0.0008
10	<i>An. mirans</i>	69	126.8805	233.2959	0.0261	0.0006	-0.0413	-0.0951	0.3469	0.0413	0.0006
11	<i>Armigeres joloensis</i>	43	70.2391	114.7197	0.0162	0.0002	-0.0290	-0.0667	0.2753	0.0290	0.0002
12	<i>Armigeres inchoatus</i>	94	185.4740	365.9514	0.0355	0.0012	-0.0514	-0.1185	0.3956	0.0514	0.0012
13	<i>Christophersomyia annularis</i>	45	74.3945	122.985	0.0170	0.0002	-0.0300	-0.0692	0.2822	0.0300	0.0002
14	<i>Christophersomyia thomsoni</i>	63	113.3584	203.9562	0.0238	0.0005	-0.0386	-0.0889	0.3325	0.0386	0.0005
15	<i>Cx. quinquefasciatus</i>	190	432.9631	986.556	0.0719	0.0051	-0.0822	-0.1892	0.4982	0.0822	0.0051
16	<i>Cx. mimulus</i>	56	97.8985	171.1248	0.0211	0.0004	-0.0353	-0.0814	0.3141	0.0353	0.0004
17	<i>Cx. pseudovishnui</i>	48	80.6995	135.6672	0.0181	0.0003	-0.0315	-0.0726	0.2913	0.0315	0.0003
18	<i>Cx. flagilis</i>	42	68.1764	110.6574	0.0158	0.0002	-0.0284	-0.0655	0.2718	0.0284	0.0002
19	<i>Cx. flavicomis</i>	95	187.8837	371.567	0.0359	0.0012	-0.0518	-0.1194	0.3973	0.0518	0.0012
20	<i>Cx. uniformis</i>	129	272.2660	574.5918	0.0488	0.0023	-0.0640	-0.1473	0.4450	0.0640	0.0023
21	<i>Downsiomyia albolateralis</i>	91	178.2727	349.2216	0.0344	0.0011	-0.0503	-0.1159	0.3906	0.0503	0.0011
22	<i>Downsiomyia nivea</i>	41	66.1241	106.6328	0.0155	0.0002	-0.0280	-0.0280	0.2691	0.0280	0.0002
23	<i>Ochlerotatus greeni</i>	54	93.5492	162.0432	0.0204	0.0004	-0.0344	-0.0794	0.3090	0.0344	0.0004
24	<i>Ochlerotatus albocinctus</i>	52	89.2321	153.1192	0.0196	0.0003	-0.0334	-0.0770	0.3030	0.0334	0.0003
25	<i>Ochlerotatus gubernatoris</i>	57	100.0848	175.7196	0.0215	0.0004	-0.0358	-0.0825	0.3169	0.0358	0.0004

26	<i>Ochlerotatus khazani</i>	112	229.5124	470.3104	0.0423	0.0017	-0.0581	-0.1337	0.4231	0.0581	0.0017
27	<i>Ochlerotatus pseudotaeniatus</i>	112	229.5124	470.3104	0.0423	0.0017	-0.0581	-0.1337	0.4231	0.0581	0.0017
28	<i>Toxorhynchites splendens</i>	78	147.5833	279.2088	0.0295	0.0008	-0.0451	-0.1039	0.3662	0.0451	0.0008
29	<i>Toxorhynchites viridibasis</i>	40	64.0823	102.656	0.0151	0.0002	-0.0274	-0.0633	0.2654	0.0274	0.0002
30	<i>Heizmannia grenii</i>	39	62.0515	98.7168	0.0147	0.0002	-0.0269	-0.0620	0.2617	0.0269	0.0002
Σ		2642	5288.5996	10712.4128	0.9093	0.0374	-1.417	3.1242	11.1069	1.417	0.0374

f_i =Abundance of species, N =total number of individuals, P_i =Proportion of individuals found in the species, \ln =the natural (Naperian) logarithms (\log_e), $(n_i/N)^2 = (P_i)^2$

