

THE PREFERRED OF ATTRACTANT PLANT AS ACTIVITY PLACE of *Aedes aegypti* IN THE LABORATORY

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ABSTRACT

This research aimed to determine the preferences and activities of *Aedes aegypti* toward attractant plants in the laboratory. This was a descriptive observational study using the visual control method. The data were analyzed using descriptive statistics. The results showed that the highest presence of male (50%) was found on *Mangifera indica*, followed by *Murraya* sp. (23%), *Ixora* sp. (11%), *Annona muricata* (11%), *Plumeria* sp. (3%) and *Pteridium aquilinum* (2%). The highest presence of female (65%) was found on *Mangifera indica*, followed by *Murraya* sp. (9%), *Ixora* sp. (9%), *Plumeria* sp. (9%), *Annona muricata* (4%), and *Pteridium aquilinum* (4%). The feeding activity of both male and female was highest on *Mangifera indica* and lowest on *Ixora* sp., *Pteridium aquilinum*, and *Annona muricata*. Resting activity was observed on all test plants except *Pteridium aquilinum*. The *Mangifera indica* has a high attraction for both male and female *Ae. aegypti* for feeding and resting activities.

Key words: *Aedes aegypti*, attractant plant, feeding activity, preference, resting activity

ABSTRAK

Tujuan penelitian ini mengetahui preferensi dan aktivitas *Aedes aegypti* terhadap tanaman atraktan di laboratorium. Penelitian ini adalah observasional deskriptif dengan menggunakan metode visual kontrol. Data dianalisis menggunakan statistik deskriptif. Hasil penelitian menunjukkan tingkat kehadiran *Ae. aegypti* jantan paling tinggi ditemukan pada *Mangifera indica* (50%), diikuti *Murraya* sp. (23%), *Ixora* sp. (11%), *Annona muricata* (11%), *Plumeria* sp. (3%) dan *Pteridium aquilinum* (2%). Kehadiran betina *Ae. aegypti* paling sering ditemukan pada *Mangifera indica* (65%), diikuti *Murraya* sp. (9%), *Ixora* sp. (9%), *Plumeria* sp. (9%), *Annona muricata* (4%) dan *Pteridium aquilinum* (4%). Aktivitas makan jantan dan betina paling tinggi ditemukan pada *Mangifera indica* sedangkan aktivitas makan terendah ditemukan pada *Ixora* sp., *Pteridium aquilinum* dan *Annona muricata*. Aktivitas istirahat ditemukan pada semua tanaman uji kecuali *Pteridium aquilinum*. Tanaman *Mangifera indica* mempunyai daya tarik tinggi terhadap nyamuk jantan dan betina *Ae. aegypti* untuk melakukan aktivitas makan dan istirahat.

Kata kunci: *Aedes aegypti*, tanaman atraktan, aktivitas makan, preferensi, aktivitas istirahat

INTRODUCTION

The *Aedes aegypti* requires three essential habitats for its life cycle; breeding place, feeding place, and resting place. Breeding place *Ae. aegypti* lays its eggs in containers that can hold water, such as water storage containers, discarded items, and natural reservoirs (Agustina and Emil 2024). Male and female of *Aedes* primarily feed on nectar and plant fluids as their main source of energy (Barredo and DeGennaro, 2020). Female mosquitoes also require blood as a supplementary nutrient (Cassone *et al.* 2024), which provides essential proteins needed for egg maturation (de Swart *et al.* 2023). *Ae. aegypti* can be found resting both indoors and outdoors, such as on vegetation near their feeding sites, feeding place, breeding containers and inside human dwellings (Agustina *et al.* 2019; Diallo and Diallo, 2020).

The breeding place of *Aedes* have been widely studied and are well known to the public. However, information regarding the resting and feeding place of adult *Ae. aegypti* remains very limited. Plants play a crucial role in supporting mosquito survival. They serve as place for feeding, resting, or hiding (Agustina *et al.* 2019). The study of *Aedes* selection and activity on nutrient source plants is essential to clarify the factors influencing their preference for specific plants.

Each mosquito species has different preferences for plant species (Pare *et al.* 2024). This preference is

influenced by the mosquito's olfactory system and the volatile compounds released by plants. Therefore, to confirm this preference, further laboratory testing is necessary (Nyasembe *et al.* 2018). The mosquito olfactory system influences its ability to search for and identify nutrient source plants. Mosquitoes can distinguish between different plant species based on the composition of volatile compounds released by the plants (Konopka *et al.* 2021).

