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Ecological Characteristics of Tree-Holes and Insect Larval Diversity in Tree-Hole Water in Mayiladuthurai Taluk

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ABSTRACT

Tree holes are formed by environmental factors and biotic pressures and provide a unique environment for the development of insect larvae. These water-filled cavities, or their equivalents, are considered natural microhabitats due to their simplicity, small size, clear boundaries, and inherent replication. This study aimed to investigate the ecology of tree holes in different tree species, along with the distribution and diversity of insect larvae. Eight tree species with tree holes were identified in the study area. *Moringa oleifera* had the highest number of tree holes, followed by *Samanea saman*. Significant differences were observed in the independent variables of tree holes across the 3 tree species. A total of 6 larvae species were recorded, representing 5 insect species and 1 annelid. These included *Culex quinquefasciatus*, *Aedes aegypti*, *Scirtidae* sp., *Chaboridae* sp., *Armadillidae* sp., and *Euborellia annulipes* from the arthropod phylum, along with *Naididae* sp. from the annelid phylum. Among the 6 species, *Culex quinquefasciatus* had the highest abundance, followed by *Aedes aegypti*. The findings indicate that tree holes and their water provide sufficient resources to support insect larvae. A long-term survey is recommended to further study their assemblages and understand their role in biodiversity.

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

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Introduction

Tree holes provide ideal environments for comparative studies on population interactions [1]. They are found from the subarctic to the equator and are common in regions with hardwood trees, even in places like the Mojave Desert [2]. These habitats vary greatly in terms of local climate, species composition, and community complexity. Filled with rainwater, tree cavities, also known as dendrothelms, serve as temporary pools where insects breed and seek shelter during the day [1]. These temporary aquatic habitats, associated with living trees, support a wide range of organisms, from bacteria to invertebrates, across the globe [3]. Numerous macroinvertebrates rely on tree holes for reproduction, with some species exclusively breeding in this specialized environment [4].

Tree holes can form through natural growth processes of the tree or due to external factors such as wind damage, forest fires, or excavation by animals like woodpeckers or insects [5]. Rainwater fills these cavities either through direct precipitation or via stem flow. Nutrients are introduced into the tree holes from the tree's foliage and bark during this process, making the chemical composition of the water dependent on the tree species and the atmospheric deposits on the bark [6]. Water-filled tree holes often feature rot holes and pans with varying frequency. Rot holes are found in tree stems, branch breaks, and stumps, where water comes into contact with the wood. Pans, typically created in branch forks or at the base of buttress roots, are lined with bark. Many tree species contain both types of water-filled cavities, but their occurrence can vary by tree species [7]. These habitats serve as primary breeding sites for numerous disease vectors, including mosquitoes and biting midges. Tree holes accumulate water along with organic material from leaves, wood, and animals. After an extensive review of existing literature, the current study was initiated to explore the insect fauna in tree holes across various tree species, focusing on the associated physicochemical factors.

Materials and Methods

The research was conducted in the Sembarnarkoil region (11º 06' N, 79º 44' E) located within Tharagambadi Taluk, Mayiladuthurai District, Tamil Nadu. This area is a densely populated semi-urban zone, primarily characterized by agricultural lands where paddy (Oryza sativa) is the main crop. In addition to the crops, the region is also home to a wide variety of tree species, including *Azadirachta indica*, *Samanea saman*, *Moringa olifera*, *Tamarindus indica*, *Pongamia glabra*, *Tectona grandis, Mangifera indica*, *Ficus religiosa*, *Madhuca longifolia*, and *Polyalthia longifolia*, among others. The local climate is divided into four distinct seasons based on rainfall: monsoon (October-December), post-monsoon (January-March), summer (April-June), and pre-monsoon (July-September). The current investigation took place during the transition from the monsoon to post-monsoon seasons. Various locations within the study area, including Thiruchampalli, Kalagasthenathapuram, Vallum, and Parasalur, were selected to examine the characteristics of tree holes and the distribution of insect fauna.