Table.4 Tree hole mosquitoes species diversity and dominance indices in Kerala state, India (April 2013 - March 2017)

S. No	Name of the Species	f_i	$f_i \log f_i$	$f_i \log^2 f_i$	P_i	$P_i \log P_i$	$P_i \ln P_i$	$P_i (\ln P_i)^2$	Shannon-Weiner Index $H = (N \log N - \sum f_i \log f_i / N)$ (or) $-(P_i \log P_i)$	Simpson's Dominance Index $C = \sum (n_i/N)^2$
1	<i>Ae. aegypti</i>	65	117.8393	213.629	0.1490	-0.1231	-0.2836	0.5400	0.1231	0.0222
2	<i>Ae. albopictus</i>	29	42.4095	62.0107	0.0665	-0.0782	-0.1802	0.4885	0.0782	0.0044
3	<i>An. stephensi</i>	79	149.9125	284.4632	0.1811	-0.1343	-0.3094	0.5287	0.1343	0.0328
4	<i>An. annularis</i>	41	66.1241	106.6328	0.0940	-0.0965	-0.2222	0.5255	0.0965	0.0088
5	<i>An. subpictus</i>	34	52.0702	79.7334	0.0779	-0.0863	-0.1988	0.5074	0.0863	0.0060
6	<i>An. culiciformis</i>	14	16.0457	18.389	0.0321	-0.0479	-0.1103	0.3796	0.0479	0.0010
7	<i>An. aitkenii</i>	31	46.2322	68.9409	0.0711	-0.0816	-0.1879	0.4969	0.0816	0.0050
8	<i>An. maculatus</i>	13	14.4812	16.1291	0.0298	-0.0454	-0.1046	0.3678	0.0454	0.0008
9	<i>An. mirans</i>	21	27.7666	36.7122	0.0481	-0.0633	-0.1459	0.4429	0.0633	0.0023
10	<i>Armigeres subalbatus</i>	10	10.0000	1.0000	0.0229	-0.0375	-0.0864	0.3266	0.0375	0.0005
11	<i>Cx. quinquefasciatus</i>	93	183.0689	360.3285	0.2133	-0.1431	-0.3295	0.5091	0.1431	0.0454
12	<i>Cx. tritaeniorhynchus</i>	22	29.5332	39.644	0.0504	-0.0653	-0.1505	0.4499	0.0653	0.0025
13	<i>Cx. vishnui</i>	28	40.5204	58.632	0.0642	-0.0765	-0.2035	0.4840	0.0765	0.0041
14	<i>Cx. pseudovishnui</i>	60	106.6890	189.696	0.1376	-0.1185	-0.2729	0.5413	0.1185	0.0189
Σ		436	902.6928	1535.9408	1.238	-1.1975	-2.7857	6.5882	1.1975	0.1547

Table.5 Tree hole mosquitoes species diversity and dominance indices in Karnataka state, India (April 2013 – March 2017)