Secondary metabolites are organic compounds produced by plants, primarily functioning to protect them from herbivore disturbances and to prevent infections caused by microbial pathogens. Secondary metabolites act as signaling agents and competition agents among plants, contributing significantly to plant-environment interactions. Signaling agents, such as volatile compounds, give plants their distinctive aroma. Volatile compounds not only directly protect plants from herbivore attacks but also serve as plant signals to attract insects (Divekar *et al.* 2022). Plants contain secondary metabolite compounds that can function as repellents and attractants for *Aedes*. House yard plants have the potential to be used as a strategy for controlling disease vector transmission (Agustina *et al.* 2022). Chemical control using insecticides such as mosquito repellents has led to mosquito resistance to these chemicals. Therefore, alternative control efforts are necessary, such as the use of attractant compounds, most of which have not been extensively explored (Yudhana *et al.* 2018).

A study on plant preferences for *Aedes* activity in home yards in Kopelma Village, Banda Aceh, identified six plant species that serve as activity place for *Ae. aegypti* and *Ae. albopictus*. These plants include *Mangifera indica*, *Annona muricata*, *Pteridium aquilinum*, *Ixora* sp., *Plumeria* sp., and *Murraya* sp. (Agustina et al. 2019). The selection of plants in this study was based on the presence of *Aedes* resting on them. However, the results of this study have not yet confirmed these plants as host plants. Therefore, further research is needed to determine the host plants and the activity of *Aedes* on the plants. Based on the previous findings, these plants were subsequently used as test plants to gain a deeper understanding of their role in attracting *Ae. aegypti* in the laboratory. The aim of this study is to conduct an in-depth laboratory analysis of the preferences and activities of male and female *Ae. aegypti* toward of attractant plants. Information on attractant plant preferences as activity place for mosquitoes is essential as a basis for developing *Aedes* control strategies.

MATERIALS AND METHODS

This study is a descriptive observational study using the visual control method. Data were obtained through direct observation of adult *Ae. aegypti* activity. The observed activities included feeding and resting behaviours on plants. The selection of plants for the experiment was based on previous studies (Agustina et al. 2019). Mosquito feeding activity is characterized by a slightly inclined body position or an angled posture, which is presumed to help apply pressure with the proboscis while piercing. Meanwhile, resting activity is identified when the mosquito's proboscis is not inserted into the leaf but rests on the leaf surface. A mosquito is considered to be resting when it remains on the plant surface for a relatively longer duration, as opposed to those that merely perch temporarily.

Rearing of *Aedes aegypti* in the Laboratory

The *Ae. aegypti* mosquito larvae collected from the field were transferred to a plastic tray measuring 30 cm × 23 cm × 4 cm, filled with 500 mL of breeding water. Mosquito maintenance was carried out at room temperature (24-28° C) with a relative humidity of 85%-90%. Once the larvae developed into pupae, they were immediately collected and transferred to a 200 ml plastic cup filled with breeding water up to three-quarters of its volume. The pupae were then placed in an adult mosquito cage measuring 40 × 40 × 40 cm. The frame of the mosquito cage was made of wood, while its walls were made of mesh. After emerging as adult, these *Ae. aegypti* were then used for the experimental stage to assess mosquito preferences for the test plants.

Provision of Plants as Places for Feeding and Resting Activities *Aedes aegypti*

Mangifera indica, *Annona muricata*, *Pteridium aquilinum*, *Ixora* sp., *Plumeria* sp., and *Murraya* sp., along with additional plants from the field with a height

of approximately 1 m, were prepared as feeding and resting sites for mosquitoes. The plants selected as places for *Ae. aegypti* feeding and resting activities had stems, leaves, and flowers, except for the *Pteridium aquilinum*, which consisted only of stems and leaves.

Observation of *Aedes aegypti* Preferences for Plants as Feeding and Resting Places

Observations of *Ae. aegypti* feeding and resting preferences were conducted in the Zoology Unit Laboratory, Department of Biology Education, Faculty of Education and Teacher Training, Universitas Islam Negeri Ar-Raniry, Banda Aceh. All prepared plants were placed inside a Peet-Grady Chamber measuring 1.50 × 1.50 × 1.50 m³. A total of 10 male and 10 female adult *Ae. aegypti* were prepared and released into the Peet-Grady Chamber. Observations on the plants in the laboratory were conducted in the morning and afternoon, specifically from 07:00-09:00 WIB, 10:00- 12:00 WIB, and 15:00-17:00 WIB. The selection of these time periods was based on nectar secretion by plants and environmental temperature, which influence *Aedes* activity (Chadee et al. 2014). Observations were carried out on all parts of the plants, including the stems, leaves, and flowers.

