FIELD EVALUATION OF SELECTED COMMON HOUSEHOLD CONTAINERS

AS OVIPOSITION ATTRACTANTS OF AEDES ALBOPICTUS (DIPTERA:

CULICIDAE)

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ABSTRACT

Dengue is a fatal arthropod-borne disease that affects humans worldwide. The

mosquito Aedes albopictus (Skuse) is the secondary vector of dengue in Sri Lanka, however,

studies on oviposition preferences of Ae. albopictus is scarce. The objective of the current

study was to investigate the oviposition attraction of Aedes albopictus to selected household

containers; black colour basins, metal cans, rain gutter parts, curd pots, coconut shells and

yoghurt cups. For this, water containers for oviposition were placed in three outdoor shady

sites and at three different heights. The mosquito larvae were collected after 5 days. The

larvae were reared to adult stage and then they were identified and enumerated. Wing lengths

of adult female mosquitoes that developed in different containers were measured. In the

meantime, temperature, pH, dissolved oxygen (DO) and total dissolved solids (TDS) were

measured in each container. The Ae. albopictus larval density was higher in coconut shells.

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The maximum mean number of mosquito larvae was observed in the containers at ground level. The oviposition attraction of *Ae. albopictus* was increased with the aging of coconut shells and old coconut shells were preferred than new coconut shells. Highest TDS level and neutral pH were observed in coconut shells which support the mosquito oviposition. The highest wing lengths were observed in female *Ae. albopictus* that developed in curd pots, representing higher fecundity. In conclusion, discarded coconut shells and curd pots should be carefully managed as means of eliminating dengue vector mosquito breeding sites.

Keywords: Aedes albopictus, oviposition attraction, mosquito density, wing length, fecundity

INTRODUCTION

Dengue is a fatal arthropod-borne disease dispersed in tropical, subtropical and temperate areas worldwide. Dengue control efforts in the country are targeted at laboratory surveillance for dengue virus (DENV) infections in patients as well as controlling the mosquito vectors (Sirisena and Noordeen, 2014, Tissera *et al.*, 2016). The mosquito *Aedes albopictus* (Skuse) is the secondary vector of DENV that is commonly found in both urban and non-urban areas (Lambrechts *et al.*, 2010; Ferreira-De-Lima and Lima-Camara, 2018).

The species *Ae. albopictus* is an outdoor resting species having outdoor breeding habitats (Rozilawati *et al.*, 2015; Nordin *et al.*, 2017; Haziqah-Rashid *et al.*, 2019). Moreover, it is majorly a container-breeding mosquito species (Thavara *et al.*, 2004; Weerasinghe, 2005; Kraemer *et al.*, 2015, Becker *et al.*, 2017; Abílio *et al.*, 2018) that prefers clean water in both natural and artificial containers. The natural breeding containers are usually water-filled fruit shells, leaf axils and tree holes (Alto and Juliyano, 2001; Lounibos *et al.*, 2001; Snow and Ramsdale, 2002; Vijayakumar *et al.*, 2014; Abílio *et al.*, 2018). The artificial containers are mainly tyres, metal cans, plastic bottles, flower vases, buckets, plastic drink cups, cans, rain gutters, ornamental ponds or bird baths.

Oviposition site selection is one of the important behaviours of mosquitoes. Female mosquitoes seeking ovipositional sites with minimum exposure to predators ensures the survival of offspring (Spencer et al., 2002; Kiflawi et al., 2003; Blaustein et al., 2004). This behaviour is dependent on several physical, chemical and biological factors (Yanoviak, 2001; Li et al., 2009; Sirisena and Noordeen, 2014; Alcalay et al., 2019). However, these factors do not play independently, instead, they interact with each other (Bentley and Day, 1989; Afify and Galizia, 2015). Rainfall, relative humidity, temperature and wind speed are physical factors that influence the ovipositional site selection in mosquitoes (Bentley and Day, 1989; Pinto et al., 2011; Tran et al., 2013). In addition, it has been reported that material of the container is one of the important physical parameters of oviposition site selection (Snow & Ramsdale, 2002; Thavara et al., 2004; Vijayakumar et al., 2014; Swan et al., 2018). Moreover, nutrient content is also a chemical parameter dependent on the type of container. The nutrient content in a water-filled breeding container depends on water spilling which often leads to removal of nutrients or resource depletion (Dieng et al., 2002). This resource depletion and overcrowding are critical factors of survival for container breeding mosquitoes. Such stress factors result in altered body size and wing length in mosquitoes (Blackmore and Lord, 2000; Armbruster and Hutchinson, 2002; Ciocchetta et al., 2017).