S. No	Name of the Species	f_i	$f_i \log f_i$	$f_i \log^2 f_i$	P_i	$P_i \log P_i$	$P_i \ln P_i$	$P_i (\ln P_i)^2$	Shannon-Weiner Index $H = (N \log N - \sum f_i \log f_i / N)$ (or) $-(P_i \log P_i)$	Simpson's Dominance Index $C = \sum (n_i / N)^2$
1	<i>An. elegans</i>	143	308.2130	664.2779	0.1313	-0.1157	-0.2665	0.5412	0.1157	0.0172
2	<i>An. stephensi</i>	63	113.3584	292.6539	0.0578	-0.0715	-0.1647	0.4697	0.0715	0.0033
3	<i>An. aitekenii</i>	78	147.5833	279.2088	0.0716	-0.0819	-0.1887	0.4977	0.0819	0.0051
4	<i>Ar. inchoatus</i>	50	84.9485	144.31	0.0459	-0.0614	-0.1414	0.4357	0.0614	0.0021
5	<i>Christophersiomyia annularis</i>	68	124.6106	196.2616	0.0624	-0.0751	-0.2327	0.4802	0.0751	0.0038
6	<i>Ch. thomsoni</i>	89	173.4957	338.1733	0.0817	-0.0888	-0.2046	0.5125	0.0888	0.0066
7	<i>Cx. quinquefasciatus</i>	42	68.1764	110.6574	0.0385	-0.0544	-0.1253	0.4084	0.0544	0.0014
8	<i>Cx. mimulus</i>	19	24.2963	31.065	0.0174	-0.0306	-0.0704	0.2855	0.0306	0.0000
9	<i>Cx. pseudovishnui</i>	25	34.9485	48.8525	0.0229	-0.0375	-0.0864	0.3266	0.0375	0.0000
10	<i>Ae. aegypti</i>	194	443.8335	1015.396	0.1781	-0.1334	-0.3072	0.5302	0.1334	0.0317
11	<i>Ae. albopictus</i>	72	133.7279	248.364	0.0661	-0.0779	-0.1795	0.4878	0.0779	0.0004
12	<i>Ae. pseudoalbopicta</i>	36	56.0268	87.192	0.0330	-0.0488	-0.1125	0.3840	0.0488	0.0001
13	<i>Uranotaenia sticklandi</i>	65	117.8393	213.629	0.0596	-0.0729	-0.1680	0.4739	0.0729	0.0003
14	<i>Ur. bicolor</i>	34	52.0702	79.7334	0.0312	-0.0469	-0.1081	0.3750	0.0469	0.0000
15	<i>Heizmannia discrepans</i>	39	62.0515	98.7168	0.0358	-0.0517	-0.1192	0.3969	0.0517	0.0001
16	<i>Lutzia halifaxii</i>	25	34.9485	48.8525	0.0229	-0.0375	-0.0864	0.3266	0.0375	0.0000
17	<i>Ochlerotatus greeni</i>	47	78.5885	131.3885	0.0431	-0.0588	-0.1355	0.4260	0.0588	0.0001
	Σ	1089	2058.7169	4028.7326	0.8993	-1.1448	-2.6971	7.3579	1.1448	0.0722

