



## Ecology of Tree-Holes and Diversity of Insect Larvae in Tree-Hole Water in Mayiladuthurai Taluk

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### ABSTRACT

Tree holes are produced due to environmental factors as well as biotic pressure. It is a unique habitat for insect breeding and survival. Water-filled tree-holes or their analogs are natural microcosms because of their relative simplicity in habitat structure, small size, discrete boundaries, and naturally replicated nature. The present investigation was carried out with aim of the ecology of tree holes from the different tree species with diversity and distribution of insect larvae. Eight species of trees have been identified with tree holes from the study area. Among the eight species of trees, *Moringa olifera* has the highest number of tree holes followed by *Samanea saman*. All the independent variable of the tree hole was significantly varied among the three species. Six species of larvae were recorded from the tree hole water analysis belonging to five insects and one annelid. Namely, *Culex quinquefasciatus*, *Ades aegypti*, *Scirtidae* sp, *Chaboridae* sp, *Armadillidae* sp, and *Euborellia annulipes* from the phylum of Arthropoda and Naididae sp from the Annelida. Among the six species of larvae, *Culex quinquefasciatus* has a large number of individuals followed by *Ades aegypti* and so on. It concluded that three holes and their water has many resources to inhabit insect larvae successfully. It needs to be a long-term survey to check their assemblage and its importance to biodiversity.

**Keywords:** Tree hole, Microhabitat, Morphometric measurements, Insects, Larvae.

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### INTRODUCTION

Tree holes are especially appropriate habitats for comparative studies of population interactions [1]. Their range extends from the subarctic to the equator; they are ubiquitous in and harbor insects at all latitudes where hardwood trees occur, even in the Mojave Desert [2]. Over this range, these habitats exhibit tremendous variation in the surrounding climatic conditions, species composition, and community complexity. Tree holes or cavities filled with rainwater act as ephemeral pools called dendrothelms, and are used for breeding and hiding during daytime [1]. The ephemeral water bodies that are associated with living plants are inhabited by a high diversity of

organisms, from bacteria to invertebrates and they occur worldwide [3]. A variety of macroinvertebrates use the tree holes as breeding sites and many species breed exclusively in this habitat [4].

Tree holes are formed by intrinsic growth patterns of the tree or extrinsic agencies such as wind breakage, forest fires, and cavity excavation by organisms such as woodpeckers or insects [5]. Using the direct method or stem flow method, rainwater fills the tree holes. Nutrients are transferred into the tree holes from foliage and bark through this process, and thus, the species of the tree and the dry atmospheric depositions on the bark are what the chemical content of the water depends on [6]. Rot-holes and pans occur with varying

frequency, in water-filled tree holes. Water is in contact with the wood in rot-holes, in which holes are found in stems, branch breaks, and stumps. The hole is lined with bark in pans, which are made by stem and branch forks as well as between buttress roots. In a lot of trees, both types of water-filled tree holes can be found, but their frequencies have differences, between different species of the trees [7]. This habitat is the primary breeding site for many disease-causing vectors, especially mosquitoes and biting midges. Tree-hole contains water and organic detritus of leaf, wood, and animal origin. After a long literature review, the present investigation was designed and carried out the insect fauna in tree holes in various trees with associated physicochemical factors.

### MATERIALS AND METHODS

The study was carried out in Sembarnarkoil (11° 06' N, latitude 79° 44' E longitude) region, Tharagambadi Taluk, Mayiladuthurai District, Tamil Nadu. The study area is a densely populated semi-urban, which predominantly has agricultural lands with paddy (*Oryza sativa*) as the major crop. Apart from the crops cultivated in this area, the tree covers are also highly distributed i.e., *Azadirachta indica*, *Moringa olifera*, *Samanea saman*, *Tamarindus indica*, *Tectona grandis*, *Pongamia glabra*, *Ficus religiosa*, *Madhuca longifolia*, *Mangifera indica*, *Polyalthia longifolia*, etc., According to the rainfall the study area are classified into four seasons viz., monsoon (October-December), post-monsoon (January-March), summer (April-June) and pre-monsoon (July-September). The present study was carried out between the seasons of monsoon to post-monsoon. Chosen various places to study tree hole properties and insect fauna distribution i.e., Thiruchampalli, Kalagasthenathapuram, Vallum, and Parasalur. Tree-holes were sampled from October 2019 to March 2020. Location of tree hole and type of tree hole were observed by direct vision. Tree hole length, width, depth, water level, and height

