BIODIVERSITAS
Volume 24, Number 9, September 2023
Pages: 4820-4828

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240923

Phytotelmata accounts for *Aedes* breeding places in Mantup Sub-district, Lamongan District, Indonesia

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Manuscript received: 8 May 2023. Revision accepted: 23 September 2023.

Abstract. *Fauziyah S, Susanti SF, Hariyono H, Fazirrah V, Novitasari AE, Fadhilah N, Sucipto TH, Naw SW. 2023. Phytotelmata accounts for* Aedes *breeding places in Mantup Sub-district, Lamongan District, Indonesia. Biodiversitas 24: 4820-4828.* Dengue infection still remains a public health problem in Indonesia, which is classified as a tropical country. Some environmental factors that contribute to the transmission of dengue infection are human migration, human activity, food supply, and climate change. The other type of breeding place is natural breeding habitats, called phytotelmata. This study aims to investigate the diversity of *Aedes* species in phytotelmata from Mantup Sub-district, Lamongan, East Java, Indonesia. The analytical observational study was conducted with a purposive sampling design. Mosquito larvae collection was carried out from February to March 2023 in Mantup Sub-district, an endemic region of dengue infection in Lamongan. Immature mosquitoes were reared in the laboratory till they merged into adult mosquitoes. This study found four groups of phytotelmata: leaf axils, bamboo joints, fallen leaves, and coconut shells. Leaf axils were the predominant type of phytotelmata (86.67%). Meanwhile, the lowest percentage was coconut shells and fallen leaves (3.33%). A total of 56 mosquito larvae consisting of 12 male *Aedes aegypti*, 21 female *Ae. aegypti*, 12 male *Aedes albopictus*, and 11 female *Ae. albopictus* were collected from 30 observation points. Eleven families of phytotelmata were found in this study. The predominant family was Araceae (33.3%), while the lowest were Agavaceae, Commelinaceae, Marantaceae, and Liliaceae (3.33%). Phytotelmata accounts for mosquito breeding places, which should be noticed and considered in dengue vector control programs.

Keywords: Dengue, Lamongan, mosquito-borne diseases, phytotelmata

INTRODUCTION

Dengue infection still remains a public health problem in Indonesia, which is classified as a tropical country. Dengue infection is caused by the dengue virus, which spreads through mosquito bytes of *Aedes* species, namely *Aedes aegypti* and *Aedes albopictus* (World Health Organization 2020). Since it has evolved to coexist closely with human habitations, *Ae. aegypti's* geographic range is limited by environmental factors, particularly temperature, which is above 22°C and below 32°C based on the lifespan and viability of the egg, larva, pupa, and fecundity phases of adults (Kraemer et al. 2015; do Nascimento et al. 2022). Female mosquitoes rest indoors, blood-feed primarily on human hosts, and lay their eggs predominantly in artificial containers found in peridomestic settings (David et al. 2021). In addition to tires, flower vases, plastic pots, drains, buckets, and water tanks, a variety of container types with a wide range of sizes, shapes, and materials will commonly be the breeding sites of *Ae. aegypti* (Gavini et al. 2018; Flaibani et al. 2020). Many socioeconomic and behavioral factors have an impact on how frequently *Ae. aegypti* colonizes different types of containers. The distribution, relative abundance, and survival of mosquito species are significantly impacted by human ecology, behavior, and habits (Schrama et al. 2020). Any place that could store water for a long time, in any amount, was considered a "potential mosquito breeding site". Then, the literature defined the criteria of mosquito breeding sites such as the bromeliad plant, tree holes, and building supplies (Yang et al. 2020).

Urbanization can increase the number of potential breeding habitats for *Aedes* due to the increasing number of used tires, which can hold the amount of water suitable for *Aedes* larvae to survive (Mukhtar et al. 2018). Some environmental factors that contribute to the transmission of dengue infection are human migration, human activity, food supply, and climate change (Mudin 2015). Another study in the dengue endemic area in North Sumatra, Indonesia, shows that the artificial breeding places, namely bathtubs, were the predominant larval habitat for *Aedes* (Siregar et al. 2017). *Aedes* survey in Malaysia shows positive breeding tanks in indoor and outdoor areas. The material type of positive rearing tanks found varies and can be classified into 6 categories, namely ceramics, cement, glass, plastics, rubber, and metals (Athaillah et al. 2020). Other reports confirmed the positive correlation between some variables (the depth and width of the containers) and the number of mosquito larvae (Ruairuen et al. 2022). Temperature and pH of the water in *Aedes* breeding places can influence the abundance of *Aedes* larvae. *Aedes* can live in water temperatures with a pH between 7.5 and 8.5, with temperatures ranging between 25.3 and 39.8ºC. Both *Ae. aegypti* and *Ae. albopictus* tend to lay their eggs in water with low turbidity (Dalpadado et al. 2022).