Data Analysis

The data were analyzed using descriptive statistics based on the frequency and percentage of male and female *Ae. aegypti* orientation toward each test plant. After analysis, the data were interpreted, and a final conclusion was drawn. Descriptive statistical analysis involves describing the data as they are, without intending to make conclusions that apply generally or can be generalized.

RESULTS AND DISCUSSION

The Preference of Male *Aedes aegypti* for Test Plants

The results indicated that the preference of male *Ae. aegypti* for test plants varies in behavior. The percentage of resting and feeding activity of *Ae. aegypti* was calculated based on the number of male and female mosquitoes that choose the test plants. Observations also showed that male *Ae. aegypti* were more active in moving or flying to search for suitable plants as their food source, whereas female *Ae. aegypti* were more passive and spent more time resting. The percentage of male *Ae. aegypti* mosquito preferences for test plants in the laboratory can be seen in Figure 1.

In Figure 1, it can be seen that male *Ae. aegypti* exhibit varying levels of preference for different household garden plants. The highest to lowest preferences were observed for *Mangifera indica*, *Murraya* sp., *Ixora* sp., *Annona muricata*, *Plumeria* sp., and *Pteridium aquilinum*. Plant nectar flora is the sole food source for male mosquitoes. The varying levels of preference among male mosquitoes for different plants were suspected to be related to the volatile compounds released and the nectar content produced by the plants. The preference for plants by mosquito species varied

greatly. This selection probably based on geographical habitat and seasonal availability. Mosquitoes can distinguish between plants with high and low nectar flora (Barredo and DeGennaro 2020).

Based on the research findings, all test plants in the laboratory were visited by male *Ae. aegypti*, but the percentage of visits varied. This variation was assumed to be due to differences in the quantity and type of plant compounds released, as well as the unique aroma of each plant (Bitwell et al. 2023). Additionally, the morphological structure of the plants was also believed to influence the attraction of male *Ae. aegypti*. Flavonoid compounds constitute the largest class of plant phenolics. Flavonoids play a role in attracting insects. Alkaloids are compounds with significant pharmacological effects on animals, and most are effective in deterring herbivorous mammals (Qasim et al. 2024).

Preference of Female *Aedes aegypti* for Test Plants

The preference of female *Ae. aegypti* for plants show varying levels of preference. The preference level of female *Ae. aegypti* for household garden plants can be seen in Figure 2. In Figure 2, *Mangifera indica* showed the highest preference level for female *Ae. aegypti*. Meanwhile, the lowest preference levels for female *Ae.*

aegypti were found in *Pteridium aquilinum* and *Annona muricata*. Female *Ae. aegypti*, in addition to consuming floral nectar for metabolic energy, also require blood protein for egg development. Female mosquitoes must select the best floral nectar that meets their metabolic and reproductive needs (Venkataraman et al. 2022).

Based on laboratory observations, released female mosquitoes tended to be passive in approaching plants. Initially, they preferred to rest on the walls of the cage. This was likely because the olfactory system of female mosquitoes is highly sensitive to human body odor. The preference of female *Aedes* mosquitoes for human blood is influenced by their sensory nervous system, which is used to detect human hosts (McBride et al. 2014). The nectar content of plants also affected the selection of specific plants by female *Ae. aegypti*. The fitness level of female *Anopheles gambiae* was found to be higher when consuming floral nectar from *Parthenium hysterophorus* and *Ricinus communis* compared to *Bidens pilosa* (Nyasembe et al. 2015). *Anopheles gambiae*, *Culex pipiens molestus*, and *Ae. aegypti* were attracted to the volatile organic compounds of flowers, specifically the chemical groups of aromatics, monoterpenes, sesquiterpenes, and fatty acid derivatives (Yu et al. 2015).