from the ground-using scale in centimeter. Leaf litter will be preserved and its dry weight will be obtained after drying. Tree holes were studied by removing their contents by hand-collection up to hole depth (depending on hole morphology) [8]. Insect fauna was collected within a week after the rainfall and collected insects preserved in ethanol (70%), After bringing laboratory the insects were counted and identified with the help of Photomicroscopy (LABOMED STC- ML 100X10X) [8].

The following physicochemical parameters were assessed by using appropriate methodologies [9] such as temperature (probe thermometer), pH (Digital pH Meter), Nitrate, Ammonium, Alkalinity, Salinity, Total hardness, Nitrite, Iron, and Calcium. The statistical analyses are diversity indices and ANOVA proved the significance of the study and it was done by using IBM SPSS version 25.

### RESULTS AND DISCUSSION

Totally 40 tree holes were surveyed which belong to eight species of trees. Namely, *Azadirachta indica* (6), *Moringa olifera* (9), *Samanea saman* (8), *Tamarindus indica* (4), *Tectona grandis* (5), *Pongamia glabra* (4), *Ficus religiosa* (3) and *Madhuca longifolia* (1). Among the eight species of trees, the *Moringa olifera* has the highest number of tree holes followed by *Samanea saman* and so on. *Madhuca longifolia* has the least number of tree holes. The tree height was measured and noted that the *Tectona grandis* was high (21m) and the least height was *Ficus religiosa* (7m). Two types of shapes were noted from the sampling trees i.e., rod (48%) and pan (52%). Morphometric measurements of tree holes were assessed. The largest opening size, depth, height from the ground, water level, and litters of the tree holes were observed in *Ficus religiosa* and least observed in *Samanea saman* (Table 1).

**Table 1.** Morphometric measurements (Mean±SD) of trees holes surveyed during the study period (n=40)

Tree species	Hole type (n)	Opening size (cmx cm)	Depth (cm)	Height from the ground (cm)	Water level (cm)	Litters (g)
<i>Azadirachta indica</i>	Pan (3)	13.5 X 18.83	17.70±1.2	157.90±8.5	9.97±0.6	2.78±0.4
	Rot (3)	17.17 X 17.4	19.43±1.5	222.67±7.5	12.63±0.8	1.49±0.3
<i>Moringa olifera</i>	Pan (3)	23.13 X 22	27.53±2.5	89.47±4.2	22.73±1.2	3.15±0.4

	Rot (6)	15.61 X 16.36	31.25±2.6	120.57±5.3	17.53±1.4	2.62±0.6
<i>Ficus religiosa</i>	Pan (2)	38.1 X 28	45.20±3.5	595.00±9.8	29.30±2.5	6.13±0.8
	Rot (1)	14.0 X 22.0	49.00±3.8	70.00±4.2	36.00±3.5	3.16±0.1
<i>Pongamia glabra</i>	Pan (2)	15.45 X 13.25	15.50±1.2	112.10±2.3	12.15±1.2	4.18±0.4
	Rot (2)	11.45 X 13	20.00±2.1	109.80±6.2	14.00±1.2	1.98±0.3
<i>Samanea saman</i>	Pan (5)	4.72 X 4.2	6.92±0.5	29.00±1.5	2.36±0.4	1.14±0.1
	Rot (3)	6.13 X 5.6	6.33±0.8	76.33±1.5	3.40±0.4	0.43±0.1
<i>Tamarindus indica</i>	Pan (2)	17.5 X 20.2	26.90±2.1	99.30±1.8	10.35±1.2	2.74±0.4
	Rot (2)	17.8 X 20.25	21.65±2.4	58.65±1.7	13.30±1.3	3.17±0.6
<i>Tectona grandis</i>	Pan (3)	20.0 X 16.96	14.07±1.4	62.67±1.2	8.70±1.2	1.92±0.1
	Rot (2)	6.4 X 8.2	19.75±1.6	110.00±2.1	8.95±1.4	1.62±0.2
<i>Madhuca longifolia</i>	Pan (1)	29 X 21	29.00±0.0	480.00±0.0	18.00±0.0	3.42±0.0