Table.6 Tree hole mosquitoes species diversity and dominance indices in Andra Pradesh state, India (April 2013 – March 2017)

S. No	Name of the Species	f_i	$f_i \log f_i$	$f_i \log^2 f_i$	P_i	$P_i \log P_i$	$P_i \ln P_i$	$P_i (\ln P_i)^2$	Shannon-Weiner Index $H = (N \log N - \sum f_i \log f_i / N)$ (or) $-(P_i \log P_i)$	Simpson's Dominance Index $C = \sum (n_i/N)^2$
1	<i>Ae. aegypti</i>	164	363.2343	804.4692	0.2316	-0.1471	-0.3387	0.4955	0.1471	0.0536
2	<i>Ae. albopictus</i>	61	108.9051	194.4192	0.0861	-0.0916	-0.2111	0.5177	0.0916	0.0074
3	<i>Ae. pseudoalbopicta</i>	48	80.6995	135.6672	0.0677	-0.0791	-0.1822	0.4908	0.0791	0.0045
4	<i>Ochlerotatus greeni</i>	37	58.0234	90.9904	0.0522	-0.0669	-0.1541	0.4550	0.0669	0.0027
5	<i>Ochlerotatus gubernatoris</i>	21	27.7666	37.3254	0.0296	-0.0452	-0.1041	0.3667	0.0452	0.0000
6	<i>Ochlerotatus khazani</i>	60	106.6890	189.696	0.0847	-0.0908	-0.2090	0.5161	0.0908	0.0071
7	<i>An. culiciformis</i>	52	89.2321	153.1192	0.0734	-0.0832	-0.1917	0.5007	0.0832	0.0053
8	<i>An. insulaeflorum</i>	51	87.0860	148.6905	0.0720	-0.0822	-0.1894	0.4984	0.0822	0.0051
9	<i>An. interruptus</i>	62	111.1282	199.1626	0.0875	-0.0925	-0.2131	0.5192	0.0925	0.0076
10	<i>An. sintoni</i>	48	80.6995	135.6672	0.0677	-0.0791	-0.1822	0.4908	0.0791	0.0045
11	<i>Armigeres flavus</i>	41	66.1241	106.6328	0.0579	-0.0716	-0.1649	0.4699	0.0716	0.0033
12	<i>Tripteroides aranoi</i>	29	42.4095	62.0107	0.0409	-0.0567	-0.1307	0.4179	0.0567	0.0016
13	<i>Uranotaenia annandalei</i>	21	27.7666	36.7122	0.0296	-0.0452	-0.1041	0.3667	0.0452	0.0000
14	<i>Uranotaeni bicolor</i>	7	5.9156	4.998	0.0098	-0.0196	-0.0453	0.2096	0.0196	0.0000
15	<i>Toxo. splendens</i>	6	4.6689	3.6324	0.0084	-0.0174	-0.0401	0.1918	0.0174	0.0000
	Σ	708	1260.3484	2030.193	0.9991	-0.986	-2.4607	6.5068	0.986	0.1027

