

RESEARCH ARTICLE

Exploration of *Aedes* breeding site with the evidence of different types of container in plant nursery, Klang, Malaysia

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Abstract:

Dengue fever which transmitted by the *Aedes* mosquitoes has been one of the most widespread vector-borne disease. The most important thing in reducing the dengue infection cases is by controlling the populations of the *Aedes* mosquito by destructing *Aedes* breeding sites. *Aedes* breeding sites was determined by conducting a larval survey. Most of the study that has been conducted are targeting the housing area since it is a resident of human. However, plant nursery can be one of the contributors for the increasing of dengue especially the one located near the housing area. This is because the potential container such as flowerpot plate, tires and bucket also could be found in plant nursery. This study helps to identify any natural or artificial breeding site that can be found in a plant nursery and if this potential breeding site can give impact to the *Aedes* population. Container survey were used to identify the potential breeding site at the plant nursery and larvae collection was conducted to depicting positive breeding site. Overall, 68 containers were found as potential breeding sites hence artificial container was found to be the main contributor to the increasing of positive breeding site in plant nursery. Henceforth, the relationship between the type of container and positive breeding site was determined by their likelihood to breed in the container with large surface area such as bucket and shaded area such as tires and bucket.

Keywords: *Aedes* breeding site, artificial container, container survey, plant nursery.

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1. INTRODUCTION

Dengue fever is considered as one of the most widespread vector-borne diseases which has affected 128 countries in this world (Brady et al., 2012). According to the Ministry of Health, the total number of dengue cases in Malaysia for 2018 is 80,615 cases being reported with 147 deaths due to dengue. Most dengue cases occurred in Selangor state with 45,349 dengue cases followed by Federal Territory of Kuala Lumpur and Pulau Pinang with 7,591 cases and 6,071 cases respectively. Due to the increase of the dengue cases in Malaysia, the most important thing in reducing the dengue infection cases is by controlling the populations of the *Aedes* mosquito since the transmission requires *Aedes* mosquito as the vectors. Hence, the control should be targeting the habitats that are more productive such as the container characteristic that lead to the breeding of the *Aedes*. *Aedes* larvae usually can be found either in natural or artificial containers. The common container for the

breeding site of the *Aedes* can be found discarded tires, flowerpots, flowerpot plates, rain barrels and other places that have stagnant water which is a suitable place for the mosquito to lay their eggs (Madzlan, 2016). Usually, the larval survey will be conducted at the housing area since it is near with the human population. Plant nursery can be an example of a potential *Aedes* breeding site as they have many artificial containers such as flowerpot plates, rain barrels and canvas sheets. Besides that, Petaling Jaya City Council (MBPJ) Health and Environment Department director Dr Chitra Davi N. Vadivellu has said that *Aedes* larvae also can be found at the plant leaves that can hold water. Some studies have proved that *Aedes* sp. especially *Ae. aegypti* can breed in plant leaves such as ornamental bromeliads (André B. B. Wilke, 2018).

In response to this problem, this study was aimed to identify the potential *Aedes* breeding site in the plant nursery and its relationship with different type of container as well as larvae

population. There were 9 plant nurseries in Klang was chosen for this study. The nurseries were inspected to identify the potential breeding site and number of positive containers.

Generally, reducing the *Aedes* breeding site is vital to reduce dengue cases in an area. Identification of the *Aedes* breeding site is usually being conducted at the residential area since it is close to the community. Gardening utensils were found as the common breeding site for the *Aedes* which include flowerpot, flowerpot plates and watering cans (Ramlee, 2013). However, these things also commonly can be found at the plant nursery. There is not much study being conducted at the plant nursery because most of the study is more focused at the community area such as residential area or institutional. Besides that, some of the ornamental plants such as Bromeliads has been proved can be a breeding site for *Aedes* (Wilke, 2018). Therefore, may increase the number of potential breeding sites at the plant nursery.

2. MATERIALS AND METHODS

A cross sectional study was carried out in determining the interested parameters. Setia (2016) has referred to the cross-sectional study as the measure of the outcome and the exposure in the study participant at the same time. Convenient sampling was used in gathering the plant nursery participation. Container survey method was used in investigating the potential breeding site and the larval was collected for identification purposes ex-situ. During the container survey, importance information such as type of container, number of potential containers, and container with positive breeding was recorded. The larvae found was collected using the dipping or pipette and was placed in universal bottle whereby each of the larvae samples was labelled with location and types of original container. Identification of *Aedes aegypti*, *Aedes albopictus* or other type of mosquito was carried out in vector laboratory.