The physicochemical factors of tree hole waters were analyzed. The temperature was high in the *Tectona grandis* and least in *Madhuca longifolia*. The pH was high in *Madhuca longifolia* and least recorded in *Azadirachta indica*. Ammonia level was high *Pongamia glabra* and least was in *Ficus religiosa*. Salinity was high in *Azadirachta indica* and least recorded in the *Tectona grandis*. As well as three species showed an absence of

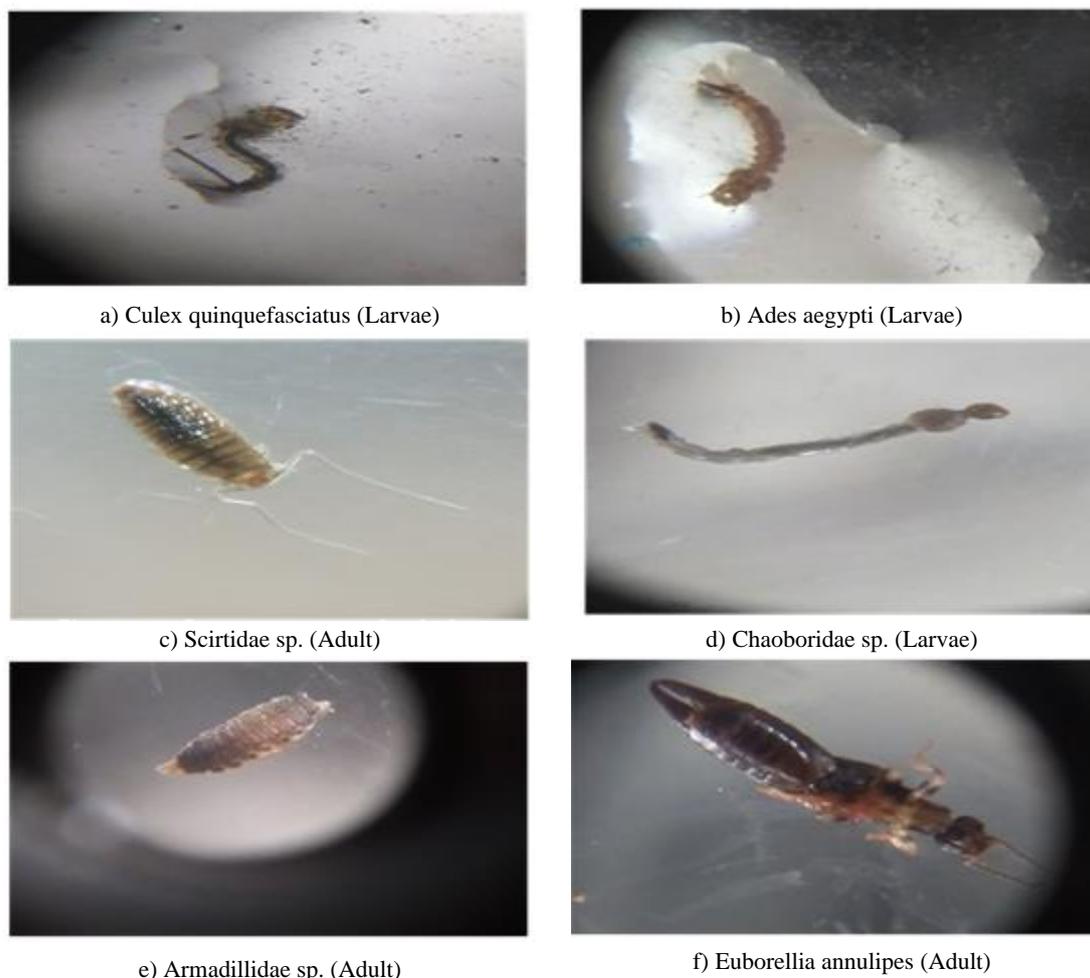
salinity (*Ficus religiosa*, *Madhuca longifolia*, and *Tamarindus indica*). Hardness and alkalinity were high in *Samanea saman* and the least was in *Ficus religiosa*. Few species of trees only have nitrate and iron. Nitrite level was assessed and it's showed absence in all the tree hole water. Calcium level was high in *Azadirachta indica* and least was recorded in *Madhuca longifolia* (**Table 2**).