The other type of *Aedes* breeding places are natural breeding habitats called phytotelmata. Phytotelmata are aquatic habitats created naturally when water is retained by whole or partial terrestrial plants, whether alive or dead (Marteis et al. 2017). Microorganisms, macroinvertebrates, which comprise most of the animal biomass, and tiny vertebrates can all live in phytotelmata, creating a highly discontinuous aquatic meta-habitat. Consequently, phytotelmata are pertinent natural microecosystems that can be comprehensively sampled and provide objective records of community-level diversity for investigating various topics. They also indicate environmental influences on community assembly (Dejean et al. 2018). Some reports have already been published about the phytotelmata distribution in Indonesia, which consists of bromeliads, coconut shells, tree holes, etc. (Fauziyah and Pranoto 2020). In Sidoarjo, *Musa paradisiaca* or banana plant, was also confirmed as the natural breeding place of *Aedes* larvae, which mainly consisted of *Ae. albopictus* (Wahidah and Rosmanida 2021). According to the author's criteria, the vast majority of known phytotelmata can be divided into five to seven major classes, with tree holes, bamboo internodes, leaf axils, flower parts, fruit peels, trunks, and modified leaves being the most prevalent types (Ramos and do Nascimento Moura 2019). Dengue infection is one of the endemic diseases in Lamongan. The latest record of dengue cases in Lamongan in 2022 was 129 cases. Based on the problems mentioned above, this study aims to investigate the distribution of *Aedes* in phytotelmata from the Mantup Sub-district, Lamongan District, East Java Province.

MATERIALS AND METHODS

Study area

This study was conducted in Mantup Sub-district, Lamongan District, East Java Province, Indonesia (Figure 1). Lamongan District has an area of ±1,752.21 km^2 or the equivalent of 175,221 ha or ± 3.67% of the total area of East Java Province. Mantup has a long coastline of 47 km and is crossed by the Bengawan Solo River for ± 68 km. Lamongan District consists of 27 districts with 462 villages. Astronomically, Lamongan District is located at coordinates 6º51'54"-7º23'6" S and 112º4'41"-112º33'12" E (Bappeda Lamongan 2022).

Study design

An analytical observational study with a cross-sectional design was conducted during the rainy season (January 2023) in an area that represented a high dengue risk in Mantup Sub-district, Lamongan. Purposive sampling was conducted in 30 sampling points distributed alongside the Mantup Sub-district. The surveyor was driven alongside the Mantup Sub-district. The determination of 30 sampling points was carried out purposively—the selection aimed at a point whose environmental condition potential was a breeding site for *Aedes* mosquitoes.