Tree holes were sampled between October 2019 and March 2020. The locations and types of tree holes were observed visually. Measurements such as water level, length, depth, width, and height from the ground were taken using a scale in centimeters. Leaf litter was collected, and dried, and its dry weight was recorded. The contents of the tree holes were carefully extracted by hand up to the hole's depth, depending on its structure [8]. Insect fauna was gathered within one week following rainfall and preserved in 70% ethanol. In the laboratory, the collected insects were identified and counted with the aid of Photomicroscopy (LABOMED STC-ML 100X10X) [8].

To assess the physicochemical parameters, appropriate methods were used [9], including measurements of temperature (with a probe thermometer), pH (using a digital pH meter), and concentrations of Nitrate, Alkalinity, Ammonium, Total hardness, Salinity, Nitrite, Calcium, and Iron. Statistical analyses, including diversity indices and ANOVA, were conducted to assess the significance of the research using IBM SPSS version 25.

Results and Discussion

A total of 40 tree holes from 8 different tree species were surveyed. These species included *Azadirachta indica* (6 trees), *Moringa olifera* (9 trees), *Samanea saman* (8 trees), *Tamarindus indica* (4 trees), *Tectona grandis* (5 trees), *Pongamia glabra* (4 trees), *Ficus religiosa* (3 trees), and *Madhuca longifolia* (1 tree). Among these, *Moringa olifera* had the highest number of tree holes, followed by *Samanea saman*, while *Madhuca longifolia* had the fewest. Tree height measurements revealed that *Tectona grandis* was the tallest species at 21 m, and *Ficus religiosa* was the shortest at 7m. Two distinct shapes were observed in the sampled trees: rod-shaped (48%) and pan-shaped (52%). Morphometric analysis of the tree holes showed that Ficus religiosa had the largest dimensions in terms of opening size, water level, depth, height from the ground, and litter accumulation, whereas Samanea saman exhibited the smallest measurements **(Table 1)**.

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

Tree species	Hole type (n)	Opening size (cm x cm)	Depth (cm)	Height from the ground (cm)	Water level (cm)	Litters (g)
Azadirachta indica	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
Moringa olifera	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
Ficus religiosa	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

Pongamia glabra	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
Samanea saman	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
Tamarinudus indica	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
Tectona grandis	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
Madhuca longifolia	Pan (1)	29 X 21	29.00 ± 0.0	480.00 ± 0.0	18.00 ± 0.0	3.42 ± 0.0

The physicochemical properties of tree-hole waters were evaluated. The highest temperature was recorded in *Tectona grandis*, while *Manduca longifolia* exhibited the lowest. pH levels were highest in *Manduca longifolia* and lowest in *Azadirachta indica*. Ammonia concentrations were highest in *Pongamia glabra*, with *Ficus religiosa* showing the lowest levels. *Azadirachta indica* had the highest salinity, while *Tectona grandis* showed the lowest. Additionally, three species—*Ficus religiosa*, *Madhuca longifolia*, and *Tamarindus indica*—did not exhibit salinity. Samanea saman had the highest hardness and alkalinity, while *Ficus religiosa* had the lowest. Only a few tree species displayed nitrate and iron presence. Nitrite was absent in all tree-hole waters. Calcium levels were highest in *Azadirachta indica* and lowest 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)
A. indica	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
F. religiosa	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
M. longifolia	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
M. olifera	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
P. glabra	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
S. saman	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
T. indica	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
T. grandis	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

The analysis of tree hole water revealed 6 species of larvae, comprising five insect species and one annelid. These species included *Culex quinquefasciatus*, *Aedes aegypti*, *Scirtidae* sp., *Chaboridae* sp., *Armadillidae* sp., and *Euborellia annulipes* from the *Arthropoda phylum* **(Figure 1)**, as well as Naididae sp. from *Annelida*. *Culex quinquefasciatus* exhibited the highest population, followed by *Aedes aegypti*. In contrast, *Euborellia annulipes* had the fewest individuals. Regarding the diversity indices, *Azadirachta indica* and *Pongamia glabra* exhibited the greatest species richness, while *Madhuca longifolia* showed the lowest. In terms of abundance and dominance, Samanea saman recorded the highest values, followed by *Tamarindus indica*, *Madhuca longifolia*, and *Ficus religiosa*. The Simpson diversity index and Shannon H’ index were also highest in *Azadirachta indica*, with *Tectona grandis* ranking next. *Madhuca longifolia* had the highest evenness index, while *Samanea saman* showed the lowest **(Tables 3 and 4)**.