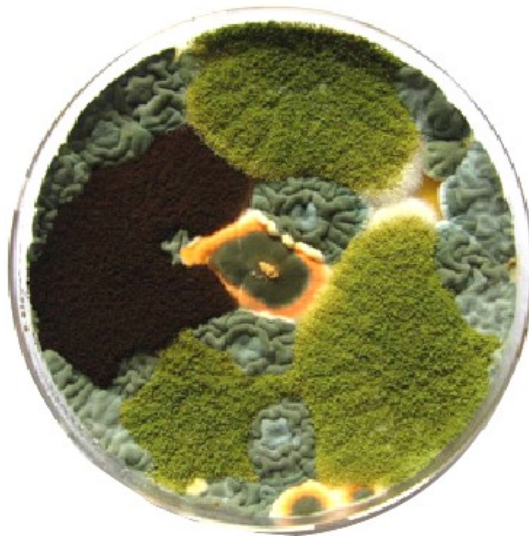
Table.3.5.1. Physico-chemical characteristics of tree hole mosquito breeding in Southern India during April 2013 – March 2017

Name of the District	Tree hole Water Temperature (°C)	pH	Conductivity (µS/cm)	Turbidity (NTU)	Total Alkalinity (mg/L)	Total Hardness (mg/L)	Magnesium (mg/L)	Chloride (mg/L)	Nitrate (mg/L)	TDS (mg/L)	Total Suspended Solids (mg/L)	Phosphate (mg/L)	DO (mg/L)
Coimbatore	28	6.8±0.94	162.9±22.3	22.7±3.08	60.36±1.46	138.55±2.28	7.01±0.3	8.36±0.13	14.0±0.2	226.15±10.27	380±19.1	0.68±0.05	6.21±0.13
Dindugal	18	7.54±0.2	6.02±1.1	80.3±0.1	41±0.3	20.2±8.1	2.2±0.3	41±1.2	22±3	132±2.4	305±6.1	0.25±0.1	4.9±0.8
Salem	32	7.5±0.66	148.6±16.3	36.2±6.7	49.17±2.58	144.72±3.11	3.6±1.7	9.57±0.72	31±2.6	258.04±7.65	178±7.3	0.58±0.38	6.8±0.06
Namakkal	27	5.89±0.5	241±92	253±27	58.02±11	67.3±21.1	6±0.3	35±10	13±0.5	124±3.2	460±28	10±2.1	5.49±0.7
Dharmapuri	25	7.1±0.14	263±41	187±19	52.36±4.5	100±5.07	5.1±1.7	26.96±1.36	16.2±2.1	561.07±6.65	368±21	2.15±0.25	3.64±0.08
Theni	19	7.2±0.52	98.1±11.03	46.81±6.18	73.75±3.25	127.38±2.72	7.1±3.2	10.45±0.28	17.6±2.8	298.26±9.57	323±12	1.48±0.64	6.18±0.04
Madurai	28	5.94±0.56	164±02.05	253±2.06	30±2.50	34±1.69	1.2±0.1	24±3.48	11±0.44	124±04.16	460±06.07	11.05±2.01	4.2±3.41
Kanyakumari	25	5.94±0.99	241±1.02	280±0.02	63±1.02	68±0.11	4.6±0.2	11±5.1	14±0.08	140±1.27	638±08.97	0.51±1.3	2.0±0.5
Nagapattinam	27	6.21±1.69	185±3.02	240±1.10	40±1.02	53±03.02	6.3±1.5	7.5±0.9	10±0.02	125±6.01	440±09.09	0.25±03.2	3.8±2.1
Virudhunagar	29	5.80±1.18	150±04.31	225±5.09	48±1.36	25±03.07	2.1±03.2	21±3.5	10±0.87	1.50±1.08	435±1.02	10.6±0.2	4.69±3.2
Nilgiris	16	6.59±1.08	350±3.09	310±1.39	55±1.61	18.90±03.56	4.3±0.2	9.1±2.1	11.3±0.5	1.50±0.12	112±3.21	1.48±0.2	2.06±0.1
Thiruvavur	30	6.35±1.04	201±4.26	245±7.52	33±1.61	48±03.02	2.6±0.4	8.6±0.6	7.3±0.8	435±9.69	580±2.09	2.51±0.7	3.08±1.1
Kancheepuram	31	7.3±2.0	225±2.8	30.6±1.5	51±1.01	100±1.64	1.2±0.1	19.9±1.0	1.44±0.3	10±1.08	260±28	0.78±0.38	6.78±2.8
Chennai	31	8.2±2.10	6.20±0.1	331±6.3	49.21±2.01	80±0.8	6.2±0.3	25.3±1.30	1.65±0.2	338.4±5.40	291.5±0.41	6.4±0.4	44.2±1.34
