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Ecological and physicochemical characteristics of tree holes as mosquito breeding habitats: A systematic review

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Abstract

Mosquitoes are a significant group of vectors that transmit fatal human diseases. In India, the most common mosquito species are *Culex quinquefasciatus*, *Aedes aegypti*, *Anopheles stephensi*, and *Aedes albopictus*. *Aedes aegypti* use both natural and human made artificial breeding habitats. Tree holes are one of the most neglected natural habitats, but they play a significant role in harbouring mosquito larvae, bacteria, and fungi. The interactions between physicochemical variables of the breeding water and organisms may cause larval mortality, a natural decline in larval abundance, or alterations in their growth. Parasitism, pathogenism, predation, and competition has a major role in the overall development of mosquito species. In an effective mosquito control programme, information on tree hole habitats and prevalent physicochemical, biotic, and abiotic factors must be investigated. This review focuses on the various species of mosquitoes that breed in tree holes, with an emphasis on understanding how ecological factors and physicochemical variables affect the abundance of mosquito larval populations in tree hole breeding habitats observed in India with special attention to Tamil Nadu for the possible development of strategies to control of vector populations and to prevent the transmission of infectious diseases.

Keywords: Mosquitoes, tree hole, parasitism, infectious diseases, Tamil Nadu

Introduction

Tree holes are the most important phytotelmata that are formed by the accumulation of rainwater in the natural cavities or in the rot holes of the trees. The presence of water-filled tree holes creates a distinct environment that is suitable for highly specialised invertebrate groups. Typically, this particular microhabitat is found only in fully developed, usually deciduous trees^[1]. These habitats are highly prevalent in numerous tropical and temperate forests. Treeholes are economically significant ecosystems due to their role as primary breeding grounds for many disease vectors, particularly mosquitoes (Diptera: Culicidae) and biting midges (Diptera: Ceratopogonidae). Mosquitoes spread a variety of parasites and pathogens^[2]. At the present, vector borne infectious diseases kill over 70,000 people each year and cause significant economic damage^[3]. *Flaviviridae* viruses cause dengue fever, West Nile fever, and yellow fever, while *Togaviridae* viruses cause chikungunya are primarily vectored by *Ae.aegypti*^[2].

Tree holes provide optimal breeding environments for insects, specifically *Aedes* mosquitoes. These inherent cavities offer the optimal environment for mosquito eggs to incubate and larvae to mature^[4]. The proliferation of mosquitoes in tree hole water is influenced by various factors, including physicochemical, biotic, and abiotic factors. The survival and development of mosquito larvae can be influenced by physicochemical elements present in tree hole water, including temperature and pH levels. Mosquito growth and development are impacted by the presence of organic matter and nutrients in the water, along with the availability of oxygen. The existence of other species (predators) in the tree hole can exert an influence on mosquito populations, since these biotic factors play a role.

Abiotic factors, such as precipitation and solar radiation, can also impact the development of mosquitoes in tree cavities. Furthermore, the size of tree holes, the amount of water present, and the vulnerability to drought can significantly influence the population and diversity of mosquito larvae in these environments [5]. This review explores the physicochemical, biotic and abiotic factors that influence the abundance of mosquito larvae in tree hole breeding habitats for the effective management of vector population and in the control of vector borne diseases.

Breeding habitats of mosquitoes

Mosquitoes are formidable disease vectors due to their global distribution and adaptation to a wide variety of environmental conditions. They are restricted to aquatic and terrestrial habitats as juveniles and adults, respectively. Mosquitoes inhabit in various habitats of the environment in both temperate and tropical regions [6]. The breeding habitats are divided into natural habitats and artificial containers. The natural habitats include phytotelmata, rock pools, burrow pits, snail cells, carb holes, wells etc. Artificial containers are man-made that store water and some of the potential containers that inhabit mosquito species include plastic containers, discarded tyres, water tanks, flowerpots etc. [7].

Despite such divergent habitats, the larval and adult stages of the mosquito life cycle are interdependent. The breeding habitats of mosquito larvae range from ephemeral to permanent, and most mosquito species are typically associated with breeding sites. *Aedes* mosquitoes, for instance, primarily breed in artificial containers and tree holes, whereas *Anopheles* and *Culex* mosquitoes breed in larger water bodies such as swamps, marshes, and agricultural fields. Consequently, during their early developmental stages, larvae are exposed to a multitude of environmental conditions and ecological factors, each of which is uniquely structured.

The phytotelmata are the natural breeding habitats of mosquito species which include tree holes, bamboo stem and sheath, fallen trees, papaya stump, reed stump, leaf axis, banana stump, mushrooms, log holes and pitcher plant [8]. Water filled tree holes are unique habitats that had fascinated scientist for a very long time. The characteristic and species composition of these specialized habitats has been reported before 100 years [9, 10]. These habitats are termed as bromeliads – “hanging aquaria” [10-12]. The initial curiosity about these habitats led to major explorations in various types of phytotelmata, such as pitcher plant flower bracts, bamboo inter-nodes, and bromeliads in different parts of the world [13]. Epidemiological importance of these habitats as breeding place for several disease vectors and its wide distribution especially in the case of tree-hole aquatic habitat transformed the initial curiosity into a hot epidemic research topic [2, 6, 7]. The autecological epidemic research on tree hole aquatic habitat then led to community level studies [13, 14] exploring into the ecology, community determinations and realization as important tools for studying these processes. Holes in trees can be formed either due to their inherent growth pattern or as a result of external influences such as wind, forest fires, bird and insect activity, or microbial attack [13, 15]. These tree holes are classified as pan tree holes or rot tree holes based on the presence or absence of inner bark lining. Further, the tree holes are classified as 1. Bowl (Fig. 1a), 2. Slit (Fig. 1b) and 3. Pan tree hole (Fig. 1 c,d,e). Pan tree holes have large opening area compared to its depth, while bowl tree holes are

high in depth compared to its orifice. The tree holes with narrow opening are termed as slit holes.

Influence of biotic and abiotic factors

Both biotic and abiotic ecological factors shape the development of aquatic larvae and influence the physiology and behaviour of adult mosquitoes (Fig. 2). Abiotic factors such as temperature, rainfall and physicochemical characteristics of the breeding water have a significant impact on the larval development and behaviour characteristics of adult mosquitoes. Like abiotic factors, biotic factors such as presence of efficient nutrition, intraspecific and interspecific competition for resources, and predation by other species influence adult survival [9, 10]. As larvae, mosquitoes, acquire and store nutrients, and the amount of nutrients stored influences how adult females seek nutrition and blood meals [2, 6, 7]. Increased larval densities reduce per capita resources due to competition, resulting in smaller sized adult [15]. In the presence of predators, behavioural changes adapted by mosquito larvae increase its survival at the expense of energy losses associated with antipredator activities, thereby decreasing the fitness of adult mosquitoes [11, 14-16]. All these larval-stage effects are reflected in adults' changes in behaviour, physiology, and reproduction, which cascade to population-level effects and, ultimately, disease transmission [7, 9, 12, 16-18].