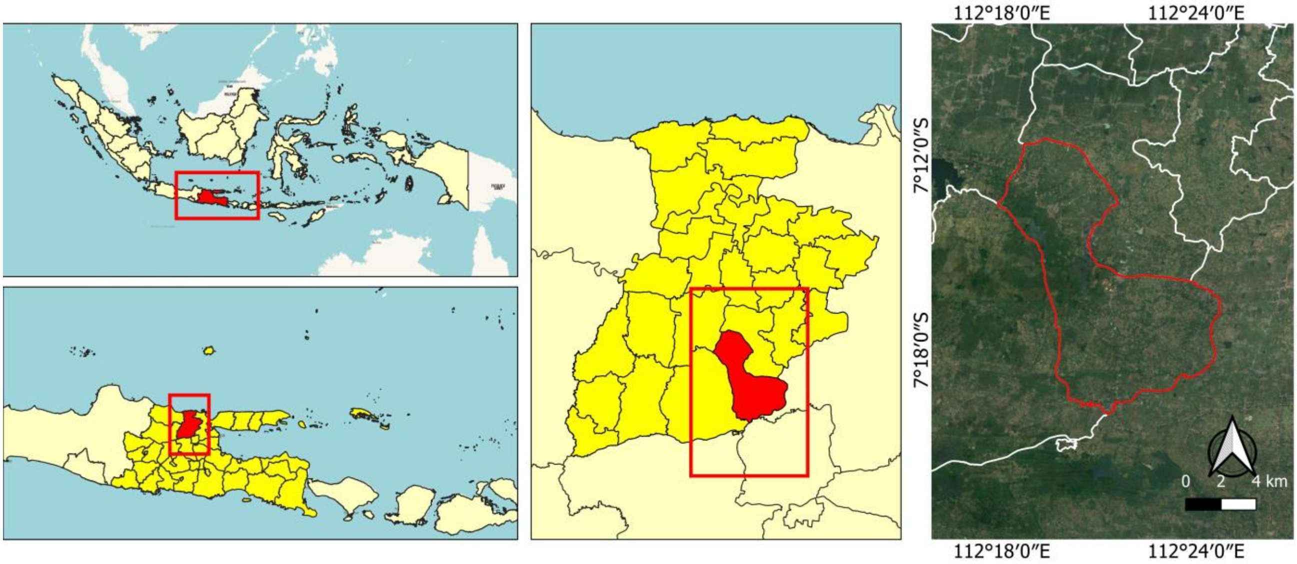

Figure 1. Satellite image of Mantup Sub-district, Lamongan District, East Java Province, Indonesia

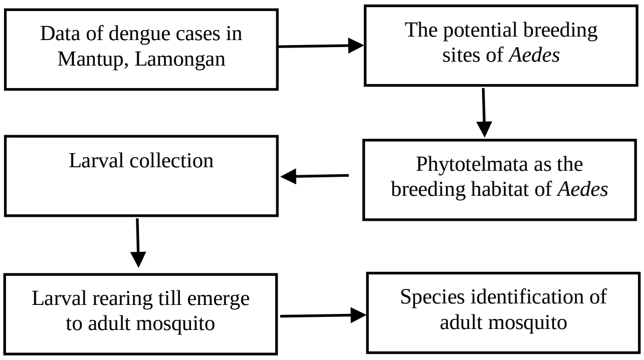

Figure 2. Schematic picture of study design

Procedures

All water held in every phytotelma was pipetted by a plastic pipette of 3 mL. Then, the mosquito larvae included in the water were reared in laboratory conditions until they emerged as adult mosquitoes, following the previous guidelines (FAO/IAEA, 2020). All of the larvae were placed in the tray with a size of 30 cm x 20 cm. Each tray was filled with water depth ranging from 1.2 to 1.5 cm. Mosquito larvae were reared in laboratory conditions at 28±1ºC temperature, 70-80% relative humidity with a 10h:14h light: dark photoperiod. Larvae were fed daily with nutritional fish food. Every day, the larvae were checked for the possibility of pupation. After developing into pupae, the pupae were moved inside the cage for preparation before emerging into adult mosquitoes. The size of the mosquito cage was 40 cm x 25 cm x 15 cm covered with transparent cloth. Adult mosquitoes were fed with a sugar solution of 10%. Based on the previous guidelines, mosquito identification was made through morphological observation (European Centre for Disease Prevention and Control 2021). All mosquitoes were separated into male and female through the morphological observation of their antennae. After the identification process, the adult mosquitoes were stored in cold storage at -80ºC for further virological examination. Bromeliads were identified by comparing their morphology with Benzing's book (Benzing 2000). Figure 2 shows the flow chat diagram of the research procedure.

Data analysis

Data were interpreted in the Frequency Table. The distribution and diversity of *Aedes* species were measured by the Shannon diversity index (H'), frequency, relative abundance, and species dominance (Odum 1971)

Diversity index (Shannon-Weiner):

$H' = \sum -Pi \text{ Ln } Pi$. where $Pi = ni/N$

Where:
ni : the number of individuals per larval species
N : total number of larvae
H' : Shanon-Weiner diversity index
Pi : abundance index

Relative abundance

The relative abundance is the ratio of the number of individual species of mosquito larvae to the total species of larvae collected and expressed as a percent. Relative abundance can be calculated as:

The number of individual mosquito larvae of certain species/total species of mosquito larvae collected x 100%

Frequency of species

The frequency of caught mosquito larvae is calculated based on the comparison between the number of collections obtained by certain mosquito larvae and the total collection of mosquito larvae.