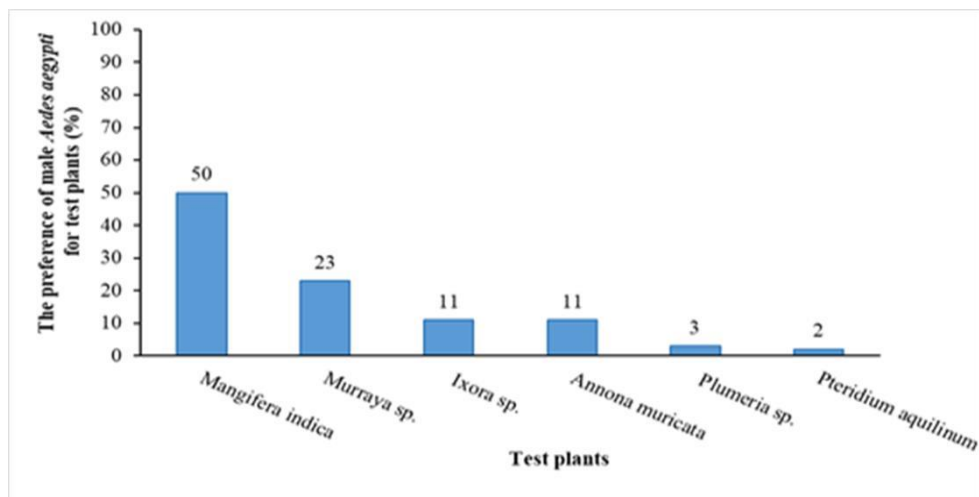


Figure 1. The percentage of male *Ae. aegypti* preference for test plants

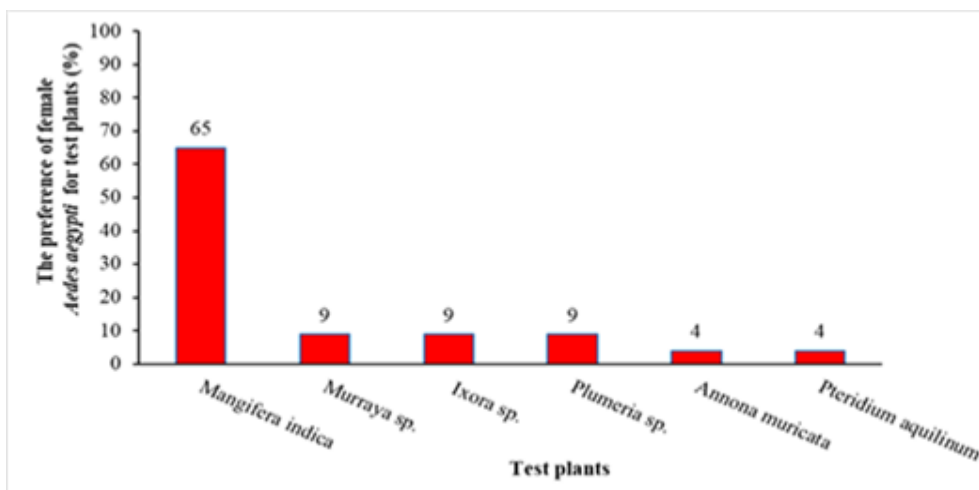


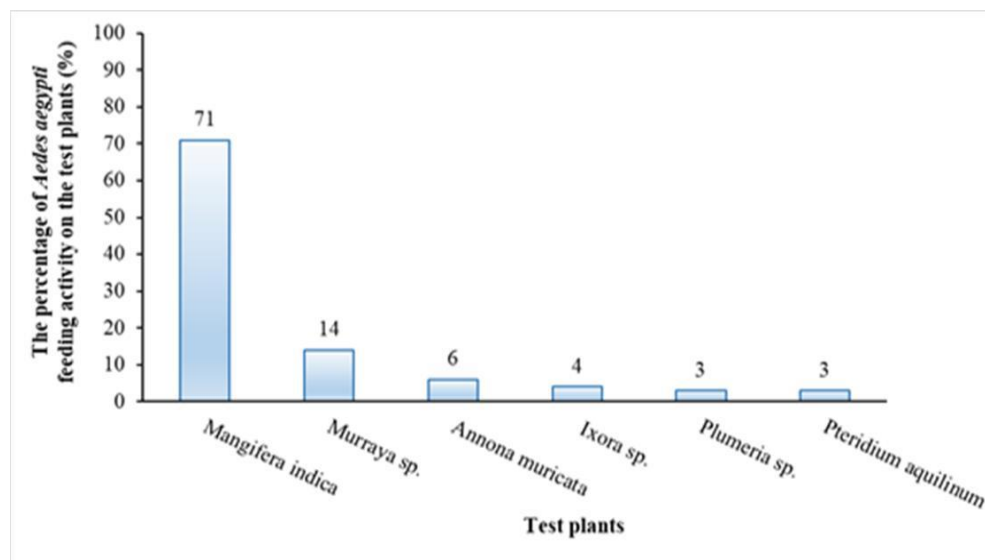
Figure 2. The percentage of female *Ae. aegypti* preference for test plants