Tree hole breeding *Aedes* species

Tree-hole mosquitoes exhibited a high degree of co-occurrence at their breeding locations (Table 1 and 2). The predominant species relationships observed were *Aedes triseriatus*, *Aedes barberi*, *Aedes triseriatus*, and *Aedes hendersoni* [19]. *Aedes aegypti* is a notorious mosquito species that is highly adapted to human-made domestic habitats and transmits fatal diseases including yellow fever, chikungunya, dengue, and zika [15, 20, 21]. *Aedes aegypti*'s evolution is highly dependent on human activities and the species maintains close contact with humans [21-24]. Five to ten thousand years ago, the species invaded human settlement and acquired domestic adaptations [22, 24]. Two major subspecies of *Aedes aegypti* were identified: *Aedes aegypti formosus* (Aaf) and *Aedes aegypti aegypti* (Aaa). Aaa are predominantly found in domestic settings, whereas Aaf inhabit both forest and domestic habitats [22, 23, 25, 26]. *Ae. aegypti* can currently be found in Africa, both in its sylvan settings where the larvae reside in tree holes and rock holes, as well as in areas surrounding human settlements such as villages and transient human dwellings. The subspecies Aaa is known as a "domestic" mosquito because it breeds in artificial containers made by humans. Aaa mosquitoes have a preference for feeding on human blood [27]. This species, over the past 400-500 years, spread to a significant extent across the tropical and subtropical regions of the world due to human migration and trade. This led to major occurrences of diseases transmitted by mosquitoes. *Aedes aegypti* are opportunistic breeders that use both natural habitats and man-made containers. The larvae of *Aedes aegypti* can tolerate a wide range of environmental conditions [21, 22, 24, 25]. *Ae. aegypti* co-exists with *Ae. albopictus* in tree hole habitats and prefers tree hole habitats that are exposed to sunlight [28].

Aedes albopictus is the second most significant vector of dengue in terms of its influence on human health, following *Aedes aegypti* [29]. It is also capable of efficiently transmitting

other arboviruses including *Dirofilaria* sp [30]. *Aedes albopictus* is a species of mosquito that typically breeds in small, confined, shaded sources of water found in tree holes. The incorporation of decomposing leaves from the surrounding trees creates chemical conditions akin to those found in tree holes, which serve as an optimal substrate for breeding. *Aedes albopictus* can also thrive and persist in non-urban environments without any man-made containers, which poses further public health problems for rural regions [31, 32]. Juliano, 2002 [33] suggested that the coexistence of these species in the same location is feasible due to warm and dry weather, which benefit *Ae. aegypti* by reducing the competition from *Ae. albopictus* through the selective death of *Ae. albopictus* eggs. This mechanism may also be responsible for the reported coexistence of species in tropical Asia. In southeast Asia, *Ae. aegypti* and *Ae. albopictus* seldom occupy the same habitats in locations where they coexist [34]. The former species is predominantly found in urban settings, where it lays its eggs indoors [35, 36]. On the other hand, the latter species is often found in less urbanised habitats, with a higher presence of outdoor larval sites [34, 35]. The displacement of *Ae. albopictus* by *Ae. aegypti* in certain Asian cities was proposed to be due to the elimination of *Ae. albopictus* habitats, accompanied by a rise in urban habitats that are more favourable for *Ae. aegypti* [34, 37].

Tree holes harbours diverse insects and microbes

Though the water filled tree holes are isolated from other water sources, they still host many aquatic and semi aquatic species including insects, amphibians, crustaceans, rotifer protozoans and nematodes. They also inhabit bacteria, fungi, and algae [17, 38, 40]. Most of the inhabitants of tree holes with water depend on decomposed organic matter [39]. In addition, the species are also influenced by stem flow water containing nutrients and chemicals [40]. The decomposition of detritus is the primary function of the tree hole ecosystem. Numerous studies have investigated the presence of various organisms in tree holes such as mites, polychaetas, gastropods, microcrustaceans amphibians, bacteria, fungi, and algae. Compared to temperate regions, studies of tree holes in the tropics have revealed the presence of amphibians and mites [41, 42]. Bacteria, nematodes, and protists were observed in tree holes in temperate region. Importantly, they serve as breeding grounds for numerous deadly mosquito species that transmit disease to humans. Many mosquito species that transmit diseases in humans undergoes their larval and pupal developmental processes in phytotelmata [43]. Mosquito prefers deep and non-draining branches and trunk holes for oviposition. The draining and shallow containers lose water due to evaporation and hence are less attractive to mosquitoes [44]. Thus, climate change could result in the spread of mosquito species that breed in tree holes [45]. In temperate regions, tree hole species may experience a harsh environment due to drought and freezing temperatures, whereas in tropical regions with year-round rainfall, survival of tree hole species is quite comfortable [39]. Anthropogenic activities such as pollution and forest management can affect tree holes and can be used as an indicator of environmental change [46-48]. The presence of microbes in breeding habitat influences the oviposition preferences of female mosquitoes. Female mosquitoes can detect and oviposit in habitats containing a specific microbial population relative to others [49]. In addition, external factors such as the availability of food

sources influence mosquitoes' oviposition site selection [50]. The microbes form an important source of food for the early developmental stages of mosquitoes. However, the identification of microbes in the midgut of larvae varies between individuals in the same habitat [51].

Energy flow in tree hole habitats

The tree hole habitats are confined microcosm that are spatially distributed and widely available. The tree hole habitats are thus discrete and patchy [40]. Tree hole habitats acquire energy from allochthonous sources such as decomposition of leaf litter and by stem flow. The heterotrophic detritus-based tree hole ecosystem is largely influenced by the quality and quantity of detritus, its processing, hydrology, and the amount of rainfall in these aquatic habitats [52, 53]. The reliance of tree hole species on detritus is due to the absence of primary producers because of low light intensity in tree holes. However, stem flow water compensates for the nutrient requirements in tree hole habitats [40]. The energy in this heterotrophic system is thus obtained from the decomposition of complex organic compounds into simpler compounds. This is achieved by two functional groups namely Particulate organic matter (POM) and dissolved organic matter (DOM) generation from coarse particulate organic matter (CPOM) [54, 55]. The leaf litters leach out inorganic and organic matter into DOM and the CPOM gets converted into Fine particulate matter (FPOM). This transformation is carried out by microbial interaction, particularly bacteria and fungi, metabolism, and the shredding by macro invertebrates [52, 56]. *Hyphomycetes* of fungi produces enzymes including cellulose, hemicellulose, and pectinase breaks down the leaf litters which is further decomposed by the action of nitrifying, denitrifying and sulphate reducing bacterial species present in the tree hole water [52, 56, 57]. Besides all these efforts by fungi and bacteria in the decomposition of leaf litters, the tree hole habitats possess a limited supply of inorganic nutrients, and this short fall is compensated by the stem flow from plants and by animal detritus [52]. Invertebrates such as *tipulidae* and *coleoptera* convert the CPOM into FPOM. The unconsumed shredder litters are thus made available to microbes and insects [56]. The stem flow helps in neutralizing the toxic metabolites of hydrogen sulphide and ammonia in the tree hole habitats. Further, the stem flow acts as the resource for nutrients such as nitrate, sulphate, and inorganic cations. The bacterial population, nutrient dynamics and mosquito population were influenced by stem flow [58]. The stem flow is directly concerned with the characteristics of tree hole structure and formation. It is evident that pan tree holes receive high amount of stem flow compared to rot hole habitats due to its structural and formation patterns. The water chemistry of the tree hole habitats has considerable impact on the life traits of mosquito species [40].