Thiruvallur	32	8.4±1.51	5.30±0.3	322±2.8	81.2±3.1	140±4.5	5.8±0.6	28.3±1.2	0.94±0.1	177.6±2.8	321±1.2	4.3±1.2	22.1±2.0
Ramanathapuram	30	6.5±0.66	248.6±16.3	36.2±6.7	49.17±2.58	244.72±3.11	3.6±1.7	9.57±0.72	31±2.6	358.04±7.65	178±7.3	0.58±0.38	6.8±0.06
Tirunelveli	28	5.89±0.5	241±92	253±27	58.02±11	67.3±21.1	6±0.3	35±10	13±0.5	124±3.2	460±28	10±2.1	5.49±0.7
Sivagangai	32	8.1±1.9	5.40±1.1	335±4.3	62.1±1.8	160±2.0	3.2±0.4	29.6±1.9	0.75±0.1	215.4±4.1	220.1±1.7	0.67±0.1	49.5±2.8
Pudukottai	29	7.21±1.69	285±3.02	140±1.1	30±1.02	43±02	5.3±1.5	7.5±0.9	9±0.02	125±6.01	440±09.09	0.25±03.2	3.8±2.1
Vellore	30	4.80±1.18	50±04.31	325±5.04	28±1.26	35±01.07	2.1±03.2	21±3.5	10±0.87	1.50±1.08	435±1.02	10.6±0.2	4.69±3.2
Tiruchirappalli	31	7.8±0.3	185.1±1.51	24.6±1.3	28.31±2.03	130±2.1	4.05±1.5	10.2±0.1	1.74±0.1	176±2.8	367±9.1	0.79±0.01	6.52±0.5
Tanjore	29	7.5±0.6	149.1±3.4	31.5±1.3	19.3±0.21	70±1.8	3.8±0.6	12.4±1.0	1.76±0.2	129±3.4	283±1.4	1.38±0.31	6.75±1.2
Krishnagiri	32	6.3±2.0	125±2.8	20.6±1.5	41±1.01	89±1.64	1.2±0.1	12.9±1.0	1.44±0.3	10±1.08	260±28	0.58±0.38	6.78±2.8
Villupuram	29	7.8±1.20	5.27±1.26	269±2.80	42.36±4.5	90±9.15	3.2±0.3	30.3±4.12	0.70±0.03	358.04±7.65	205±6.1	0.25±03.2	40.9±1.02
Perambalur	31	7.9±1.6	5.29±3.1	340±4.8	63.75±3.25	140±2.7	5.6±1.7	30.5±1.3	0.67±0.01	221±3.2	78±7.3	10.6±0.2	38.1±1.5
Ariyalur	31	8.3±1.4	5.15±2.10	370±6.2	43±1.36	120±2.7	8±0.3	20.8±0.90	8.77±2.5	361.07±4.65	360±28	1.48±0.2	49.8±2.1
Tuticorin	29	5.21±1.69	85±3.02	140±1.10	35±02	33±03.02	4.3±1.5	6.5±0.9	11±0.02	325±5.01	240±09	0.35±02.2	3.8±2.1
Thiruvannamalai	30	7.9±2.5	5.38±1.6	312±4.02	28±1.03	110±6.4	6.1±3.2	31.7±2.10	0.69±0.01	225±6.01	278±7.3	0.15±0.1	39.2±1.2
Karur	30	7.3±2.0	125±2.8	50.6±1.5	51±1.01	100±1.64	1.2±0.1	19.9±1.0	1.44±0.3	10±1.08	260±28	0.78±0.38	6.78±2.8
Erode	27	7.1±1.3	6.90±2.15	294.1±3.5	22±1.6	120±1.6	3.6±0.2	13.7±0.2	1.12±0.1	150±0.12	168±21	9±2.1	36.5±1.1
Tirupur	29	5.1±0.1	10.1±1.02	180±0.02	23±1.03	48±0.41	3.2±0.1	10±4.1	11±0.04	120±1.27	438±11	0.11±0.03	2.0±0.5
Cuddalore	31	8.1±0.2	6.13±1.31	280±1.02	41.2±2.1	90±2.1	2.03±0.1	21.6±0.6	0.50±0.01	125±6.01	240±04	0.15±03	46.3±2.3