**Table 2.** Physico-chemical properties of tree hole water (Mean±SD) (n=40)

Tree species	Temperature (C°)	pH	Ammonia (ppm)	Salinity (g/L)	Total hardness (ppm)	Total alkalinity (ppm)	Nitrate (ppm)	Iron (ppm)	Calcium (ppm)
<i>A. indica</i>	30.33±1.2	7.75±0.5	1.85±0.2	0.83±0.2	141.67±5.2	143.33±3.2	0	0.12±0.1	51.67±1.2
<i>F. religiosa</i>	29.00±1.3	7.93±0.6	0.10±0.0	0	66.67±3.2	46.67±1.2	0	0	23.33±1.3
<i>M. longifolia</i>	28.00±0.0	8.00±0.0	0.30±0.0	0	100.00±0.0	90.00±0.0	0	0	20.00±0.0
<i>M. olifera</i>	29.56±1.2	7.86±0.4	2.11±0.2	0.22±0.1	194.44±5.6	148.89±2.3	1.11±0.2	0.03±0.0	52.22±2.5
<i>P. glabra</i>	30.25±1.2	7.85±0.4	3.03±0.3	0.50±0.2	187.50±7.4	190.00±3.4	7.50±0.6	0.18±0.0	50.00±2.9
<i>S. saman</i>	29.88±1.2	7.85±0.4	2.53±0.4	0.75±0.3	243.75±8.4	203.75±4.5	7.50±0.7	0.04±0.0	56.25±2.7
<i>T. indica</i>	28.75±1.3	7.80±0.4	1.50±0.2	0	100.00±3.2	175.00±4.2	0	0	35.00±2.2
<i>T. grandis</i>	31.80±1.5	7.76±0.4	0.60±0.1	0.20±0.1	160.00±4.5	124.00±1.5	2.00±0.3	0.14±0.0	40.00±3.1

Six species of larvae were recorded from the tree hole water analysis belonging to five insects and one annelid. Namely, *Culex quinquefasciatus*, *Ades aegypti*, *Scirtidae* sp, *Chaboridae* sp, *Armadillidae* sp, and *Euborellia annulipes* from the phylum of Arthropoda (**Figure 1**) and Naididae sp from the Annelida. Among the six species of larvae, *Culex quinquefasciatus* has a large number of individuals followed by *Ades aegypti* and so on. The very least number of individuals was recorded in *Euborellia annulipes*. The diversity indices also calculated

among the various tree species hole water, that shows species richness of fauna was high *Azadirachta indica* and *Pongamia glabra* and least in *Madhuca longifolia*. The abundance and dominance index of fauna was high in *Samanea saman* followed by *Tamarindus India*, *Madhuca longifolia*, *Ficus religiosa*, and so on. The Simpson diversity and Shannon H' index were high in *Azadirachta indica* followed by *Tectona grandis* and so on (**Table 3**). The evenness index was high in *Madhuca longifolia* and the least was in *Samanea saman* (**Table 3**).



**Figure 1.** Insects and larvae recorded from tree hole water during the study period (100X10)

**Table 3.** Diversity indices of Insect fauna recorded from tree hole water (n=40)

Indices	AI	FR	ML	MO	PG	SS	TI	TG
Species richness	6	4	3	4	6	5	5	4
Abundance	33	58	56	38	36	75	71	34
Dominance index	0.358	0.401	0.406	0.403	0.374	0.697	0.524	0.366
Simpson Diversity index	0.642	0.599	0.594	0.598	0.627	0.303	0.476	0.635
Shannon H' index	1.215	1.065	0.997	1.057	1.187	0.610	0.973	1.185
Evenness index	0.562	0.726	0.903	0.719	0.546	0.368	0.529	0.818