Tree hole communities

Numerous organisms representing simple fauna mostly dominated by insects are the inhabitants of the tree hole habitats [59]. The microbial inhabitants of tree hole habitats are litter-detritus based communities which includes fungi, bacteria, algae, rotifers, protozoa, nematodes, crustaceans, insects, amphibians, helminths etc (Table 3). These organisms are grouped into three types such as accidental, facultative and specialist based on the utilization of tree hole habitats [59].

^{60, 61}. These microbial communities are heterotrophic in nature. Bacterial species belonging to 29 families from *Bacteroidetes*, *Proteobacteria*, *Verrucomicrobia*, *Actinobacter* and *Firmicutes* phyla were identified in tree holes ^[62]. Similarly, Kaufman *et al.* 1999 ^[63] reported the presence of 45 fungal species in tree hole habitats. About 40 protozoan species have been identified in European beach tree holes. The most preferred bacterial species by mosquito larvae in the tree hole habitat was found to be *Flavobacteriaceae* ^[64]. The protozoan community in tree hole habitats are dominated by *Parameciidae*, *Erionellidae*, *Tetrahymenidae*, *Colpodidae*, *Discocephalidae* and *Chilodonellidae* species ^[65]. Nematodes, rotifers and Diptera were dominant in the tree hole habitats in Czech Republic ^[66].

The tree holes lack the presence of photosynthetic algae due to the prevailing extreme conditions of nutrient concentration in these habitats ^[67]. The stability of the tree hole habitats in harbouring mosquito species dependent on the size of the habitat and the amount of rainfall that fills the habitat ^[68]. Thus, large size and the availability of high food resources in tree holes lead to species richness and biomass. Height of the tree holes from the ground has negative influence in species richness due to evaporation or seepage of tree hole water. Thus, the temperature and rainfall directly influence the availability of water in tree hole habitats ^[57, 59, 61, 69]. Leaf litter diversity influences the pupal development in tree hole habitats. High leaf litter composition favored the growth of *Ae. pseudotaniatus* species. Further, surplus nutrient availability lead to larval mortality in *Ae. aegypti*. Under controlled experimental conditions, the combination of *Spondia spinnata*, *Acacia caesia* and *Pacetta indica* leaf litters resulted in high production of mosquito larvae ^[61].

Tree hole aquatic community interaction

The interaction between communities of tree hole habitats includes facilitation, inter and intra specific competition and predation ^[70]. The heterotrophic community of tree hole aquatic habitat are involved in the unidirectional conditioning of detritus resources in which the higher trophic depends on the lower trophic organisms. This process of chain interaction is common in community interactions ^[39, 71]. Processing chain interaction between coarse detritus browsers and *dipterans* Bradshaw *et al.* 1992 ^[72] *coleopterans* and *mites* Paradise *et al.* 1999 ^[73] *helotid* beetles and Ceratopogonid midges, shredders, and mosquito population Paradise *et al.* 1999 ^[73] are previously reported. Competition is considered as an important characteristic in community structuring ^[71, 61]. The availability of resources and the 'mosquito population density cohorts' competitive effect on the overall performance of mosquito species ^[74]. This intra specific competition among mosquitoes of tree hole habitats in relation to resource availability reduces the larval survival, rate of pupation, pupal density, and adult emergence ^[57] (Fish 1982). The community interaction is primarily determined by the presence of predators in the tree hole habitats ^[71, 75].

Mosquito larvae are an important part of tree hole habitats though it serves a link in the food cycle. The tropical structure of aquatic tree hole habitats consists of food web with omnivorous species that are top and intermediate predators (ciliate and flagellate). They serve as prey for mosquito larvae ^[63]. The top-down effects of predators on the diversity and abundance of tree hole species increases with decreasing water volume ^[76]. The colonist species prefer larger containers

for oviposition and deposit eggs depending on the availability and volume of water ^[77]. The interesting aspect of the food chain is that mosquito larvae *Megarhinus*, are cannibalistic and fed on other mosquito larvae such as *Orthopodomyia*, *Anopheles berbers* and *Aedes triseriatus*. *An. barberi* are occasional predators of *Or. signifera* and *Ae. triseriatus*. Other predators of mosquito larvae in tree hole habitats are *Bezziavaricolor* and tree hole beetles of the genus, *Cyphon* ^[75]. *Toxorhynchites* larvae, *dystiscid* beetles and *odonatan* are some of the important predators inhabiting tree hole habitats. *Toxorhynchites* are regarded as appropriate to manage medically significant vector species, owing to their nectar-feeding habits and absence of blood-feeding behaviour. They do not, therefore, appear to raise the danger of mosquito-borne illness transmission ^[78]. Similarly, density dependent facultative predations also exist. The predation was observed to be high in low resource habitats compared to habitats with high resource availability. This pattern of predation was affected by high population of mosquito species and selective predation on large mosquitoes. The interaction between the availability of resources and predation is detrimental in the survival and co-existence of different types of mosquitoes in the same habitat. The survival and co-existence of *Aedes albopictus* and *Ochlerotatus triseriatus* was successful because of increased predation effects ^[79].

Aedes aegypti mosquitoes prefer to oviposit in containers where *Toxorhynchites* mosquitoes were present, possibly because of the high bacterial cues ^[80]. Evidence indicates that, *Ae. aegypti* consciously select aquatic settings with abundant bacterial food for their larvae, despite the risk of predation on their progeny. *Toxorhynchites'* feeding activities or predatory behaviour might directly or indirectly enhance the presence of bacteria in oviposition sites. Additionally, remains of dead larvae serve as a food source for bacteria. This characteristic enhances the support for utilising *Toxorhynchites* in the management of *Aedes* species.

Fungi coexist with mosquito larvae in various habitat types. Therefore, it is evident that fungi may interact with mosquitoes throughout its life. Fungi are important food source for mosquito larvae which provides essential nutrients for its development ^[81]. The predominant fungal order isolated from mosquito larvae was *Blastocladales*, *Erotiales*, *Hypocreales*, and *Saccharomycetes*. *Aedes triseriatus*, feed fungal population present in decaying oak leaf matter through browsing and grazing ^[57, 61]. The feeding behaviour of mosquitoes establishes a commensal, where four different fungal species belonging to genus *Smittium* sp were identified to replicate in hind gut without causing any deleterious effect on the survival and growth of mosquito larvae ^[82-84]. Entomopathogenic fungus such as *Smittium morbosum*, *Metarhizium anisopliae* and the fungal pathogen, *Actinomyces clavus* spores cause infections and toxicity in the gut of mosquito larval upon ingestion [85, 86]. Fungus such as *Coelomomyces opifexi*, *C. sorophorae*, *M. anisopliae* and *Beauveria bassiana* infect mosquito larvae through contact and cuticular penetration ^[87].