Frequency = the number of collections obtained by certain species of mosquito larvae/total mosquito larvae collection.

The number of species dominance is calculated based on the multiplication results between relative abundance and the frequency of caught by the species at one time of capture.

Species dominance relative abundance x frequencies captured (Odum 1971).

RESULTS AND DISCUSSION

Entomological surveys were carried out, and Figure 3 shows the distribution of the phytotelmata that was interpreted using the Geographical Information System. This figure also shows the composition of *Aedes* in phytotelmata found alongside the Mantup Sub-district. There were two species of *Aedes*, namely *Ae. aegypti* and *Ae. albopictus*. The detail of phytotelmata deciphers is in Table 1.

Table 1 interprets the classification of phytotelmata according to its family. Araceae is the predominant phytotelmata family found in this study (33.3%). Meanwhile, the lowest families were Agavaceae, Commelinaceae, Marantaceae, and Liliaceae (3.33%).

Figure 4 shows the distribution of phytotelmata based on the classification of Kitching's guidelines (1971), which shows that leaf axils were the predominant type of phytotelmata (86.67%). Meanwhile, coconut shells (Cocos nucifera) and fallen leaves (3.33%) were the lowest percentages.

In every phytotelma, we found many mosquito larvae, which can be classified into two species: *Ae. aegypti* and *Ae. albopictus.* Of these, female *Ae. aegypti* was the predominant species found (37.5%) (Figure 5).

Table 2 shows the distribution of *Aedes* in every type of phytotelmata, female *Ae. aegypti* was the predominant species that was found, with a total of 18 larvae (32.14%), which were found in leaf axils.

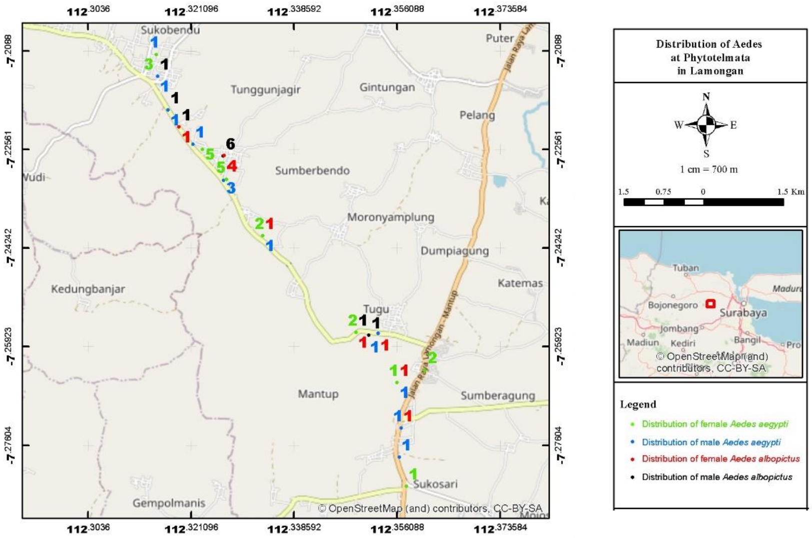

Figure 3. The distribution of *Aedes* larvae was found in phytotelmata in Mantup Sub-district, Lamongan, East Java Province, Indonesia. The red numbers indicate the number of female *Aedes albopictus*, black numbers indicate male *Aedes albopictus*, green numbers indicate the number of female *Aedes aegypti*, and blue numbers indicate the number of male *Aedes aegypti*

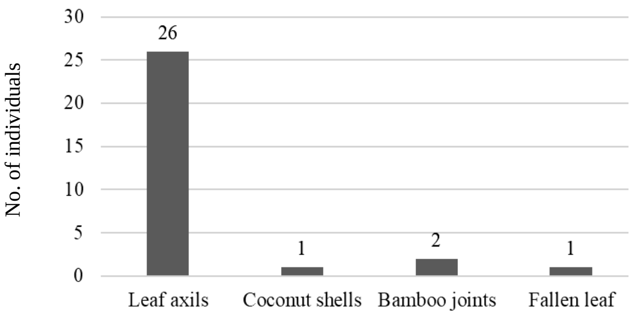

Figure 4. The distribution of phytotelmata type based on classification guidelines (Kitching 1971)