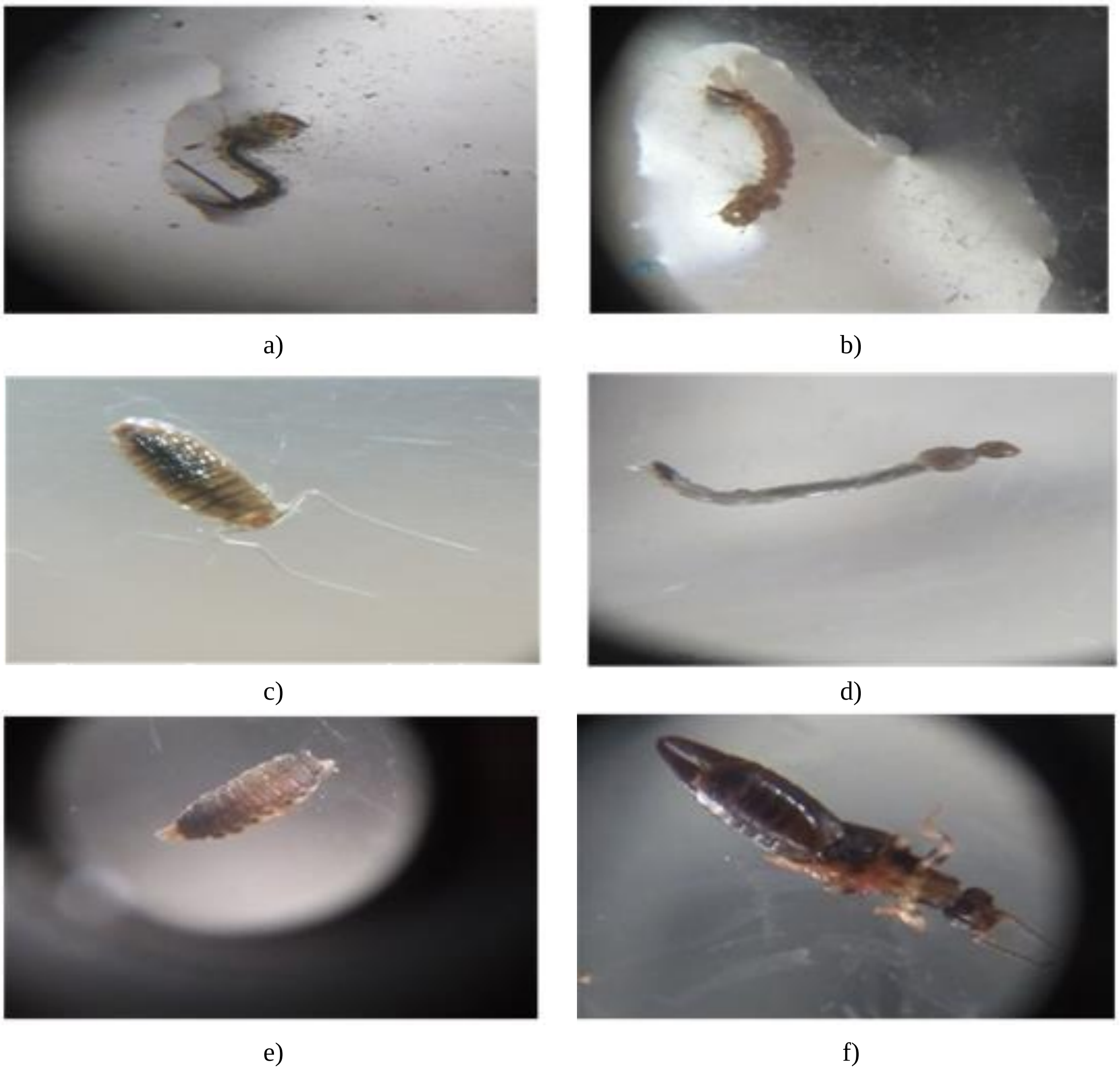

Figure 1. Insects and larvae recorded from tree hole water during the study period (100 X 10), a) *Culex quinquefasciatus* (larvae), b) *Ades aegypti* (larvae), c) *Scirtidae* sp. (adult), d) *Chaoboridae* sp. (larvae), e) *Armadillidae* sp. (adult), f) *Euborellia annulipes* (adult)

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- *Tamarinudus 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-value)**
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

Tree holes are naturally occurring cavities formed in the trunks or branches of trees, which can be found in both live and dead trees. These holes may arise due to a range of factors such as wind, fire, extreme heat, lightning, rain, or damage from insects, bacteria, or fungi, which expose the heartwood of the tree [10]. As trees mature, they may shed their lower branches, leading to exposed areas where the branches were once attached. Animals, such as birds or mammals, may then expand these natural cavities using their beaks, claws, or teeth. Among the trees studied, *Moringa olifera* contained the most tree holes, while *Madhuca longifolia* had the fewest. *Moringa olifera*, a softwood species, excretes a gum-like substance when damaged, which may help retain water inside the tree hole after rain. However, the water in Moringa olifera's tree holes supports only 4 species of insect larvae, likely due to the specific chemical properties of the water. The largest tree holes, in terms of opening size, height from the ground, depth, water level, and litter, were found in Ficus religiosa, while Samanea saman had the smallest. Despite the large size of Ficus religiosa's tree holes, it also hosted just four insect larvae species but with a rich abundance. The characteristics of the hole, such as its height and volume, varied significantly depending on the host tree species [4]. These variations in the tree hole environment, influenced by tree species and the water's physicochemical properties, likely explain the differences in insect larvae diversity across species.