Table.3.6.1 Structural characteristics of tree holes in southern states (Tamilnadu, Kerala, Karnataka, Andra Pradesh and Puducherry Union Territory) of India

S. No	Tree Species	Common Name	No. of trees surveyed	No. of tree holes with water	No. of Positive tree holes with Mosquito Immature	Mosquitoes Collected	Tree-hole structural characteristics			Water characteristics	
							Height (cm) From Ground	Diameter (cm)	Depth (cm)	Water volume (ml)	Water Quality Appearance
1	<i>Polyalthia longifolia</i>	Nettilingam	36	49	28	146	151	5	25	20-25	Turbid
2	<i>Delonix regia</i>	Flamboyant	341	458	324	2820	166	9	10	800-1160	Clear/straw
3	<i>Magnifera indica</i>	Mango	80	91	78	98	178	6	9	40-60	Turbid
4	<i>Azadirachta indica</i>	Neem	87	52	41	76	132	9	13	35-60	Turbid
5	<i>Anacardium occidentale</i>	Cashew	41	61	37	81	111	12	9	190-200	Clear/straw
6	<i>Eugenia jambolana</i>	Indian Jamun	19	25	19	420	98	6	15	90-110	Clear/straw
7	<i>Pinusrox burgii</i>	Pine	84	72	66	398	124	8	13	2000-2100	Clear/straw
8	<i>Eucalptus globulus</i>	Eucalyptus	76	71	60	581	165	7	25	155-160	Turbid
9	<i>Acacia arabica</i>	Gum Arabic	111	90	69	129	121	10	26	40-80	Turbid
10	<i>Terminalia catappa</i>	Indian almond	70	53	48	142	94	6	12	90-170	Clear/straw
11	<i>Ficus bengalensis</i>	Banyan	63	73	65	60	124	12.5	18	10-40	Turbid
12	<i>Emlica officinalis</i>	Goose berry	54	67	51	71	184	5	25	20-45	Turbid
13	<i>Albizia saman</i>	Rain tree	71	88	72	109	124	5	29	10-65	Turbid
14	<i>Ziziphus jujube</i>	Jujube tree	33	47	30	63	120	10	20	80-130	Clear/straw
15	<i>Salix alba</i>	White willow	49	61	55	132	138	13	46	400-500	Turbid
16	<i>Milletia pinnata</i>	Pungai	129	140	81	164	172	5	12	90-140	Turbid
17	<i>Pungamia pinnata</i>	Pongamia	199	268	109	927	143	9	34	200-270	Turbid
18	<i>Tectona grandis</i>	Teak	84	95	74	91	165	14	25	200-350	Turbid
19	<i>Caesalpinia ferra</i>	Ironwood	63	71	59	55	116	6	21	500-650	Turbid
20	<i>Alanthus excels</i>	Prumaram	67	88	72	696	79	8	10	30-80	Clear/straw
21	<i>Delonix elata</i>	Vathanarayan	219	311	158	1545	93	7	9	40-70	Turbid
22	<i>Ficus religiosa</i>	Peepul	75	68	49	110	121	8	13	15-45	Turbid
23	<i>Murraya koenigii</i>	Murungai	81	84	59	277	194	9	26	600-850	Clear/straw
24	<i>Millingtonia hortensis</i>	Indian cork tree	64	29	19	198	132	11	15	20-45	Clear/straw
25	<i>Kaya senegalensis</i>	Senegal Mahogany	72	39	31	76	154	14	13	30-65	Turbid
26	<i>Plumeria rubra</i>	Champka	25	45	37	91	128	5	24	80-120	Turbid
27	<i>Peltophorum pterocarpum</i>	Copperpod	32	56	42	62	96	26	25	10-25	Clear/straw
28	<i>Moringa pterygosperma</i>	Drumstick tree	53	74	62	359	137	6	11	550-700	Turbid
Total			2378	2726	1895	9977					

Plate.19. Identification of Micro-organisms from Tree hole water



Fungi



Bacteria



Actinomycetes

SUMMARY OF THE PROJECT

The report embodies results of an investigation carried out during April 2013 – March 2017 gather information on taxonomic diversity and ecology of tree hole mosquitoes from thirty two different districts of Tamilnadu, Karaikkal, Pondicherry and selected places of Andra Pradesh, Karnataka, Kerala, of India and to identify vector mosquitoes breeding in tree holes.

Mosquito surveillance data accounted totally 12,314 (larvae and pupae 6834; 3143 adults) were collected during April 2013 – March 2017 in Tamilnadu and Puducherry (9977), Kerala (540), Karnataka (1089) and Andra Pradesh (708) India. The district Coimbatore (618) having high prevalence of tree hole mosquitoes, and the district Karur having less in number (96). The genus *Aedes* was the overall dominant species compared with other species and lowest genus collected was *Fredwardisus*. The highest prevalence of mosquitoes were in the months of November (1803) and December (2652) and least in April (169) and May (104). Twenty eight different tree species of tree holes were selected, among them 1895 were identified as mosquito breeding tree holes with water and 9977 immatures as well as adult mosquitoes were collected from tree holes by following standard protocols for a period of four year from April 2013 – March 2017. A total of 2642 mosquitoes were collected from Coimbatore (618), Dindugal (456), Salem (458), Namakkal (409), Theni (386), and Dharmapuri (315) which includes 30 different species. The species, *Aedes pseudoalbopictus* was the most diversified and recorded 7.30 per cent in these six districts. The highest population of mosquitoes were recorded during the month of December.

The species diversity and abundance of species indices of mosquitoes collected from each districts during the study period were determined by two different indices viz., Shannon-Weiner index and Simpson's dominance index. Based on the results of the two indices, *Ae. aegypti* was the most predominant species in almost all the districts of Tamilnadu followed by *Culex quinquefasciatus* and *An. stephensi*. The highest Shannon-Weiner diversity index (0.0805) and Simpson's dominance (0.0048) value were recorded in Coimbatore district of Western Ghats. Among the collected mosquito species *Ae. aegypti* recorded as abundant diversity and

abundance indices in 32 different districts of Tamilnadu, followed by *Cx. quinquefasciatus* and *An. stephensi* species.