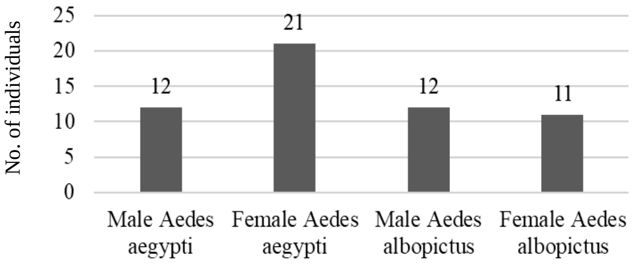

Figure 5. The distribution of *Aedes* species in phytotelmata

Table 1. The distribution of the phytotelmata family, which was founded alongside Mantub Sub-district, Lamongan, East Java Province, Indonesia

Family	N	Percentage (%)	Species
Araceae	10	33.3	*Aglaonema commutatum* *Alocasia cucullata* *Colocasia esculenta* *Dieffenbachia sequine* *Homalomena rubescens* *Philodendron cipoense* *Philodendron crassinervium* *Philodendron crassinervium* *Xanthosoma sagittifolium*
Musaceae	2	6.67	*Musa paradisiaca*
Arecaceae	2	6.67	*Cocos nucifera* *Dypsis lutescens*
Agavaceae	1	3.33	*Sansevieria trifasciata laurentii*
Bromeliaceae	2	6.67	*Alcantarea imperialis* *Neoregelia carolinae*
Pandanaceae	3	10	*Pandanus amaryllifolius* *Pandanus tectorius*
Asparagaceae	5	16.67	*Agave americana* *Cordyline fruticosa*
Poaceae	2	6.67	*Bambusa* sp. *Gigantolochloa apus*
Commelinaceae	1	3.33	*Tradescantia spathacea*
Marantaceae	1	3.33	*Calathea lutea*
Liliaceae	1	3.33	*Scadoxus multiflorus*
	30	100	

Table 2. The number of *Aedes* in every phytotelmata type

Type of phytotelmata	Male *Ae.aegypti*	Female *Ae.aegypti*	Male *Ae.albopictus*	Female *Ae.albopictus*	Total
Leaf axils	11 (19.64)	18 (32.14)	10 (17.85)	10 (17.85)	49
Coconut shells	1 (1.78)	0 (0)	0 (0)	0 (0)	1
Bamboo joints	0 (0)	1 (1.78)	2 (3.57)	1 (1.78)	4
Fallen leaf	0 (0)	2 (3.57)	0 (0)	0 (0)	2
Total	12	21	12	11	56

Discussion

We recorded 56 *Aedes* larval in various phytotelmata alongside Mantub District, Lamongan. They consist of 33 *Ae. aegypti* and 23 *Ae. albopictus*. As revealed by another study, the predominant phytotelmata was leaf axil type (Fauziyah and Pranoto 2020). Our previous study indicated that the Bromeliaceae family is the most phytotelmata in the dengue-endemic area, followed by Arecaeae and Arecaceae (Fauziyah and Pranoto 2020). Both *Ae. aegypti* and *Ae. albopictus* are the dengue vector in Indonesia. *Ae. aegypti* is the primary vector, while *Ae. albopictus* is the secondary vector. Due to their quick geographical expansion and rising disease burden, dengue is raising more and more worries about global public health (Kraemer et al. 2015). The occurrence of mosquito larvae in breeding sites was affected by some factors, including the water's salinity, DO, temperature, pH, and turbidity. They had a substantial impact on the *Aedes* breeding sites and mosquito density (Mbanzulu et al. 2022). These water parameters have an impact on some fundamental mosquito traits, including survival, body size, and biting behavior. These traits are connected to mosquito vectorial ability, which determines the scope and severity of disease transmission. In fact, a lack of food during the stage of immature mosquito development results in the birth of little mosquitoes with smaller adults and shorter lifespans (Gutiérrez et al. 2020).