Descriptive analysis was used to summarize the number of larvae collected in the different location and types of container found. Percentage of the potential breeding sites found, and positive breeding sites was determined to identify the highest type of container that could be the most favoured breeding site for the *Aedes* mosquito. In addition, the Index of Available Container (IAC) was calculated for any potential breeding sites in every artificial container found and Breeding Preference Ratio (BPR) was calculated for any positive breeding site using above formula:

Breeding Preference Ratio (BPR) =

$$\frac{\text{Index of Contribution to Breeding Sites (ICBS)}}{\text{Index of Available Container (IAC)}}$$

Index of Contribution to Breeding Sites (ICBS) =

$$\frac{\text{Amount of positive container for each type}}{\text{Total amount of positive container}}$$

Index of available container (IAC) =

$$\frac{\text{Amount of potential container for each type}}{\text{Total amount of potential container}}$$

3. RESULTS AND DISCUSSION

3.1. Cleanliness and the distance of the plant nurseries from residential area

The survey was conducted at 9 plant nurseries that have been identified in Kapar, Klang by using the convenience sampling. Most of the plant nursery is located near the housing area. During the survey, information such as distance from the housing area and cleanliness were gathered from the observation during the visit (Table 1). The cleanliness of the plant nursery was based on the housekeeping and the arrangement of the place.

Table 1. Distance of Nurseries and It's Cleanliness Status

No.	Plant Nursery	Distance from nearest housing area (m)	Cleanliness
1	Plant Nursery 1	32	Good
2	Plant Nursery 2	22	Good
3	Plant Nursery 3	29	Good
4	Plant Nursery 4	45	Poor
5	Plant Nursery 5	118	Good
6	Plant Nursery 6	29	Good
7	Plant Nursery 7	44	Poor
8	Plant Nursery 8	20	Poor
9	Plant Nursery 9	69	Good

Based on the observation during the survey, the cleanliness of the plant nursery observed was ranging from poor to good. The cleanliness of the plant nursery observation were based on the housekeeping and the arrangement of the equipment respectively. Some of the plant nursery had a poor housekeeping, hence it might encourage the favourable environment for the *Aedes* mosquito to rest and breed. According to study by Rahman (2021), containers located in the outdoor of vegetation area has a higher chance for harbouring and producing larvae. This is because this environment may enhance the mosquito breeding by enabling a resting site for the female mosquitoes to lay eggs and provide sugar feeding resource for larvae from the flora within their flight range.

3.2. Determination of potential *Aedes* breeding sites at plant nurseries

Table 2 shows the potential breeding site accordingly to the different types of container, artificial or natural in the respective nurseries. The data tabulated include type of container, number of potential container and container with positive breeding. The type of container was divided into two types which are natural container such as bromeliads and artificial container such as flowerpot, flowerpot plate, tires, canvas sheet and others.

Based on the result, the natural container, the bromeliads plant could only be found in two of the nurseries in the study area. All nurseries inspected were found to have a lot of artificial container and favour as mosquito potential breeding sites. Among the mostly found artificial container include flowerpot, flowerpot plate, tires, canvas sheet, bucket, plastic barrel, water tank, watering can and plastic bottles.

The inspection also provides the evidence of potential artificial container was capable in bearing the rainwater collection hence becoming the most favourable place for *Aedes* breeding site. There were also unused flowerpots in the nurseries spotted almost everywhere, which was left unattended becoming a potential breeding site of *Aedes* mosquito as it was left untouched. On top of that, flowerpot plates were also found to be a *Aedes* larvae bearing container in the nurseries. Since the plate was placed to capture excess water from the flowerpot, its capacity in holding the water has become major issues for *Aedes* breeding since much effort is needed to attend all the flowerpot plates cleaning and close observation in nursery so as to keep such potential *Aedes* breeding place under control. Other than that, a total of 9 unused tires also could be found at some of the plant nurseries and some of the tires contain stagnant water in it. However, there were no breeding found in those tires.

Other artificial container such as bucket were commonly found at the plant nurseries. Most of the bucket were used to keep the water for planting or being placed unused. Besides that, other container such as watering can also be a potential breeding sites for the mosquitoes.

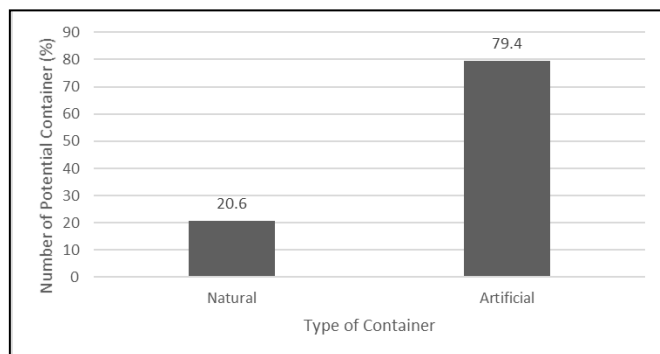


Figure 1. The percentage of potential artificial and natural container

Based on the data obtain, artificial container has a higher potential as an *Aedes* breeding site since it contains about 79.4% while there is 20.6% of natural container that could be a potential for a breeding site at the plant nursery (Figure 1). Based on the observation at the plant nursery, the natural container that can act as a potential breeding site for the *Aedes* are the bromeliads plant.