The influence of climatic factors such as temperature, relative humidity and rainfall on the distribution and diversity of mosquitoes were studied for the period of one year in all districts. It was observed that climatic conditions were favourable during the month of October, November and December, 2014, in which maximum number of mosquitoes were collected in all locations. The wider and higher distribution of mosquitoes was recorded during the post monsoon period when compared to pre monsoon period. Physico-chemical parameters viz., pH, conductivity, turbidity, total alkalinity, total hardness, chloride, nitrate, magnesium, total dissolved solids, total suspended solids, phosphate and dissolved oxygen of the water samples collected from tree holes of all locations of thirty two different districts were analysed. It was observed that the species diversity was influenced by some of the physico-chemical parameters. The types of trees present in each district were identified. It was noted that the most prevalent species in Tamilnadu was *Delonex regia*, followed by *Delonex eleta* and *Pongamyia pinnata*. The least mosquitoes were collected from the tree holes of *Caesalpinia ferra* and *Ficus bengalensis*. There was no correlation between the types of tree species and distribution of mosquitoes. PCA analysis clearly illustrated the change of tree hole mosquito community structure with spatial and temporal aspect. Corresponding analysis concluded the highest number of mosquitoes was recorded from Western Ghats hills stations of Coimbatore district. Temporal analysis was revealed that a few environmental factors could be affecting mosquito population which includes humidity and elevation etc.

CONCLUSION

Knowledge of the taxonomic and functional biodiversity of both endemic and invading vector mosquito species as well as the factors driving change is missing in Tamilnadu, India. Acquiring this knowledge is an essential step towards understanding current risk and preparing for future threats. Tree hole mosquito fauna shows similarities with respect to geographical zones. Western Ghats hills area of Coimbatore district has similar mosquito fauna from rest of the study areas (based

on diversity index analysis). Study demonstrated that the tree hole mosquito community in Tamilnadu, India strongly respond to physico-chemical parameters and land use changes. The results displayed that there is a diverse mosquito community in tree holes of Tamilnadu, India, but more importantly that the community composition varies considerably between forests and plain tree hole habitats. This strong influence of various parameters and land use changes on mosquito communities could have potential implications for pathogen transmission to humans and wildlife. Historically, mosquito studies have been predominantly focused on single species lifecycles in association with the urban environment, we suggest further ecological studies are necessary to understand how land use changes will influence disease dynamics of the whole community in order to predict and prevent future health threats.

List of Publications

1. Senthamarai Selvan, P., Jebanesan, A and Reetha, D., 2016. Entomofaunal diversity of tree hole Mosquitoes in Western and Eastern Ghats hill ranges of Tamilnadu, India. *Acta Tropica*, 159; 69-82.
2. Senthamarai Selvan, P., Jebanesan, A., Divya, G and Ramesh, V., 2015. Diversity of mosquitoes and their larval breeding preference based on the physico-chemical parameters in Western Ghats, Tamilnadu, India. *Asian Pacific Journal of Tropical Disease*, 5(Supplement 1); S59-S66.
3. Senthamarai Selvan, P., A. Jebanesan and C. Makesh Kumar, 2014. Diversity and distribution of tree hole mosquitoes in Puducherry Union Territory, India. *Journal of Coastal Life Medicine*, 4(1); 930-933.
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5. Senthamarai Selvan, P., and Jebanesan, A., 2016. Tree hole mosquitoes in Western and Eastern Ghats of Tamilnadu, India. *Atlas of Science*, 3; 1-3.

6. Senthamarai Selvan, P and Jebanesan, A., 2016. Studies on the Mosquito diversity with special reference to dengue vectors in Vellore district, Tamilnadu, India. ***International Journal of Zoology and Applied Biosciences***, 1(1); 32-39.
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