The oviposition site selection by female mosquitoes is usually dependent on high nutrient levels in the sites. Some species prefer sites with organically rich, polluted water while others prefer clean water with optimum organic matter content (Foot, 1970; Urbinatti, 2001). The chemical composition of breeding water also significantly affects the oviposition of mosquitoes (Afify and Galizia, 2015; Shaalan and Canyon, 2018). For instance, the oviposition site selection of *Ae. albopictus* is influenced by their larval holding water (Thavara *et al.*, 2004). The chemical substance that is produced by the larval stages in a container is a stimulant to the gravid females to attract to the ovipositional site (Tilak *et al.*,

2004). Furthermore, it has been shown that chemical exudates of fish in water bodies also determine the mosquito oviposition site preference (Van Dam and Walton, 2008).

The present study investigated the oviposition preference of *Ae. albopictus* to different types of containers (colour, nature of the surface, aperture size, and volume). The physico-chemical parameters of ovipositor containers, age and height from the ground level were also examined towards the oviposition preference. Interestingly, the impact of type of container on the fecundity of mosquitoes was tested through comparison of mosquito wing lengths.

METHODOLOGY

Study sites

The experiments were carried out in the premises of the University of Kelaniya, Sri Lanka. Three outdoor shady sites were selected in the University premises as Site A (6° 58′ 20.91″ N; 79° 54′ 52.83″ E), Site B (6° 58′ 21.72″N; 79° 54′ 53.27″) and Site C (6° 58′ 22.71″ N; 79° 54′ 52.84″E) (Garmin etrex®). Furthermore, three available heights including 0 m (ground level), 6 m and 16 m were selected in the premises to test the effect of height from ground level to the mosquito oviposition.

Selected containers

The current experiment was carried out with six selected containers that are commonly discarded by people. Those containers were black plastic basins, curd pots (made of clay), metal cans, parts of rain gutters, coconut shells and yoghurt cups. Each container type had different nature of surface, colour, aperture size (size of the opening) and volume (Table 1). Since the plastic basins, curd pots, metal cans and yoghurt cups had their own uniform sizes, a set of equal sized coconut shells was utilized. The parts of rain gutters were prepared with 25 cm long PVC gutter parts. Both ends were sealed by two PVC end caps. The end caps were fitted to both sides and fixed with S-lon glue (S-lon Lanka (Pvt) Ltd).

These gutter parts were air dried for one day and soaked in water for one week to remove chemical odour of the glue.

Experimental design and data collection

The experimental design was a randomized complete block. A set of 60 containers with 6 different container types (10 of each) were kept on the floor (0 m level) in the preselected locations mentioned above. Each container was filled with aerated tap water up to 3.5 cm height (the lowest container height of yoghurt cups was 5 cm). These water-filled containers were allowed for mosquito oviposition.

Table 1: Physical parameters of selected ovipositor containers

Container	Nature of the surface	Colour	(circumference of the opening) (cm)	Water volume (L) at 3.5 cm height
Plastic basin	Smooth	Black	74	0.92
Curd pot	Rough	Brown	50	0.40
Coconut shell	Rough	Brown	30	0.14
Metal can	Smooth	Silver	25	0.15
Rain gutter part	Smooth	Grey	66	0.68
Yoghurt cup	Smooth	White	24	0.01

Another three sets of the same six different types of containers (60 from each) were placed at three different heights; 0 m, 6 m and 16 m. The containers were randomly arranged in each location. In agreement with the results of a preliminary survey, the first instar larvae were observed after 2–3 days. The larval duration was ranging from 3–4 days under 28-30 °C temperature. Therefore, larval count (fourth instar) was taken on the sixth day after setting up the experiment.

During the study period, physico-chemical parameters in each container were recorded between 7.30 - 8.00 am every day. Dissolved oxygen (DO), temperature (0 C), pH

and total dissolved solids (TDS) were measured using DO meter (HI 9813-5), thermometer, pH meter (pH 315i) and TDS meter (Cond 340i), respectively.