The findings of the current study suggest that water-filled tree holes serve as vital, distinctive lentic microhabitats for various faunal species, providing a suitable environment for aquatic organisms. They also play a role as breeding sites for pests, particularly mosquitoes, which rely on these habitats for reproduction and survival [11]. These insects, while disruptive, pose significant health risks as vectors for diseases like dengue, malaria, yellow fever, and the more recently identified Zika virus [12, 13]. The aquatic macroinvertebrate community inhabiting water-filled tree holes is primarily composed of species from the phylum Arthropoda, especially those in the order Diptera [14]. Yanoviak [4] reported finding 54 macroinvertebrate species and 5 vertebrate taxa associated with water-filled tree holes on BCI. Dipteran larvae are the most diverse and common residents of these habitats [12]. Research by Blakely *et al*. [15] further confirmed that more than half (56%) of the species present in these tree holes belong to the order Diptera.

The relationship between the number of species and individuals in tree holes and their size has been the subject of numerous studies, with many suggesting that larger holes tend to house more macrofauna due to the higher availability of nutrients. However, a larger volume of tree-hole water doesn't always correlate with an increase in species diversity. Nishadh and Anoop Das [5] observed five aquatic insect orders—Diptera, Coleoptera, Heteroptera, Odonata, and Trichoptera—in tree-hole habitats. Similarly, Blakely *et al*. [15] found that in New Zealand's temperate rainforests, the majority of species (51%) were Dipteran larvae, out of a total of 20 recorded species. Majumder *et al*. [8] collected 918 insect specimens from 19 water-filled tree holes out of 32 studied, representing 17 families across 6 insect orders. Diptera was the most abundant, while Hemiptera appeared the least.

Conclusion

Tree holes, or cavities, serve as microhabitats for both aquatic and terrestrial insect species, providing essential resources for their survival. These cavities act as effective breeding sites for mosquito vectors. The study found various insect larvae, demonstrating that tree holes and their water offer ample resources for successful larval habitation. However, long-term monitoring is necessary to further assess the assemblage and ecological significance of these habitats in maintaining biodiversity.

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