This study did not find *Culex* larvae in all the phytotelmata. Here are some possible reasons why *Culex* larvae are not found in phytotelmata because the place where they develop is unsuitable. Usually, water saved in phytotelmata comes from rainwater (Almeida et al. 2020). The cleanliness of rainwater can vary depending on the location where it falls (Khayan et al. 2019). When compared to clean water, *Culex* larvae are found more in oily/rusty water (Nanjul et al. 2018). Actually, it was discovered that the Culex was the most prevalent and plentiful mosquito in some places like Sri Lanka, Saudi Arabia, Cameroon, and Indonesia (Chathuranga et al. 2018; Nchoutpouen et al. 2019; Wibawaning 2019; Mohammed et al. 2021).

Various phytotelmata, which are representative of leaf axils, coconut shells, bamboo joints, and fallen leaves, were found in this study (Figures 6-9). However, our study also supported a previous study, which confirmed that bamboo joints are the potential phytotelmata that can act as mosquito breeding places, including *Ae. aegypti* and *Ae. albopictus*. The unique structure of bamboo joints and its capability to hold water could attract mosquitoes to lay their eggs. Some mosquito species are associated with bamboo, and bamboo breeding sites have been shown to maintain the diversity of mosquitoes (Müller et al. 2022).

The volume of water in the breeding sites can also affect the persistence and abundance of mosquito species (Chaves et al. 2019). Fallen leaves can also be the breeding sites for mosquitoes due to the reasonably murky water and high organic content of the fallen leaves and other debris in breeding areas, which may serve as suitable artificial breeding habitats for *Aedes* mosquitoes (Dom et al. 2013). Coconut shells can provide a breeding site for *Aedes* mosquitoes, especially when rainwater is added, as the resources are primarily autochthonous with nutrients leaching from the endodermis (Banerjee et al. 2013). Empty coconut shells were identified as natural breeding sites favorable to *Ae. aegypti*, possibly due to their rich nutrient content, low illumination, and small orifice (Chatterjee et al. 2015). In another study, coconut shells were found to be a risk factor for the occurrence and abundance of *Ae. aegypti* mosquitoes (Kampango et al. 2021).

In Figures 6 and 7, it can be shown that many bromeliads account for mosquito breeding places due to the size of leaf axils, which can hold much water from rainfall or watering. An observation study in Bogor, Indonesia, also showed bromeliads as the predominant phytotelmata with a percentage of 74.4%. Interestingly, the mosquito species composition was different from this study, which consisted of *Armigeres subalbatus, Ae. aegypti, Ae. albopictus, Aedes* spp., and *Cx. quinquefasciatus* (Ikhsan et al. 2020). Bromeliads are a member of the 50-genus family *Bromeliaceae*, with more than 2,500 species (Ikhsan et al. 2020). However, only two species of Bromeliads are found in Mantup. The amount of water in bromeliads is correlated with the number of larvae (Cardoso et al. 2015). The location of the plant, its size, or its species can all have an impact on how many bromeliads contain water. In a contained environment, bromeliads have relatively little water. Because they are tiny and contain very little water, bromeliads may dry in open, shady, or enclosed spaces. The mosquitos' larval growth and survival were also affected by dissolved substances that come from rainwater or metabolites within the containers (Ikhsan et al. 2020)

The types of mosquitoes present in a bromeliad might vary depending on its location, species, and size. A study investigating the oviposition preferences of gravid *Ae. aegypti* found that they tended to deposit eggs in the leaf axils of Neoregelia more than in the central bowl or tank (Brown 2021). Our study indicates that different mosquito species can live together in one of the phytotelmata. Urbanization may alter the ecology of *Aedes* mosquitoes by altering species dynamics and composition, as well as increasing the number of breeding sites owing to environmental changes, which might lead to arbovirus epidemics (Li et al. 2014). Previous research discovered that additional food sources significantly impacted the developmental time and survival rate of immature mosquitoes when compared to the control group. Those suggest that the availability of nutrients in various habitat types may have a different impact on larval development depending on location. The impacts, however, were more noticeable in urban regions compared to suburban and rural areas, suggesting that other factors, including water temperature, may be more significant than food supply in urban areas. These findings showed that urbanized regions are better for larval development and survival; in other words, *Aedes* larvae are more suited to urban environments (Li et al. 2014).