Randomly selected containers (5 of each type) were kept covering with a small sized (1 mm) net until the emergence of mosquito adults. The adults were observed under low power stereo microscope (Meiji EMZ 5 Microscope, Meiji Techno Corporation) for identification to the species level. Moreover, larvae from other samples were observed under a compound microscope (Olympus CX21 Microscope, Olympus America Inc.). Both the adults and larvae were identified using standard larval identification keys (Rueda, 2004).

Preliminary observations indicated that there is an effect of the age of the coconut shell to the oviposition of *Ae. albopictus* mosquitoes. To estimate this, a set of 120 new coconut shells (within 20 days of scraping) and 120 old coconut shells (nine months after scraping) were randomly placed in the study sites. They were filled with aerated tap water and allowed to lay eggs. The number of mosquito larvae in each container was counted after 5 days. The larvae were reared until adult stage in the same container by covering the container with a small net. This experiment was repeated 5 times.

The water level in all the containers were kept constant by adding water during the experiments. The right wing of each reared adult female was removed, and wing length was measured to the nearest 0.01 mm, from the axial incision to the apical end of the wing using a compound microscope (Schneider *et al.*, 2004).

Data Analysis

Data analysis was performed with MINITAB 14.0 and PRIMER 5 statistical software packages. Mosquito larval density (larvae per 1 L volume) in each container was calculated. Mosquito densities of different containers were tested with one-way ANOVA. Furthermore, two-way ANOVA was carried out to investigate the effect of height from the ground level and the type of container. Log X+1 data transformation was followed prior to analysis.

Principal component analysis (PCA) was carried out to determine the effect of physicochemical parameters of containers as oviposition attractants. The mean wing lengths of adult female mosquitoes (reared in different containers) were compared with one-way ANOVA. The mosquito densities in new and old coconut shells were compared with the t-test. Furthermore, the wing lengths of adult female mosquitoes developed in new and old coconut shells were compared with the t-test.

RESULTS AND DISCUSSION

Out of the total 4709 of collected mosquito larvae, 93.8% (n = 4417) were *Ae. albopictus* and the remains were *Ae. aegypti*. Therefore, *Ae. albopictus* data were taken to the present analysis. *Ae. aegypti* is responsible for dengue epidemics in Sri Lanka, but its secondary vector, *Ae. albopictus* is abundant in most of the areas in the country, maintaining the dengue virus in the environment (Sirisena and Noordeen, 2014). Additionally, *Ae. albopictus* is the major vector of chikungunya transmission in Sri Lanka (Weeraratne *et al.*, 2013).

The present results demonstrate that the mosquito larval densities in the containers were significantly different (One-way ANOVA, $F_{5, 1619} = 56.77$, P < 0.05) (Figure 1). The current experiment was also conducted under the natural conditions to find the oviposition attraction to different types of containers that are commonly discarded by humans. These containers had different physical parameters such as aperture size, volume, colour and texture (Table 1). Therefore, the number of larvae per unit volume (volume density) was calculated for each container type. According to the results, peak mosquito larval density was observed in coconut shells (Figure 1).

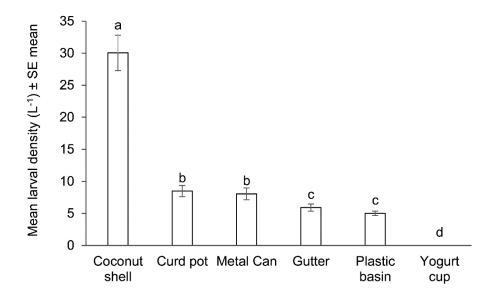


Figure 1: *Aedes albopictus* Larval density in different types of water containers (n=60). The mean number of mosquito larvae was determined per 1 Liter volume. Data are expressed as mean larval density \pm standard error (SE). Columns with the similar letters are not significantly different from each other (P > 0.05)

The PCA revealed that there was a collective influence of different physico-chemical parameters to the mosquito oviposition in different containers. The variables were DO, TDS, pH and temperature. Eigenvalues, eigenvectors and principal component scores are indicated in Table 2. The two-dimensional plot shows the position of different containers according to their PCA scores (Figure 2). According to the PCA, it is evident that the TDS level is the significantly higher factor that influences the mosquito oviposition in coconut shells (Figure 2 and Table 2). This may be due to the material of the coconut shell and according to the observations, small shell particles were loosed from the coconut shell wall and they were added into the water. This process induces the TDS level in coconut shells to higher levels. Consequently, this organically enriched medium acts as a stimulant for oviposition of *Ae. albopictus*. Considering these factors, coconut shells have a significant effect on the high oviposition rate of these mosquitoes. Day (2016), stated that the organically enriched containers were suitable oviposition substrates for *Culex* mosquito oviposition (Day, 2016).