Feeding Activity of *Aedes aegypti* on Test Plants.

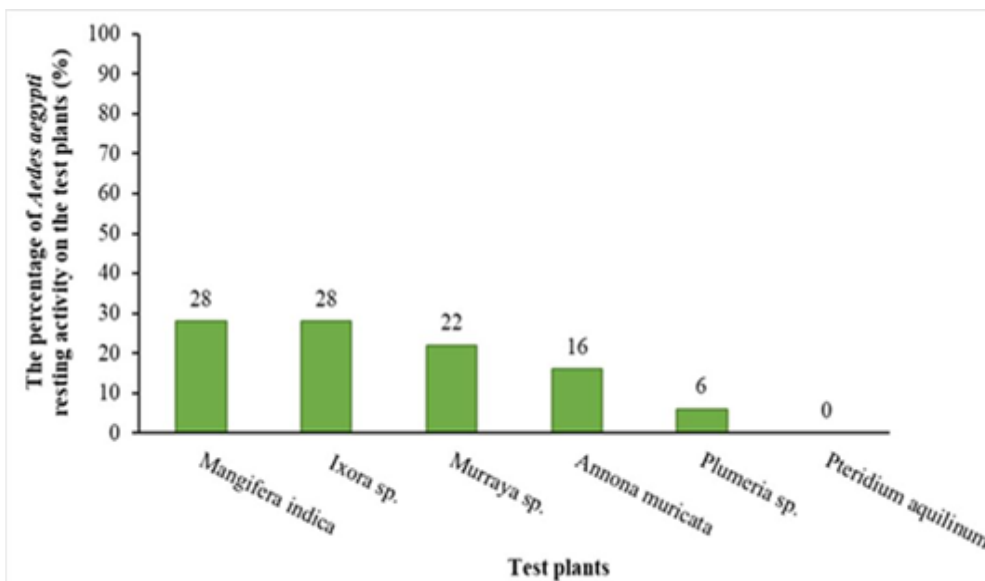
One of the roles of plants for mosquitoes is to serve as a source of energy. The feeding activity of mosquitoes on a particular plant is influenced by various factors, such as volatile compounds that attract mosquitoes, plant morphological structures, physicochemical environmental factors, and the concentration of plant compounds required by mosquitoes. The percentage of feeding activity of male and female *Ae. aegypti* on household garden plants in the laboratory can be observed in Figure 3.

Based on Figure 3, *Mangifera indica* was the plant with the highest feeding activity (71%) of male and female *Ae. aegypti*. The volatile compounds and nectar content produced by *Mangifera indica* were more distinctive and stronger in attracting the presence of *Ae. aegypti*. Besides being attracted to plants due to nectar, mosquito fitness also plays a role. The body fitness of female *Anopheles gambiae* is significantly better when consuming the sweet secretions (Nyasembe et al. 2015).

A higher fructose content was found in flowering *Mangifera indica* (Anacardiaceae). The attraction of male *Anopheles gambiae* mosquitoes to *M. indica* is influenced by olfactory cues that guide their preference in selecting plants as an energy source. *M. indica* contains 23 volatile compounds, including alcohols, aldehydes, alkanes, benzenoids, monoterpenes, sesquiterpenes, and oxygenated terpenes. The antennae of *Anopheles gambiae* exhibit a strong response to four compounds: humulene, (E)-caryophyllene, terpinolene, and myrcene (Meza et al. 2020). *M. indica* has the potential to be a food source because it contains sucrose, which is essential as an energy source (Agustina et al. 2019). Sucrose in plants is produced through photosynthesis. The production of sugar is secreted by the plant's nectary glands, which can develop on flowers, stems, and leaves (Kottapalli et al. 2018). In addition to being a food source, *M. indica* can be utilized as an alternative for reproductive control due to its mangiferin content, which has antifertility properties (Handayani