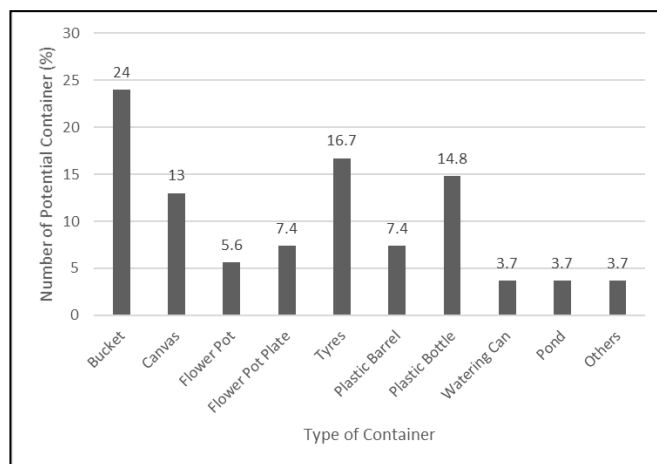


Figure 2. The percentage of potential artificial container at plant nursery

Table 2. Potential breeding sites at plant nurseries

Nursery	Type of Container												No. of Potential Container	No. of Container with Positive Breeding
	Natural Container		Artificial Container											
	Bromeliads Plant	Others	Flower pot	Flower pot plate	Tires	Canvas	Bucket	Plastic Barrel	Plastic bottle	Watering Can	Pond	Others		
1	10	-	-	-	1	5	-	3	-	-	2	1	22	-
2	-	-	-	-	-	1	-	-	-	-	-	-	1	-
3	4	-	2	-	5	-	-	-	-	-	-	-	11	-
4	-	-	-	3	-	-	3	-	-	-	-	-	6	-
5	-	-	-	-	-	-	3	-	-	-	-	1	4	-
6	-	-	-	-	-	-	2	1	-	-	-	-	3	1
7	-	-	-	1	-	-	-	-	-	2	-	-	3	-
8	-	-	1	-	2	1	3	-	8	-	-	-	15	-
9	-	-	-	-	1	-	2	-	-	-	-	-	3	-

From the result obtain for the container survey (Figure 2), bucket showed the highest percentage for artificial container that may acting as a potential breeding site with 24% out of all. This is because most of the plant nurseries observed will have bucket in their premise. This bucket usually was used as a watering media for the plant at the premise. Improper storage of the bucket could lead to higher chance to be a breeding site for the *Aedes*. During the inspection, it was noted clearly that this type of container was proven physically as *Aedes* breeding place as several of them were positive container with *Aedes albopictus* larvae found. Tires were found to be the second highest as possible breeding places for *Aedes* in this study with the percentage of 16.7% out of all containers found.

Even though there were no positive breeding for this type of container in plant nursery, study by Lenhart (2006) has recommend being more aware on this type of container since it can produce up to 70% of *Aedes* pupae especially during the rainy season. Besides that, study by Gautam (2012) has proved that discarded tires can be a high-risk container since it also can be a breeding site for *Aedes* during pre-monsoon and post monsoon season. Next, plastic bottle show the percentage of 14.8% to be accommodate in the plant nursery. The high percentage of plastic bottle in the plant nursery are due to the half-cut plastic bottles use at the legs of the shelves (Figure 3). This is a method used by the nursery owner to prevent the shelf leg from sinking into the muddy grown. Besides that, improper disposal of plastic bottle in the premise also contribute to the high percentage of plastic bottles.



Figure 3. Plastic bottle use in plant nursery to prevent the potting shelves sinking to into the soil.

There is 13% of the potential place for breeding are from the canvas used to cover the soil at the plant nursery. The water accommodate on the canvas are due to the rainwater. However, this situation might be differed during hot season since the water can be evaporated. Improper placement of flowerpot plate can lead to water stagnant in it. The owner should place the flowerpot plate at a closed area. Other than that, plastic barrel used in the plant nursery to keep water for the plant can be a potential container since the plastic barrel does not been closed properly. Lastly, container such as watering can, pond and other categories has the lowest percentage which are 3.7%. However, there is low possibility for the *Aedes* mosquitoes to breed if the container is regularly clean and practice a proper storage for the container to avoid any possibility for the *Aedes* mosquitoes to breed.