A similar relationship between organic matter in the water containers and oviposition attraction of *Ae. aegypti* and *Ae. albopictus* was reported by Chua *et al.* (2004) and Allgood and Yee (2017).

The results of the current study showed that the dissolved oxygen level (mg/L) significantly differed with the container type. Plastic basins and curd pots showed significantly higher dissolved oxygen level whereas other containers exhibited lower levels (One-way ANOVA, $F_{5,594}$ = 15.26, P < 0.05). The mean dissolved oxygen values in each type of containers are given in Table 3.

The temperature values in the current study were in the range of 28-30 °C. However, there was no significant difference of temperature across the type of container (P > 0.05). According to Akram and Lee (2004), *Ae. albopictus* mosquito population sizes reach their maximum level during May, where the temperature increases up to 27 °C. Therefore, environmental conditions during the study period were in better level for *Ae. albopictus* species. The least pH was observed in coconut shells (7.64 \pm 0.05) which is closer to neutral pH level (Table 3). The present study suggests that favourable pH for *Aedes* larvae was closer to neutral pH level (7.54 \pm 0.04). Similarly, Adebote *et al.*, (2008) and Duchet *et al.*, (2019) have concluded that neutral pH values are optimum for mosquito oviposition media.

The current study revealed that there was a correlation between the container type and the wing length of female mosquitoes (P < 0.05). The average wing lengths of female mosquitoes developed in different containers are shown in Figure 3. Wing lengths of female mosquitoes were significantly different across the type of container (One-way ANOVA, F $_{5,144} = 1272.32$, P < 0.05). According to the results, the highest wing lengths were observed in female mosquitoes developed in curd pots (3.02 \pm 0.27 mm) and the lowest wing lengths were observed in the mosquitoes developed in black basins.

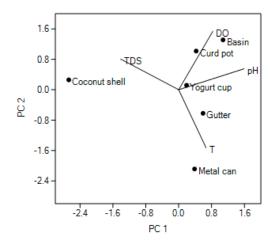


Figure 2: Two-dimensional representation of Principal Component Analysis indicating the effect of different physico-chemical parameters to the oviposition in different container types. The abbreviations are as follows, dissolved oxygen (DO), temperature (T), total dissolved solids (TDS).

Table 2: Eigenvalues, eigenvectors and scores of the Principal Component Analysis (PCA)

	PC 1	PC 2	PC 3
Summary			
Eigenvalue	1.81	1.51	0.46
% variance	45.15	37.90	11.50
Cumulative variance	45.15	83.05	94.55
PC Loadings			
Dissolved oxygen (mg/L)	0.349	0.647	0.433
Total dissolved solid (mg/L)	-0.593	0.339	0.550
рН	0.669	0.232	-0.037
T (°C)	0.280	-0.642	0.713

At the first gonotrophic cycle of the female mosquitoes, the wing lengths have been shown to have a positive relationship with their fecundity (Blackmore and Lord, 2000; Ciocchetta *et al.*, 2017). The fecundity of female *Ae. albopictus* strongly depends on the blood meal taken prior to egg development. Adequate blood feeding is so important to female mosquitoes to allocate more nutrients and energy for their oogenesis (Blackmore and Lord,

Table 3. Water quality parameters in different containers (TDS – total dissolved solid, DO – dissolved oxygen, T- temperature and pH)