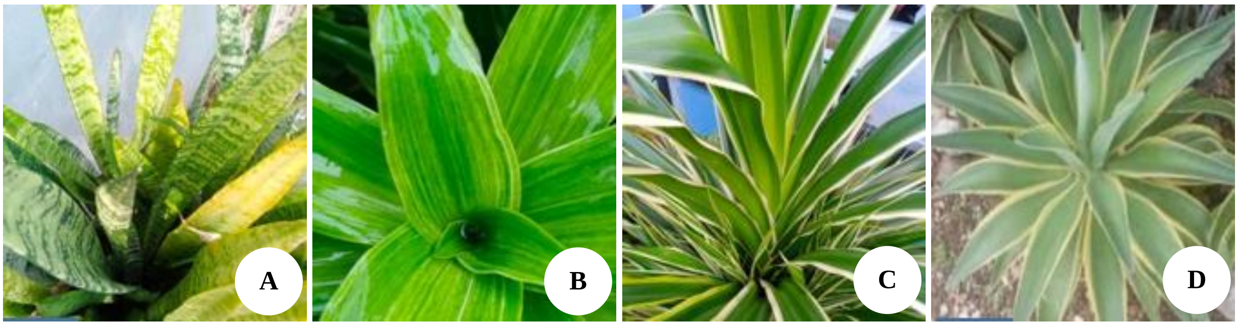

Figure 6. The representative of phytotelmata which was classified as: leaf axils: A. *Sansevieria trifasciata laurentii*; B. *Alcantarea imperialis*; C. *Neoregelia carolinae*; D. *Agave americana*

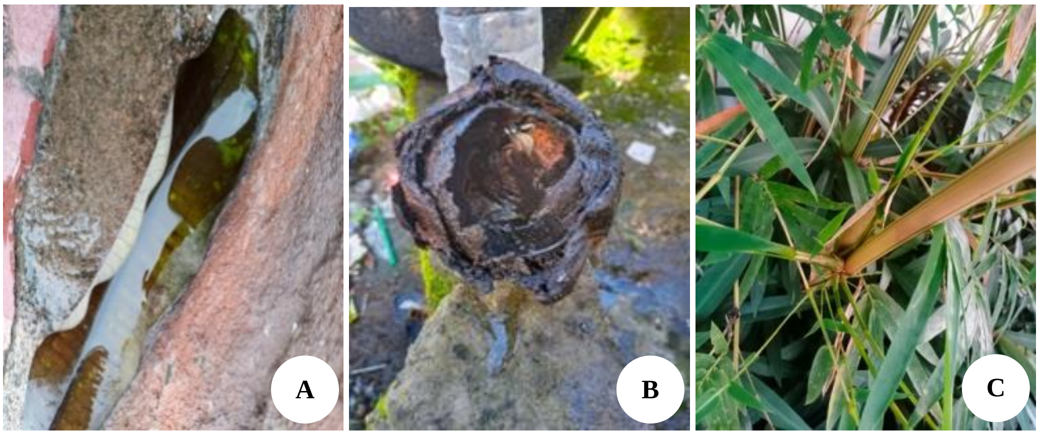

Figure 7. The representative of phytotelmata which was classified as: A**.** Fallen leaf: *Philodendron crassinervium*, B**.** Coconut shells: *Cocos nucifera*, C. Bamboo clumps: *Bambusa* sp.

The number of habitat surface areas, water depth, the occurrence of the canopy, the turbidity of the water, and the kind of substrate were all significant factors that influenced the occurrence of mosquito larvae. Mosquitos have a preference for oviposition in urban areas (Delatte et al. 2013), outdoor containers (Kroth et al. 2019), clear water (Gopalakrishnan et al. 2013), water with vegetation (Kroth et al. 2019), and more significant surface areas (Reiskind and Zarrabi 2012; Gopalakrishnan et al. 2013). The elaboration of phytotelmata as *Aedes* breeding places is still limited. Most of the studies focused on artificial breeding places. An observational study in Riau inspected as many as 401 houses shows 138 containers positive for mosquito larvae. Most of the containers were unclosed and located inside the house. Of these, some physiochemical factors were significantly correlated with the positivity of mosquito larvae, including water pH, location, type, container closure, and water temperature ($p<0.05$) (Daswito and Samosir 2021). Other inspection of mosquito larvae in Curug Village, Tangerang, also shows the disposable containers were predominated by *Ae. albopictus* larvae (Yuliani et al. 2021). Disposable containers include used bottles, cans, tires, ponds, buckets, and drums (Yuliani et al. 2021). In this study, we did not observe the occurrence of aquatic carnivores in water, which is possible as the predators of mosquito larvae. Other reports show that *Utricularia macrorhiza* has the capability as the *Aedes* larvae predator and can reduce up to 100% of mosquito larvae under laboratory conditions (Couret et al. 2020). Another protist with the capability as the predator of mosquito larvae is *Chilodonella* sp. However, the study about its capability only proved to decrease *Culex* larvae, not *Aedes* larvae. Further research to elaborate on its potential should be conducted (Wijesinghe and Amarasinghe 2023).