3.2. Calculation of Epidemiological Indexes by Container Classification

During the larval survey, one of the containers which is bucket had been identified as a positive container that contain mosquito larvae. The larvae were identified as *Aedes* species as it has 2 lateral spines on each side of the thorax and a straight row of 7 to 12 comb scales at the 8th abdominal segment. The larvae were identified as *Aedes albopictus* since the comb scales exhibit a medical spine without subapical spines. In this study, the index of available container (IAC) for bucket and bromeliads plant has the highest value with 0.2 (Table 2). Even though bucket has highest value for IAC, the breeding preference ratio for bucket indicate that the probability for the *Aedes* to breed is low.

However, previous study related to container preference showed that bucket is known as the most frequent breeding sites. This is because the bucket has the capacity to contain considerable volume of water for the *Aedes* to breed (Nicolás Flaibani, 2020). Besides that, area of the opening surface for the container is large enough for the female mosquitoes to rest and breed on it while for bromeliads plant, even though it has the highest IAC, the probability for the mosquitoes to breed is low due to insufficient volume of water that contain in it. This can contribute to larval mortality due to lack of food, overcrowding and other sources of environmental stress (Shultis, 2009).

Other than that, tires also can contribute to increase of *Aedes* population. This statement can be proved by the previous study by Dila (2020) and Rahman (2021) where found that unused tires are among the highest for the contribution of *Aedes* breeding sites. This is because water that stored in used tires are protected from the sunlight which make it more suitable places for the *Aedes* to breed since the female *Aedes* mosquitoes preferred to rest in less lighting area or shaded area (Dejene et al.,2015). Next, container such as plastic bottles found in the plant nursery also has a high IAC with 0.12. However, in this study there is no positive breeding found for this type of container. This statement can be proved by previous study where the bottles were found to be the most frequent potential container even, so it was least found to have positive breeding as the small opening surface of the bottles lead to less detectable containers (Nicolás Flaibani, 2020).

Therefore, it can be concluded that some container may become a potential breeding site in plant nursery especially artificial container such as bucket, tires, plastic barrel, and watering can. This situation could lead to risk increment of dengue cases since it can increase the population of dengue vector in the area. Based on the location of the plant nursery being observed, most of the plant nurseries were located about 20 to 120 meters from the nearest residential area. According to who, *Aedes* mosquito could fly up to 400 meters to look for any water-filled containers to lay their eggs. This situation could be the cause for any dengue cases

in the area. So, in order to strength the preventive and control of dengue, identification of any container that contain water is important to help reducing the population of the *Aedes* in the area. Besides that, giving awareness and education regarding the type of container that can act as a breeding site for *Aedes* to the plant nursery owner is important for them to be more aware thus help to reduce the amount of potential breeding site in the plant nursery.

Table 2. Index of available container by container classification

Characteristic	Type of container	Total number of potential containers	Total number of breeding sites	IAC
Natural container	Bromeliad's plant	14	-	0.2
Artificial container	Bucket	13	1	0.2
	Canvas	7	-	0.1
	Flowerpot	3	-	0.04
	Flowerpot plate	4	-	0.06
	Tires	9	-	0.13
	Plastic barrel	4	-	0.06
	Plastic bottle	8	-	0.12
	Watering can	2	-	0.03
	Pond	2	-	0.03
	Others	2	-	0.03

4. CONCLUSION

This study helps to identify any natural and artificial container that has a potential to act as a breeding site for *Aedes*. Some of the container that can be found in the plant nursery include bucket, discarded tires, plastic barrel, flowerpot plate, flowerpot, watering can, pond, plastic tray and water tank. Artificial container are found in the plant nursery has a potential to be a contributor for the increasing of *Aedes* population. Most of the potential container detected are due to the rainwater or container that used to keep water for the used in plant nursery which is the best condition for *Aedes* to breed. Besides that, the relationship between the type of container and positive breeding site was affirmed in which they are most likely to breed in the container that has large surface area such as bucket and shaded area such as tires and bucket. The limitation for this study is the target population for the study which is the plant nursery is low since it only covers the plant nursery in the study area which is Klang. Besides that, the limitation on the time frame for the study to be conduct. This study is conducted during rainy season since previous study shows that the population of *Aedes* larvae are the highest. However, this study shows an opposite result where not much positive breeding site could

found in the plant nursery during rainy season. Further study can be conducted by including the season and larger scale of sample size to have a better observation and result regarding the topic.

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