	Total dissolved solid	Dissolved oxygen	T (°C)	
Container	TDS (mg/L)	DO (mg/L)		pН
Plastic basin	140.3 ± 4.28	7.35 ± 0.06	28.8 ± 0.4	7.64 ± 0.05
Curd pot	116.1 ± 2.24	7.22 ± 0.06	28.0 ± 0.6	8.00 ± 0.04
Rain gutter part	120.4 ± 2.64	6.99 ± 0.04	28.9 ± 0.4	7.93 ± 0.04
Yogurt cup	125.6 ± 2.32	6.98 ± 0.03	28.3 ± 0.4	7.94 ± 0.04
Coconut shell	176.7 ± 6.47	6.96 ± 0.04	28.2 ± 0.4	7.87 ± 0.04
Metal can	109.5 ± 3.55	6.92 ± 0.04	29.1 ± 0.9	7.80 ± 0.04

2000). For the current study, wing lengths of the adult female mosquitoes were taken just after emergence but without any blood feeding. Therefore, these adult female mosquitoes directly reflect the nutrient content at their ovum and larval stages. The environmental factors such as temperature and chemical composition of water may influence the embryo development which in turn directly affects the wing length at adult stage. The relationship between the container type and wing length of *Ae. aegypti* mosquitoes was prominent where the wing lengths of female mosquitoes were decreased significantly in unmanaged containers (water containers without manual water filling) and containers with high larval densities (Yoshioka *et al.*, 2012; Wong *et al.*, 2012). According to Wong *et al.* (2012), the wing lengths of female mosquitoes ranged from 1.85- 3.23 mm (median = 2.51 mm). In the current study, the containers were manually filled with water to provide a better breeding habitat for the mosquitoes. It was revealed that maximum wing length was recorded in the female mosquitoes developed in curd pots (Figure 3). A possible reason for this observation may be the presence of larger constant water volumes in curd pots with minimum larval

density. Limited larval density reduces the intra-species competitions enhancing the fecundity of female *Ae. albopictus* mosquitoes. These results further support the observations reported by Wong *et al.* (2012).

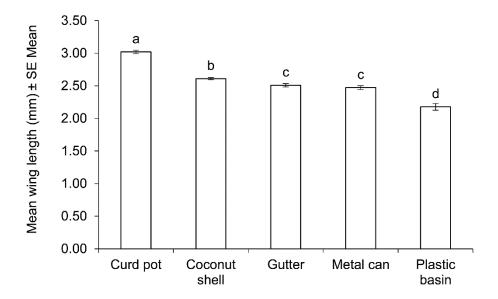


Figure 3. The variation of mosquito wing length to type of container. The mean wing lengths of female *Aedes albopictus* mosquitoes developed in different types of containers were determined. Data are expressed as mean wing length \pm standard error (SE). Columns with the similar letters are not significantly different from each other (P > 0.05).

According to the preliminary results, there was a significantly different relationship between age and the number of larvae in coconut shells (One-way ANOVA, $F_{8,\,261}=10.27$, P<0.05). The number of mosquito larvae were increased in aged coconut shells. The lowest mosquito larval numbers were observed in new coconut shells (after scraping) and these numbers gradually increased with time. This observation was tested with another experiment which revealed that higher numbers of mosquito larvae were observed in old coconut shells (nine months after scraping) than newer ones (within 20 days of scraping) (t-test, P<0.05). Furthermore, as shown in table 4, the wing lengths of female mosquitoes reared in new coconut shells were higher than that in the old coconut shells (t-test, P<0.05).

The new coconut shells had an oily texture on the water column after they were filled with water, however, this layer was not observed in old coconut shells. This layer could be a barrier to the mosquito oviposition and the development of mosquito larvae. In addition, the mean wing lengths of female mosquitoes developed in new coconut shells were significantly higher (t-test, P < 0.05) (Table 3). The low number of larvae in new coconut shells support to maintain low intra–specific competition resulting in female mosquitoes with larger wing lengths. Opposingly, the higher intra–specific competition in old coconut shells owing to higher mosquito larval density may have resulted the reduced wing length of female mosquitoes.

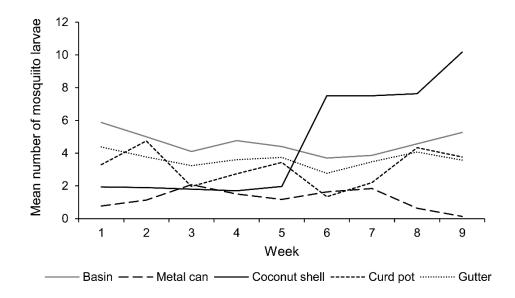


Figure 4. The variation of *Aedes albopictus* larvae in different containers during the study period. The mean numbers of *Ae. albopictus* mosquito larvae were determined in basins, metal cans, coconut shells, curd pots and gutters during the nine weeks of the study period.