Food supply was also playing an important role in the survival of mosquito larvae, as stated by the previous study. Some flora can be used as a food source for mosquito larvae, such as grasses, spirogyra, and algae (Hafeez et al. 2022). Competition between two species, *Ae. aegypti* and *Ae. albopictus* can also occur when both species are placed in the same habitat. A laboratory study explored the larval development time of *Aedes* species, especially *Ae. albopictus*. Those species have larval developmental times shorter than *Ae cretinus* (Giatropoulos et al. 2022). An observation in semi-field conditions revealed that *Toxorhynchites splendens* was a potential predator for *Ae. albopictus* larvae, when it was provided together with *Tubifex*, *Toxorhynchites* tended to consume *Aedes* larval as its food source (Malla et al. 2023). The increase in container size was correlated with the larval developmental time (Qureshi et al. 2023).

Table 3. Species diversity index, relative abundance, and frequency

Species larvae	Total	H'	RA(%)	Frequency
Ae. aegypti	33	0.58	58.92	73.33
Ae. albopictus	23	0.41	41.07	60

Note: H': Diversity index (Shannon-Weiner), RA: Relative Abundance

Aedes aegypti is the dominant species found in phytotelmata, followed by *Ae. albopictus*. However, the Shanon-Wiener index is classified as low (H': 0.58<1), and the relative abundance is 58.92% (Table 3). Biological control could be introduced to the community to prevent dengue transmission. Some biological control that rapidly progressed are using plant extract as the larval insecticide. Carbon-based nanoparticles show positive effects on *Aedes* larvae, which can accumulate in its gut and respiratory system and may lead to disturbing the larval movement system (Rodríguez et al. 2022). The other natural bioinsecticides that can trigger myelinization in mosquito larvae is fatty acid methyl esters (FAME) (Neto et al. 2023). One of the genetic modifications introduced to eliminate dengue in Indonesia is the use of *Wolbachia*, which had been applied in Yogyakarta. However, some risk assessments still need to be evaluated, and the current study showed that the release of *Wolbachia* in Yogyakarta led to negligible risk (Buchori et al. 2022).

The occurrence of phytotelmata in Mantup Sub-district Lamongan should be considered. Additionally, community awareness and education may be critical in preventing the proliferation of *Aedes* mosquitoes in the Mantup Sub-district. Residents should be urged to frequently check their gardens and surrounding areas for potential breeding places and take action to eliminate them. Using larvicides or mosquito traps is another efficient mosquito control measure that local authorities might assist residents in implementing.

It can be concluded that various phytotelmata found alongside Mantup Sub-district account for mosquito breeding places, specifically *Aedes* sp. Eleven families of phytotelmata were found in this study. The predominant family was Araceae (33.3%), while the lowest were Agavaceae, Commelinaceae, Marantaceae, and Liliaceae (3.33%). Phytotelmata accounts for mosquito breeding places, which should be noticed and considered in dengue vector control programs.

ACKNOWLEDGEMENTS

The authors would like to thank *Direktorat Akademik Pendidikan Tinggi Vokasi* (DAPTV) and *Direktorat Jenderal Pendidikan Tinggi Vokasi* (DIKSI), Indonesian Ministry of Education, Culture, Research and Technology for the funding of this research (Contract no. 199/SPK/D.D4/PPK.01.APTV/VI/202). We also thank Akademi Analis Kesehatan Delima Husada, Gresik, Indonesia students who helped collect the mosquitoes.

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