AT- *Azadirachta indica*; FR- *Ficus religiosa*; ML- *Madhuca longifolia*; MO- *Moringa olifera*; PG- *Pongamia glabra*; SS- *Samanea saman*; TI- *Tamarindus indica*; TG- *Tectona grandis*

**Table 4.** Analysis of Variance of variables studied during the study period in tree hole water (n=40)

Name of the variable	Sum of Squares	df	Mean Square	F	Sig (p)
Tree height (m)	375.592	7	53.656	6.352	.000
Hole depth (cm)	2802.700	7	400.386	5.020	.000
Tree hole height from the ground(cm)	487855.598	7	69693.657	5.858	.000
Water level (cm)	1657.611	7	236.802	4.763	.000
Total Litter (g)	27.512	7	3.930	1.666	.136
Number of species	15.874	7	2.268	2.963	.010
Number of individuals	28090.091	7	4012.870	1.464	.198

The tree hole is a partially enclosed cavity, which has naturally formed in the trunk or branch of a tree. They were founded not only in

old trees, whether living or not. The tree holes may form as the result of physiological stress from natural forces like wind, fire, heat,

lightning, rain, attack from insects, bacteria, or fungi causing the excavating and exposure of the heartwood of the tree [10]. The trees may self-prune, dropping lower branches as they reach maturity, exposing the area where the branch was attached. Many animals further develop the hollows using instruments such as their beak, teeth, or claws. The tree species *Moringa olifera* has the highest number of tree holes and *Manduca longifolia* has the least number of tree holes. *Moringa olifera* is a softwood tree and it releases the gum-like substance during the injury. The gum-like substance might be used for holding the water inside the cavity for a long time after rain. But, this type of tree hole has only four species of insect larvae. It might be due to the Physico-chemical properties of water in the cavity. The largest opening size, depth, height from the ground, water level, and litters of the tree holes were observed in *Ficus religiosa* and least observed in *Samanea saman*. *Ficus religiosa* has also only four species of insect larva and has a rich abundance. The height of the hole above the ground, maximum volume, and host tree species was significantly varied [4]. Based on the species of trees the physico-chemical properties might be changed and the insect larva variations occur.

The current study result indicates that water-filled tree holes provide important and unique lentic microhabitats for the faunal species within the trees and are a suitable obligate for aquatic species and it also act as a pest. In particular mosquito vectors are used for breeding centers for their survival [11]. These pests not only disturb people but they are also an important health hazard as diseases' vectors that spread malaria, dengue, yellow fever, and an important virus of the recent years Zika [12, 13]. Water-filled tree holes supported an aquatic macroinvertebrate community dominated by phylum Arthropoda species especially order Diptera [14]. Yanoviak [4] recorded Fifty-four macroinvertebrates and five vertebrate taxa were found in association with water-filled tree holes on BCI. Larvae of true flies (Diptera) are the most common and diverse inhabitants of this system [12]. Blakely *et al.*, [15] have been proved that more than half of the species (56%) were in the insect order Diptera.

The number of species and individuals in a hole was correlated with hole size, most of the studies revealed that the large hole has more macro-fauna because those holes have more amount nutrients. But large tree-hole volume does not necessarily result in higher numbers of species. Five orders of aquatic insects namely Diptera, Coleoptera, Heteroptera, Odonata, and Trichoptera have been recorded from tree-hole aquatic habitat by Nishadh and Anoop Das [5]. Blakey *et al.*, [14] have been reported that 20 species in the temperate rainforests of New Zealand were dominated by Dipteran larvae (51%). Majumder *et al.*, [8] were found a total of 918 insect specimens were collected from 19 water-filled tree holes out of 32 tree holes surveyed. The recorded Insect specimens belonged to 17 families under 6 orders. The most abundant order was Diptera and the least was Hemiptera.

#### CONCLUSION

The tree holes or cavity might be acting as a microhabitat for aquatic and terrestrial insect fauna and its survival. It includes successful breeding sites for vector mosquitoes. The present investigation revealed that larvae of various insects were recorded and enumerated. It concluded that the tree holes and their water has many resources to inhabit insect larvae successfully. It needs to be a long-term survey to check their assemblage and its importance to biodiversity.

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