Figures 3. Percentage of feeding activity of male and female *Ae. aegypti* on test plants



Figures 4. Percentage of resting activity of male and female *Ae. aegypti* on test plants

and Ghofur 2016). The mangiferin compound found in *M. indica* can be used for controlling the population of *Ae. aegypti*. In addition, other compounds found in all plants, such as alkaloids, saponins, flavonoids, and tannins, can be used as anti-spermatogenic and anti-fertility agents (Setiawan et al. 2021). In mammals, tannin compounds in plants have been shown to cause sperm agglutination, negatively affecting sperm quality (Thasmi et al. 2022). Alkaloid compounds found in plants such as neem have cytotoxic effects that affect germ cells and spermatogenic cells (Aulia et al. 2023). This presents an opportunity to further investigate whether these compounds also have an impact on insects. Therefore, further research is needed to determine the effects of this compound on the mosquito's reproductive system.

Observations on feeding posture behavior showed that *Ae. aegypti* mosquitoes took time to initiate feeding. After finding the appropriate plant part, such as a leaf, the mosquito inserted its proboscis into the leaf tissue while positioning its body at a slight angle, with the hind legs raised upward. The mosquito's body posture during feeding is slightly inclined or angled to apply pressure on the proboscis while piercing (Agustina et al. 2019).

Resting Activity of *Aedes aegypti* on the Test Plants

Resting activity is an important behavior for all animals. It is needed to renew energy and maintain body quality. The observation of resting activity in male and female *Ae. aegypti* mosquitoes on the test plants showed varying percentages. The percentage of resting activity ranged from the highest to the lowest, with no activity observed on some plants at all: *Mangifera indica* (28%), *Ixora* sp. (28%), *Murraya* sp. (22%), *Annona muricata* (16%), *Plumeria* sp. (6%), and *Pteridium aquilinum* (0%). Laboratory observations revealed that female mosquitoes were more likely to rest on the walls of the cage, while male mosquitoes rested more on the test plants. The abundance of male mosquitoes was more frequently found (Sukendra et al. 2020). The percentage of resting activity of *Ae. aegypti* on the test plants in the laboratory can be seen in Figure 4.

Temperature affects the resting and feeding activities of adult *Aedes* (Agustina et al. 2021). This is because, at certain temperatures, adult *Aedes* prefer to rest in specific locations, such as on plants. A particular temperature range influences host-seeking and resting behavior. At temperatures below 10° C, *Ae. aegypti* have difficulty flying and choose to rest instead (Reinhold et al. 2018). *Aedes* prefer to rest in relatively low temperatures and cool air during the morning and evening (Aikpon et al. 2019). Mosquitoes use various cues to locate their hosts, including temperature, visual signals, and chemical compounds. Host-seeking behavior is also closely related to the mosquito's ability to move and fly (Reinhold et al. 2018).

Mosquitoes utilize plants as places for resting and hiding. The presence of diverse plants in residential areas provides mosquitoes with many choices for suitable resting spots. Additionally, the density of plants in the yard also makes the temperature and humidity ideal for

the comfort of mosquitoes while resting. *Aedes* utilize vegetation as a place for breeding and resting because of the shaded conditions (Brown et al. 2018).

Dense vegetation obstructs sunlight around residential yards. Thick vegetation is highly potential as a resting place for mosquitoes (Ferraguti et al. 2016). Generally, both male and female *Ae. aegypti* rest on the undersides of leaves. This is believed to help them avoid being seen by predators and being exposed to direct sunlight. When resting, the mosquito's body lies flat on the surface of the leaf. Observations of mosquito resting activity showed that their posture and position were different from when they were feeding. The behavior of mosquitoes while resting can be identified by the proboscis not piercing into the plant organ, with the proboscis aligned with the mosquito's head, and all the legs firmly gripping the surface of the plant organ, resting for a relatively long period of time. Mosquito resting activity is also heavily influenced by environmental factors such as wind and light (Agustina et al. 2019).

CONCLUSION

Based on the research results, it can be concluded that determining a plant as a host or attractant for *Ae. aegypti* requires further investigation through observation of the preferences and activities of adult male and female *Ae. aegypti* towards a specific plant. *Mangifera indica* has the highest preference and attraction for both male and female *Ae. aegypti* to engage in feeding and resting activities.

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