Environmental factors

The diversity in the distribution of mosquito species in tree holes depends on the altitude and ecological factors prevailing in that area ^[88]. The quantity of water and the size of the tree hole have significant effects on the tree hole communities. The size of tree holes is substantial as a proxy for drought risk

at high temperatures [48]. The larval abundance of mosquito species was observed high in tree holes that had a water volume of 50 to 250 ml (52%). The abundance of larvae decreased with increase in water volume. Only 9% of larvae were observed in tree holes that hold water above 500 ml [28]. Further, the study also discovered that tree hole communities were more abundant, and diverse in larger tree holes. Species that breed in small holes are adapted to drought because they are either rapid colonisers or have a brief larval development period. However, these species may be susceptible to predation [48].

Though rainfall forms the major source of water in tree holes, heavy rainfall may have a negative impact on the eggs of mosquitoes [88]. The number of eggs per batch laid by mosquitoes depends on the availability of resources and larval density. Narimah *et al.* 1984 [89] observed that rainfall is responsible for the abundance of *Ae.ablopictus* in tree hole habitat. Turbulence of water in tree hole habitats because of rainfall makes the larvae exhaust due to its attempts to escape the water surface from being affected or being flushed out of the habitat by rainfall. *An.gambiae*, when not shielded, the attempt to escape from rainfall even kills the larvae due to loss of energy and the inability to obtain oxygen from the water surface – air interface [90].

Rainfall pattern strongly influences the population density of *Ae. aegypti* and *Ae. albopictus*. Maximum population density of these species was observed during the pre-monsoon season and declined slowly towards post-monsoon [88]. But Yamana *et al.* 2013 [91] reported that the larval population density of *Ae. aegypti* in the tree hole habitats showed an increasing trend during post-monsoon immediately after rainfall compared to pre-monsoon and decreases gradually in summer. Generally, the *Ae. aegypti* larvae show a non-seasonal fluctuation pattern in Tamil Nadu due to the presence of water filled containers throughout the year [92]. High larval abundance of *Ae. albopictus*, and *Cx. quinquefasciatus* was observed with rainfall range of 21.7 to 78.3 mm and low during summer. Temperature plays an important role in the development of mosquito larvae and accounts for most variations observed during larval development. The larval development of *Culex* species increases by 2.9 times when the temperature ranges between 16 and 32°C [93]. However, an increase in temperature beyond its thermal preference results in characteristics changes such as decrease in egg hatchability, fecundity, and longevity. Elevated temperatures also result in the diminution of adult mosquito size, longevity, and fertility [48, 94]. Regardless of the position of tree holes (different altitudes), temperature, precipitation, humidity and, the amount of detritus in tree holes influences the abundance of tree hole communities [48]. The tree holes of *Delonix regia*, *Delonix elata*, *Millettia pinnata*, *Kaya senegalensis*, *Tamarindus indica* and *Ficus benghalensis* had a maximum average humidity of 78.3% and a minimum humidity of 21.7%. The average temperature ranged from 22.8 to 31.8°C [92]. While high humidity favours survival and abundance of mosquito species, low humidity restricts its survival [91, 95]. Precipitation in tree hole habitats reduces drought conditions and permits tree hole species to utilize detritus more efficiently. But high precipitation might also limit the larval growth by overflowing the nutrients from the breeding habitats [96].

Physicochemical properties of water in tree holes

The physicochemical characteristics of tree hole water vary depending on the atmospheric inputs. Temperature, pH,

salinity, conductivity, and total dissolved solids show significant involvement in the abundance of mosquito larvae. The high concentration of hydrogen ions in tree holes is attributed to the stem flow and this high hydrogen ion concentration limits the growth and development of mosquito larvae [40, 52]. Further, the pH of the breeding water influences the osmoregulation and oxygen transportation in mosquitoes and hence plays a significant role in the survival of mosquito larvae. The tree hole habitats at Coimbatore, Tamil Nadu, India recorded pH in the range of 6.80 to 7.63 [87]. The average pH of tree hole water in Eastern and Western ghats was in the range between 5.89 and 7.54 [94]. Generally, the pH in the range between 6.02 to 7.8 favour the larval development of *Ae. aegypti* [96-98]. The larval abundance was observed in tree hole water with a pH of 7 [16]. It is evident that the mosquito larvae are capable of adaptation to varied pH of the breeding habitats. But extreme low and high pH range of breeding water disturbs the larval development. Loss of water volume significantly increases the ionic concentration and alters the pH and salinity of water. This alteration directly affects the ionic and osmotic regulation of aquatic organisms [99].

The development of mosquito larvae depends on the prevailing temperature and salinity of the habitat. But alterations in pH and DO affects the micro fauna and flora present in the breeding habitats [94]. The concentration of dissolved oxygen in tree holes has a negative effect on the abundance of mosquito species. Mosquito larvae rely primarily on atmospheric oxygen in their environment, though some mosquito larvae have been reported to supplement atmospheric oxygen with dissolved oxygen [100, 101]. Because atmospheric oxygen is readily available dissolved oxygen reduction has no effect on mosquito larvae [102, 103]. Mosquito larvae use dissolved oxygen in the absence of atmospheric oxygen. It has been reported that mosquitoes survive for several days even when they are denied access to atmospheric oxygen, [104] and the survival rate decreases as dissolved oxygen in the concealed habitat decreases. The DO content of tree hole water was between 1.11 and 2.11 ppm [90]. Yanoviak *et al.* 1999 [53] previously reported that mosquito larvae can survive in habitats with total dissolved solids ranging from 8 to 87 ppm. Insolation leads to loss of water due to evaporation and changes in primary production. This affects the dissolved oxygen (DO) concentration and turbidity of tree hole water. The macro invertebrates of tree hole utilize atmospheric oxygen through siphons and undergo morphological adaptations due to the low concentration of DO in the tree holes [53]. Fluctuations or variations in tree hole water temperature affects the concentration of DO and influences the development and metabolic processes of organisms [99]. The concentration of dissolved oxygen in tree holes has a negative effect on the abundance of mosquito species. Similarly, the correlation between pH, conductivity, species richness and abundance were negative [48].

Salinity and conductivity of breeding water is an indicator for the presence of mosquito larvae. Increase in salinity leads to decrease in species diversity. Mosquito larvae lend to resist the fluctuations in salinity and *Aedes* species show increased tolerance to salinity [14, 41]. In the tree hole habitats, salinity was observed to be between 0.18 and 0.34 ppt. High TDS of 302.3 and low TDS of 112.7 ppm were recorded. A high variation in the concentration of TDS was observed in tree hole habitats that ranged between 124 and 561.7 [87].

The quality of the breeding water can be determined with EC. A strong positive association between mosquito larvae and EC ranging between 140 and 557 $\mu\text{S}/\text{cm}$ has been reported. Othaman *et al.* 2020^[105] reported that electrical conductivity is directly proportional to nutrient concentration. The highest conductivity recorded in tree hole water was 162.9 $\mu\text{S}/\text{cm}$ and lowest conductivity was 6.02 $\mu\text{S}/\text{cm}$ ^[87].