The mean number of larvae in different heights are given in Figure 5 (main graph) and the percentage number of mosquito larvae in different containers in each height are shown in Figure 5 A, B and C. The number of *Ae. albopictus* mosquito larvae found at

ground level was significantly higher compared to 6 m and 16 m height from the ground level. The lowest larval numbers were recorded at 16 m (Turkey Pair-wise test). The results indicated that the type of the container and the height from the ground level had a significant effect on *Ae. albopictus* mosquito oviposition (Two-way ANOVA, container type: $F_{5, 449}$ = 83.85, P < 0.05, height: $F_{2, 449}$ = 138.20, P < 0.05, container type X height: $F_{10, 449}$ = 16.16, P < 0.05). Similar results have been reported in previous studies that female mosquitoes prefer ground level or closer to ground level for oviposition as well as feeding (Derraik *et al.*, 2008; Obenauer *et al.*, 2009; Johnston *et al.*, 2014).

These results suggest that water collecting places at roof level (i.e. gutters, pipes, etc.) are favourable places for *Ae. albopictus* oviposition (Figure 5; A, B and C). Contribution of gutters (10.7 %) to the oviposition of *Ae. albopictus* is substantial under these conditions (Figure 5 B). Due to favourable physical and chemical nature, coconut shells obtain maximum oviposition attraction at all heights tested (Figure 5; A, B and C). According to the observations, the high wind speed at 16 m height was a disturbing factor to these mosquitoes. Therefore, it can be suggested that in local dengue controlling programmes, more attention should be given to the breeding habitats at ground level as well as at roof level.

Table 4. The *Aedes albopictus* larvae numbers and wing lengths of female mosquitoes in different coconut shell types (New – within 20 days of scraping, Old – Nine months after scraping)

Coconut Shell	Mean number of mosquito larvae	Mean wing length (mm)	
Type	± SE	± SE	
New	0.17 ±0.06	2.79 ±0.016	
Old	12.0 ± 1.01	2.48 ± 0.011	

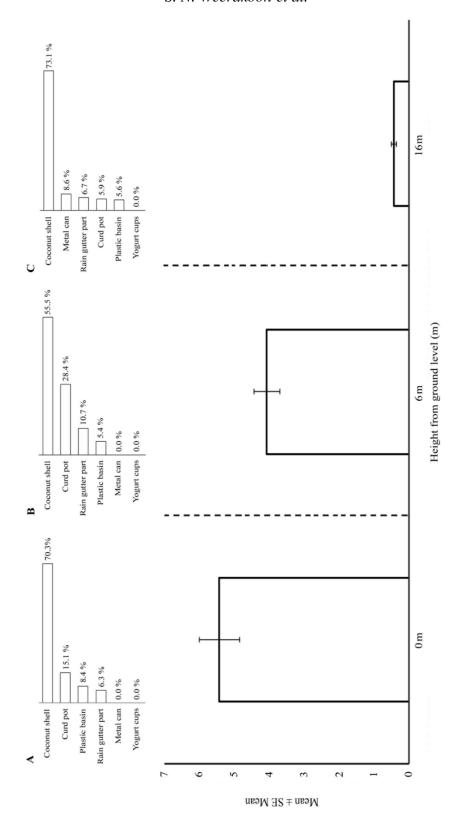


Figure 5. Mean number of *Ae. albopictus* larvae \pm standard error (SE) in 3 different heights (Main graph) with mosquito larval percentages in different containers at each height (A – at 0 m height, B – at 6 m height and C – at 16 m height).

CONCLUSIONS

The current study revealed that oviposition preference of *Ae. albopictus* varies with the type of container. Water-filled coconut shells provide a better oviposition substrate releasing significantly higher numbers of mosquitoes to the environment. Furthermore, the older the age of coconut shells higher the oviposition preference of *Ae. albopictus*. Discarded coconut shells should be highly concerned in the terms of controlling dengue vector mosquitoes. Mosquitoes developed in curd pots showed higher fecundity due to low intra–specific competition. Besides, these mosquitoes seek the breeding habitats at ground level than higher levels.

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