Turbidity in tree holes ranged between 20.7 NTU and 58.5 NTU^[87]. Turbidity plays a limiting role in the selection of breeding habitats by female mosquitoes. The *Ae. aegypti* species were found to breed in clear water^[28]. Turbidity was found to be 22.7 NTU at its lowest and 253 NTU at its highest level observed in the hills of Eastern and Western ghats. It is to be noted that the biotic and abiotic factors not only affect mosquito larval population but also the pathogens that are vectored by the mosquitoes. The *Aedes* mosquitoes thrives in tree hole habitats characterized by specific environmental conditions. These include a temperature range of 28 to 31.80 degrees Celsius, a pH range of 7.75 to 8, salinity levels ranging from 0 to 0.83 g/l, total hardness between 66.67 and 243.75, and alkalinity within the range of 46.67 to 203.75. These parameters play a crucial role in the mosquito's habitat selection and reproductive success^[106].

Tree hole breeding mosquitoes

Azadirachta indica, *Delonix rigia*, *Mangifera indica* and *Bambusa vulgaris* inhabit *Aedes*, *Culex* and *Anopheles* genera mosquito larvae^[87, 107]. Tree hole habitats of these plants showed 14 different species of larvae in Chidambaram, Tamil Nadu, India. *Aedes* genera included species such as *Ae. aegypti*, *Ae. albopictus*, *Ae. africanus*, *Ae. simpsoni* and *Ae. taylori*. The *Culex* species observed were *Cx. quinquefasciatus*, *Cx. pseudovishnui*, *Cx. pipens*, *Cx. tritaeniorhynchus*, and *Cx. nebulosus*. *An. subpictus*, *An. stephensi*, *An. fluviatilis* and *An. culcifacies* were the *Anopheles* species present in the tree holes at Chidambaram. The study reported that 60% of tree hole breeding mosquito species was *Ae. aegypti*. The abundance of mosquito species in these tree hole habitats were in the order of *Aedes*, followed by *Culex* and *Anopheles*^[107]. *Ae. aegypti*, *Ae. albopictus* and *Ae. vittatus* larvae along with *Cx. quinquefasciatus* were found in tree holes habitats at Tiruchirappalli, Tamil Nadu, India^[8]. *Ae. aegypti* and *Cx. quinquefasciatus* were the predominant mosquito species that were identified from the tree holes of *Azadirachta indica*, *Moringa olifera*, *Samanea saman*, *Tamarindus indica*, *Tectona grandis*, *Pongamia glabra*, *Ficus religiosa*, *Madhuca longifolia*, *Mangifera indica*, *Polyalthia longifolia* in Mayiladuthurai, India^[106]. Tree holes of *Plumeria* sp contained *Ae. albopictus*, *Ae. albopictus*, *Ae. kromneini*, *Ae. phagomyia*, *Ae. pseudotaeniatus*, *Armigeres subalbatus*, *Cx. brevipalpis*, *Heizmannia* sp and *Tripteroides* sp^[28]. Tree holes of *Delonix rigia* and *Kigelia pinnata* in Kolli hills, Tamil Nadu, India was observed to contain *Ae. aegypti*, *Ae. albopictus*, *Cx. uniformis*, *Orthopodomyia anopheloides*, *Toxorhynchites viridibasis* and *Toxorhynchites rutilus*. Among these species, *Ae. aegypti* and *Ae. albopictus* were found to be dominant^[107].

The surveillance of mosquito species in tree holes from high altitudes of Ramanathapuram district showed *Ae. aegypti* and *Ae. albopictus* to be the dominant species. Further, these high altitude tree hole habitats harboured more numbers of female population. The other most important breeding habitats that

showed maximum larval population were water tanks, discarded tyres, pots and coconut shells^[92]. Six genera comprising 23 species of mosquito larvae including *Culex* (6 species), *Aedes* (4 species), *Ochleratus* (4 species), *Anopheles* (4 species), *Heizmannia* (3 species) and *Toxorhynchites* (2 species) were identified in the tree holes at Coimbatore, Tamil Nadu, India. Among the species identified *Ae. aegypti* and *Ae. albopictus* were the predominant species^[87]. *An. subpictus*, *Cx. gelidus*, *Cx. nilgricus*, *Cx. tritaeniorhynchus*, *Cx. quinquefasciatus*, *Ae. vittatus*, *Ae. aegypti* and *Ae. albopictus* were reported in the tree hole habitats at Sitheri hills, Dharmapuri District, Tamil Nadu, India^[8]. *Ae. aegypti* dominated the tree hole mosquito species in Vellore, Tamil Nadu, India^[92].

Ae. albopictus, *Ae. aegypti*, *Ae. vittatus*, *An. stephensi*, *Cx. quinquefasciatus* and *Tx. splendens* were identified from the tree holes of *Azadirachta indica* and *Peltophorum pterocarpum* present in the Bharathidasan University campus, Tiruchirappalli, India. *Aedes* species, *Ae. aegypti* dominated the *Peltophorum pterocarpum* rot holes and *Ae. albopictus* were abundant in *Azadirachta indica* rot holes. The study observed that *Peltophorum pterocarpum* harboured maximum number of larval populations in the University campus^[108]. Suganthi *et al.* 2014^[8] reported the absence of *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus* from the tree hole habitats in Western Ghats. The study identified 231 species from 30 genera. The absence of *Ae. aegypti* and *Ae. albopictus* and the dominance of *Ae. vittatus* was identified in tree hole habitats at Nilgiris, Tamil Nadu, India^[109].

Reegan *et al.* 2018^[110] and Viswan *et al.* 2020^[111] reported the dominance of *Ae. aegypti* in tree hole habitats at Chhattisgarh, India. *Cx. quinquefasciatus*, *Ae. albopictus*, *Cx. brevipalpis* and *Cx. gelidus* were dominant species that were observed during winter season in tree holes^[112]. *Ae. albopictus* and *Armigeres subalbatus* dominated the tree hole communities in Erankulam district, Kerala^[113]. *Ae. aegypti* and *Ae. albopictus* were the dominant species identified from the tree hole habitats in Angul district, Odisha^[114]. *Ae. aegypti* was the major mosquito vector in the tree hole habitats in Thiruvananthapuram, Kerala^[115]. Seven species belonging to 5 genera were identified in the tree hole habitats located in Wayanad district of Kerala, India. The species identified were *Ae. albopictus*, *Ae. chrysolineatus*, *Armigeressu balbatus*, *Cx. brevipalpis*, *Cx. quinquefasciatus*, *Heizmannia chandi*, *Toxorhynchites splendens*^[116]. A total of 12 species from 3 genera were identified in Karaikal and Puducherry. The tree hole community showed high prevalence of *Ae. aegypti* and *Ae. albopictus*. The other mosquito larvae identified in the tree hole habitats were *Ae. stokesi*, *Ae. simpsoni*, *An. subpictus*, *An. stephensi*, *An. culciformis*, *An. maculatus*, *Cx. quinquefasciatus*, *Cx. pseudovishnui*, *Cx. tritaeniorhynchus* and *Cx. decens*^[107].

Joshi *et al.* 2014^[117] revealed the presence of *Ae. aegypti* and *Ae. vittatus* mosquitoes in tree hole habitats in Rajasthan, India. In Kerala, India the mosquito species identified in tree hole breeding habitats include *Ae. albopictus*, *Heizmannia chandi*, *Cx. brevipalpis*, *Armigeres subalbatus*, and *Tx. splendens*^[118]. *Ae. aegypti* and *Ae. albopictus*^[119] *Ae. scatophagoides*, *Ae. aegypti*, *Ae. vittatus*, and *Ae. albopictus*^[120]. Paily *et al.* 2013^[121] found *Ae. albopictus* in tree holes of rubber plantations that were less than 15 years old at Aimcombu, Kerala, India. Kanojia and Jamgaonkar 2008^[122] discovered several species of mosquitoes, including *Ae.*

albopictus, *Ae. annulirostris*, *Ae. micropterus*, *Ae. novalbopictus*, *Ae. subalbopictus*, *Ae. periskeletus*, *Ae. unilineatus*, and *Culex uniformis* in tree hole habitats in Karnataka, India ^[123]. documented the presence of various mosquito species, namely *Armigeres aureolineatus*, *Ar. subalbatus*, *Ar. flavus*, *Cx. brevipalpis*, *Cx. infantulus*, *Cx. uniformis*, *Hulecoeteomyia chrysolineata*, *Downsiomyia niveus* group, *Phyagomyia prominens*, *Stegomyia albopicta*, *Toxorhynchites splendens*, and *Tripteroides aranoides*, in tree holes located in Karnataka, India. Biswas *et al.* 2023 ^[124] and Sharma *et al.* 2021 ^[125] documented the occurrence of *Ae. albopictus* in tree holes in Tripura and Rajasthan, India. Tripathi *et al.* 2017 ^[126] determined that, *Ae. albopictus* and *Ae. aegypti* were the most common mosquito species found in tree hole habitats in Madhya Pradesh, India. *Delonix regia*, *Delonex elata*, *Millettia pinnata*, *Kaya senegalensis*,

Tamarindus indica and *Ficus benghalensis* in Eastern and Western ghats were observed to inhabit 30 species mosquito larvae from 9 genera. The altitude of the tree hole habitats was observed from 340 m to 7940 m and the maximum population density of the mosquito larvae were observed between 1000 and 3500 m height. Larvae of *Ae. pseudoalbopictus* and *Cx. quinquefasciatus* were the dominant species observed in tree holes of *Delonix regia*, *Delonex elata*, *Millettia pinnata*, *Kaya senegalensis*, *Tamarindus indica* and *Ficus benghalensis* ^[92]. Understanding the preferred habitat of these disease-carrying mosquitoes can aid in developing effective prevention and control strategies. Therefore, it is crucial to identify and monitor these conditions in potential breeding sites to reduce the risk of mosquito-borne illnesses.

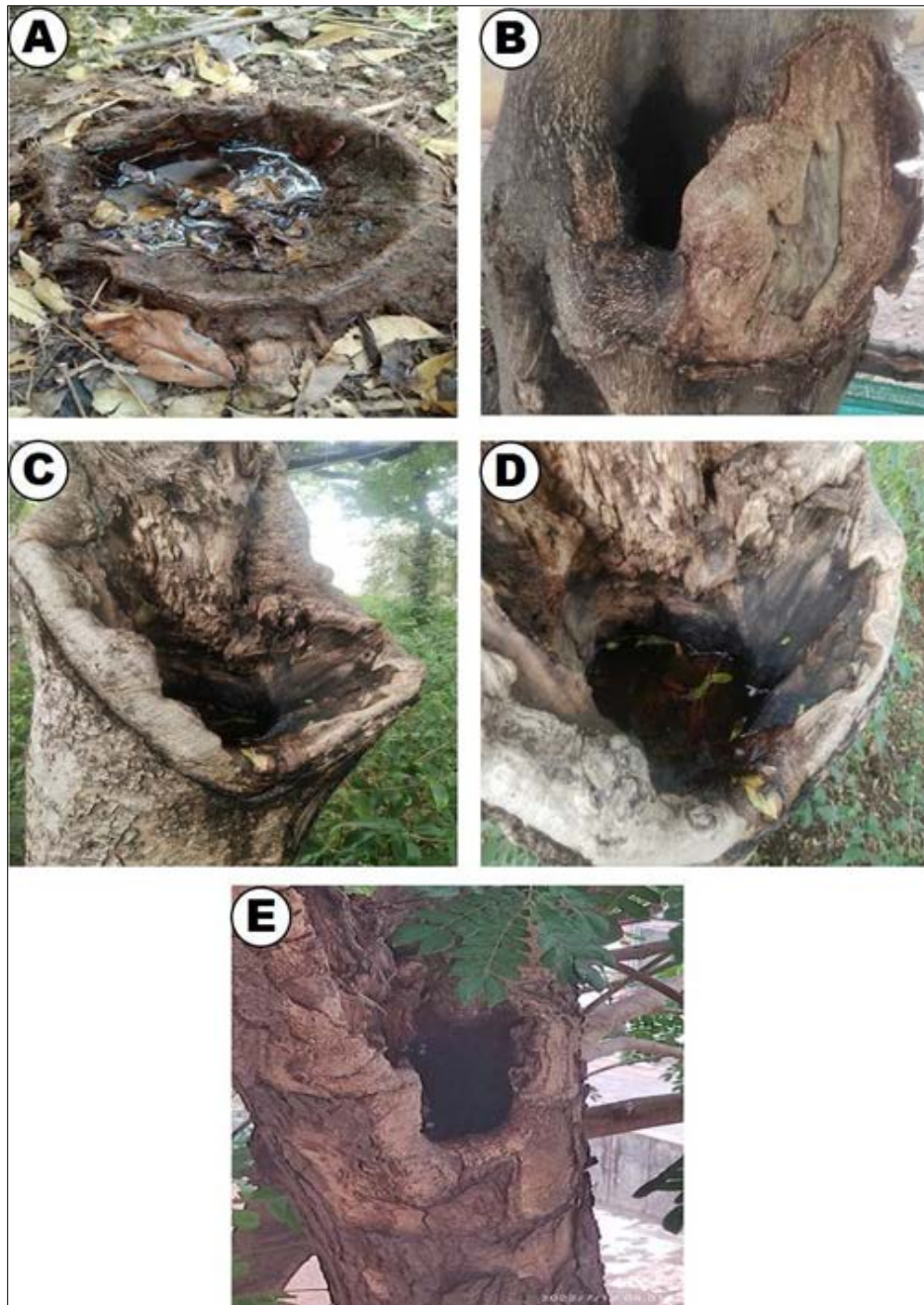


Fig 1: Tree holes habitats (A) Bowl treehole (B) Slit tree hole (C, D, E) Pan tree hole.

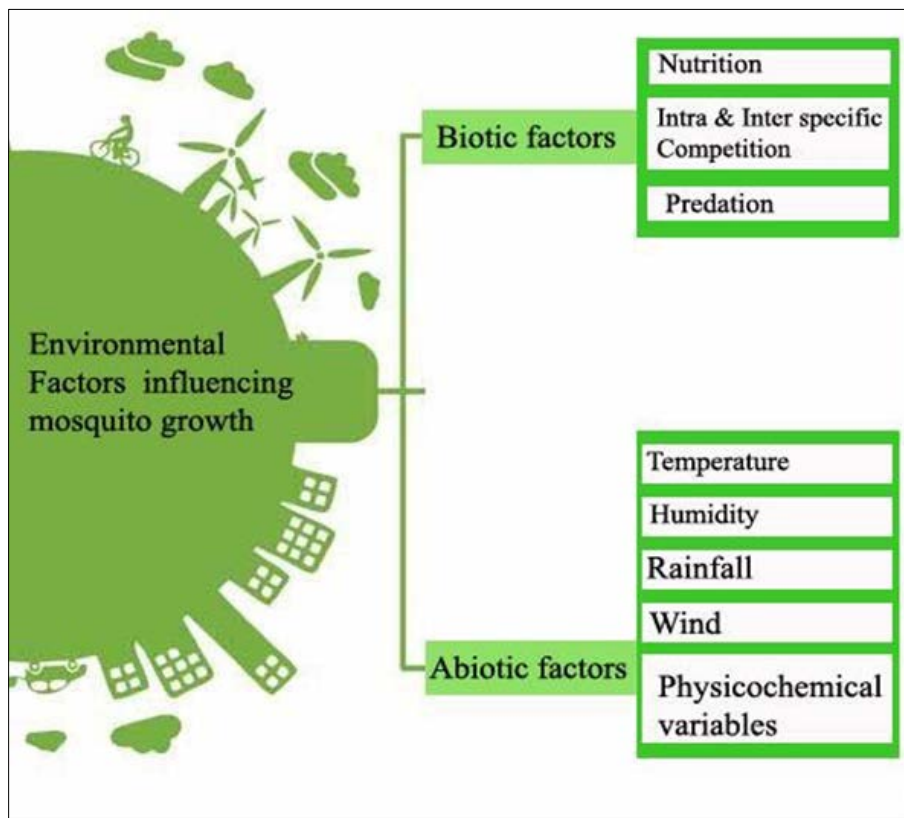


Fig 2: Environmental factors influencing mosquitoes' growth.

Table 1: Mosquito species identified in tree hole habitats in India

S. No	Tree hole breeding mosquitoes						Area of surveillance	Reference
	<i>Aedes</i>	<i>Culex</i>	<i>Anopheles</i>	<i>Toxorhynchites</i>	<i>Armigers</i>	<i>Downsiomyia</i>		
1	<i>Ae. aegypti</i>	—	—	—	—	—	Chattisgarh, India	[84]
2	<i>Ae. Albopictus</i> <i>Ae. chrysolineatus</i>	<i>Cx. brevipalpis</i> <i>Cx. quinquefasciatus</i>	<i>An.stephensi</i>	<i>Tx. splendens</i>	—	—	Wayanadu, Kerela, India	[87]
3	<i>Ae. aegypti</i> <i>Ae. vittatus</i>	—	—	—	—	—	Rajasthan, India	[88]
4	<i>Ae. albopictus</i> <i>Ae. vittatus</i>	—	—	—	—	—	Erankulam, Kerala, India	[86]
5	<i>Ae. aegypti</i>	—	—	—	—	—	Thiruvananthapuram, Kerala, India	[87]
6	<i>Ae. albopictus</i>	<i>Cx. brevipalpis</i>	—	<i>Tx.splendens</i>	<i>Ar. subalbatus</i>	—	Kerala, India	[89]
7	<i>Ae. aegypti</i> <i>Ae. albopictus</i>	—	—	—	—	—	Kerala, India	[90]
8	<i>Ae. scatophagoides</i> <i>Ae. aegypti</i> <i>Ae. vittatus</i> <i>Ae. albopictus</i>	—	—	—	—	—	Kerala, India	[91]
9	<i>Ae. albopictus</i>	—	—	—	—	—	Kerala, India	[91]
10	<i>Ae. albopictus</i> <i>Ae. annulirostris</i> <i>Ae. micropterus</i> <i>Ae. Novalbopictus</i> <i>Ae. subalbopictus</i> <i>Ae. Periskeletus</i> <i>Ae. unilineatus</i>	<i>Cx. uniformis</i>	—	—	—	—	Karnataka, India	[92]
11	—	<i>Cx. brevipalpis</i> , <i>Cx. Infantulus</i> <i>Cx. uniformis</i>	—	<i>Tx. splendens</i>	<i>Ar. aureolineatus</i> <i>Ar. subalbatus</i> <i>Ar. flavus</i>	<i>Do. niveus</i>	Karnataka, India	[93]
12	<i>Ae. albopictus</i>	—	—	—	—	—	Tripura, India	[94]
13	<i>Ae. albopictus</i>	—	—	—	—	—	Rajasthan, India	[96]
14	<i>Ae. Albopictus</i> <i>Ae. aegypti</i>	—	—	—	—	—	Madhya Pradesh, India	[95]
15	<i>Ae. albopictus</i>	<i>Cx. quinquefasciatus</i>	<i>An. jamesii</i> , <i>An. minimus</i> , <i>An. willmori</i>	—	—	—	Mizoram, India	[97]

16	<i>Ae. aegypti</i> <i>Ae. albopictus</i> <i>Ae. vittatus</i>	-	-	-	-	-	Koramangala, Bengaluru, India	[83]
17	<i>Ae. aegypti</i> <i>Ae. albopictus</i>	-	-	-	-	-	Angul, Odisha, India	[80]
18	<i>Ae. aegypti</i> <i>Ae. albopictus</i> <i>Ae. pseudoalbopictus</i> <i>Ae. subalbata</i> <i>Ae. krombeini</i> <i>Ae. stokesi</i>	<i>Cx. quinquefasciatus</i> <i>Cx. mimulus</i> <i>Cx. pseudovishnui</i> <i>Cx. flagilis</i> <i>Cx. flavicomis</i> <i>Cx. vishnui</i> <i>Cx. tritaeniorhynchus</i>	<i>An. stephensi</i> <i>An. aitekenii</i> <i>An. culiciformis</i>	-	-	<i>Do. albolateralis</i> <i>Do. nivea</i>	Eastern and Western Ghats, India	[69]

Table 2: Mosquito species identified in tree hole habitats of different trees in Tamil Nadu and Puduchery, India

S. No	Tree Name	Tree hole breeding mosquitoes					Area of surveillance	Reference
		<i>Aedes</i>	<i>Culex</i>	<i>Anopheles</i>	<i>Ochlerotatus</i>	<i>Toxorhynchites</i>		
1	<i>Azadirachta indica</i> <i>Delonex rigia</i> <i>Mangifera indica</i> <i>Bambusa vulgaris</i>	<i>Ae. Aegypti</i> <i>Ae. albopictus</i> <i>Ae. africanus</i> <i>Ae. simpsoni</i> <i>Ae. taylora</i>	<i>Cx. quinquefasciatus</i> <i>Cx. nebulosus</i> <i>Cx. tritaeniorhynchus</i> <i>Cx. pseudovishnui</i> <i>Cx. pseudovishnui</i> <i>Cx. pipiens</i>	<i>An. stephensi</i> <i>An. subpictus</i> <i>An. fluviatilis</i>	-	-	Chidambaram, Tamil Nadu	[79]
2	-	<i>Ae. albopictus</i> <i>Ae. aegypti</i> <i>Ae. vittatus</i>	<i>Cx. quinquefasciatus</i>	-	-	-	Trichirappalli, Tamil Nadu	[81]
3	-	<i>Ae. aegypti</i>	-	-	-	-	Ramanathapuram, Tamil Nadu	[69]
4	-	<i>Ae. aegypti</i> <i>Ae. albopictus</i> <i>Ae. edwardsi</i> <i>Ae. krombeini</i>	<i>Cx. mimulus</i> <i>Cx. pseudovishnui</i> <i>Cx. quinquefasciatus</i> <i>Cx. vishnui</i> <i>Cx. khazani</i> <i>Cx. uniformis</i>	<i>An. aitenii</i> <i>An. barbirostris</i> <i>An. culiciformis</i> <i>An. maculatus</i>	<i>O. anureostriatus</i> <i>O. albotaeniatitis</i>	<i>Tx. minimus</i> <i>Tx. splendens</i>	Coimbatore, Tamil Nadu	[64]
5	<i>Delonex rigia</i> <i>Kigelia pinnata</i>	<i>Ae. pseudoalbopictus</i>	<i>Cx. quinquefasciatus</i>	<i>An. elegans</i>	-	-	Kolli Hills, Tamil Nadu	[79]
7	-	<i>Ae. vittatus</i> , <i>Ae. aegypti</i>	-	-	-	-	Sitheri hills, Tamil Nadu	[8]
8	<i>Azadirachta indica</i> <i>Peltophorum pterocarpum</i> <i>Pongamia pinnata</i> <i>Eucalyptus</i> <i>Mangifera indica</i> <i>Ficus benghalensis</i> <i>Ficus religiosa</i>	<i>Ae. Aegypti</i> <i>Ae. albopictus</i> <i>Ae. vittatus</i>	<i>Cx. brevipalpis</i> <i>Cx. quinquefasciatus</i>	<i>An. stephensi</i>	-	<i>Tx. splendens</i>	Trichirappalli, Tamil Nadu	[81]
9	<i>Delonix rigia</i> <i>Kigelia pinnata</i>	<i>Ae. aegypti</i> <i>Ae. albopictus</i> <i>Ae. Stokes</i> <i>Ae. simpsoni</i>	<i>Cx. quinquefasciatus</i> <i>Cx. pseudovishnui</i> <i>Cx. tritaeniorhynchus</i>	<i>An. stephensi</i> <i>An. culiciformis</i> <i>An. subpictus</i> <i>An. maculatus</i>	-	-	Karaikal, Puduchery	[64]
10	-	<i>Ae. albopictus</i> <i>Ae. aegypti</i> <i>Ae. vittatus</i>	<i>Cx. quinquefasciatus</i> <i>Cx. gelidus</i> <i>Cx. nilgricus</i> <i>Cx. tritaeniorhynchus</i>	<i>An. subpictus</i>	-	-	Western ghats, Tamilnadu	[8]
11	-	-	<i>Cx. brevipalpi</i> <i>Cx. gelidu</i> <i>Cx. tritaeniorhynchus</i>	<i>An. barbirostris</i>	-	-	Villupuram district, Tamil Nadu	[84]
12	<i>Spondias pinnata</i> <i>Acacia caesia</i> <i>Pacetta indica</i>	<i>Ae. aegypti</i>	-	-	-	-	Madurai, Tamil Nadu	[97]
13	-	<i>Ae.</i>	-	-	-	-	Nilgiris, Tamil	[85]

		<i>albopictus</i> <i>Ae. vittatus</i>					Nadu	
14	<i>Azadirachta indica</i> <i>Moringa olifera</i> <i>Samanea saman</i> <i>Tamarindus indica</i> <i>Tectona grandis</i> <i>Pongamia glabra</i> <i>Ficus religiosa</i> <i>Madhuca longifolia</i> <i>Mangifera indica</i> <i>Polyalthia longifolia</i>	<i>Ades</i> <i>aegypti</i>	<i>Cx.</i> <i>quinquefasciatus</i>	-	-	-	Mayiladuthurai	[78]

Table 3: Co-existing insect and microbial species with mosquito larvae in tree hole breeding habitats

S. No	Mosquito species	Coexisting species			Reference
		Insects	Bacteria	Fungi	
1	<i>Aedes geniculatus</i> <i>Anopheles plumbeus</i>	<i>Prionocyphon niger</i>	-	-	[25]
2	<i>Aedes terrens</i> spp. <i>Anopheles stephensi</i> <i>Culex mollis</i> <i>Culex urichii</i> <i>Haemagogus (H.)</i> spp. <i>Toxorhynchites theobald</i>	<i>Orthopodomyia Copelatus</i> <i>Laccophilus Prionocyphon serricornis</i> <i>Gynacantha membranali</i> <i>Triacantha gynadentata</i> <i>Libellula Mecistogaster linearis</i>	-	-	[41]
3	<i>Aedes</i> spp. <i>Tripteroides</i> spp. <i>Culex</i> spp.	<i>Eristalis</i>	-	-	[30]
4	<i>Aedes albopictus</i> <i>Aedes desmotes</i> <i>Aedes malikuli</i> <i>Tripteroides aranooides</i> <i>Tripteroides bambusae</i>	-	<i>Betaproteobacteria Neisseriales</i> <i>Chromobacteriaceae</i> <i>Gammaproteobacteria</i> <i>Pseudomonadales Moraxellaceae</i> <i>Acinetobacter Enterobacteriaceae</i> <i>Pseudomonadaceae Bacillaceae</i> <i>Chryseobacterium Flavobacteriia</i>	-	[30]
5	<i>Aedes triseriatus</i>	-	-	<i>Coelomomyces opifexi</i> <i>Coelomomyces psorophorae</i> <i>Metarhizium anisopliae</i> <i>Beauveria bassiana</i>	[63]
6	<i>Culex pipiens</i> <i>Culex restuans</i> <i>Culex salinarius</i> <i>Aedes vexans</i> <i>Ochlerotatus triseriatus</i> <i>Ochlerotatus trivittatus</i>	-	-	<i>Smittium</i> Spp.	[98]
7	<i>Ochlerotatus triseriatus</i>	-	-	<i>Letiomyces Dothideomycetes</i> <i>Mitosporic Ascomycota</i> <i>Saccharomycetes Chytridiomycot</i> <i>Blastocladales Erotiales</i> <i>Hypocreales</i>	[42]
8	<i>Aedes aegypti</i>	-	-	<i>Smittium morbosum</i>	[62]

Conclusion

Tree holes with water in them are crucial. These microcosms serve as an effective ecological model system. Many opportunities exist to explore the responsibilities of studying how communities react to environmental perturbation in water-filled tree holes. With the help of an evolutionary connection to mosquitoes that live in containers, their significance in the broad forest ecosystem needs to be investigated. It is worthwhile to explore these tree whole habitats due to their significance as a vital habitat, their ability to house a wide range of species of epidemiological importance, and their application as an effective tool for ecosystem experiments. Investigating the physicochemical and environmental factors of tree hole breeding habitats of the *Ae. aegypti* mosquito species is essential for efficiently controlling vector-borne illnesses. Comprehending the precise circumstances that facilitate the breeding and endurance of these mosquitoes is crucial for developing focused control

strategies. Through the examination of the physical and chemical attributes of tree whole habitats, vital knowledge can be acquired regarding the factors that impact the dynamics of mosquito populations. In addition, analysing the environmental factors, can assist in identifying potential sites for breeding and determining the order of importance for implementing control measures.

Ethics Declarations

Not applicable

Conflict of Interest

The authors declare that they have no conflict of interest.

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