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Oviposition and vertical dispersal of *Aedes* mosquitoes in multiple storey buildings in Colombo district, Sri Lanka

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ABSTRACT

Background & objectives: The Colombo City in Sri Lanka is experiencing tremendous development and construction of multiple storey buildings and high rise apartments. The change in housing types and microhabitats might have altered the flight and breeding behaviour of *Aedes* mosquito population. This study was carried out to determine the vertical dispersal and abundance of *Aedes* mosquitoes in multiple storey buildings in the Colombo district, with respect to abiotic factors such as rainfall, humidity and wind speed. Hence, this study is of paramount importance, particularly for planning and implementation of control measures against *Aedes* mosquitoes.

Methods: An ovitrap based study was carried out at four selected multiple storey buildings in four residential areas located in Colombo, Sri Lanka, from August to December 2013. Results were analyzed using four indices; ovitrap index, mean number of larvae, mean number of eggs and mean number of larvae per ovipaddle.

Results: The results implied that *Aedes* mosquitoes could be found in different elevations from ground floor to the highest floor (130 ft). There was a significant difference between height and ovitrap index ($p < 0.05$), and height and mean number of larvae per recovered ovipaddle ($p < 0.05$). The highest index value for mean number of eggs was observed as 3.492 ± 0.655 at the 6th floor (60 ft high from ground level). At the same height (60 ft height) other indices (ovitrap index, mean number of larvae and mean number of larvae per ovipaddle) also displayed higher values, i.e. $13.19 \pm 2.98\%$, 1.366 ± 0.527 , and $2.070 \pm 0.421\%$, respectively. Abiotic factors such as wind speed, coastal nature, etc. displayed a significant effect to the vertical dispersal of *Aedes* mosquitoes ($p < 0.05$).

Interpretation & conclusion: The study suggested that *Aedes* mosquitoes are able to breed at any level of the buildings and not restricted by their height. The indices (mean number of larvae, mean number of eggs) representing the vertical dispersal with respect to abundance seemed to be statistically non-significant ($p > 0.05$) with height which indicates high abundance of *Aedes* mosquitoes at higher floors. Abiotic factors also seemed to cause significant effect to the vertical dispersal of *Aedes* mosquitoes in high rise buildings.

Key words *Aedes* mosquitoes; Colombo district; dengue; multiple storey buildings; oviposition; Sri Lanka; vertical dispersal

INTRODUCTION

Dengue has become a major public-health problem throughout tropical and sub-tropical regions of the world and is the most rapidly spreading mosquito-borne viral disease, with a 30-fold increase in global incidence over the past 50 years¹. Hence, in 2012, dengue was ranked as the most important arboviral disease in the world². Dengue fever and the dengue haemorrhagic fever (DF/DHF) have become epidemic in Sri Lanka with progressively large and more frequent outbreaks in this millennium. The dramatic increase in the incidence of dengue and its severe manifestations has made this infectious disease a major public health problem even in Sri Lanka³⁻⁴.

The disease is caused by the dengue virus, which belongs to the genus *Flavivirus*, family Flaviviridae. There are four genetically related, but antigenically distinct, dengue virus serotypes designated as DEN-1, DEN-2,

DEN-3 and DEN-4 that form the dengue (DEN) antigenic complex, all of which can cause DF or DHF⁵.

Viruses are transmitted from viremic to susceptible human beings by various species of *Aedes* mosquitoes. Among them, subgenus *Stegomyia* contains the most important vectors of dengue viruses, namely, *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) (Diptera: Culicidae)⁵⁻⁷. These two species are the prevalent vectors in Sri Lanka, of which *Ae. aegypti* is the primary vector and has been considered to be responsible for the major epidemics that are reported in Sri Lanka while *Ae. albopictus* is the secondary vector of dengue⁸⁻⁹.

Since vaccines discovered to date, do not work effectively on all three clinical phases (febrile, critical and recovery) and as vaccine development is still under research, showing no immediate prospect or success, preventing dengue transmission through vector control seems to be the only effective remedy to control this deadly disease¹⁰. Control of dengue vectors has mainly

been approached by source reduction through the elimination of discarded containers that are favourable sites for oviposition and development of the immature stages². The necessity of a community based Integrated Vector Control Strategy is vehemently emphasized, since source reduction alone could not help to eradicate dengue¹¹⁻¹². Since, *Aedes* mosquito eradication is not the only goal, ongoing surveillance will be essential for the success of the new dengue control strategy.

Vector surveillance is the most effective early warning system for detecting a potential outbreak of dengue and, thus has a great demand¹³. Vector surveillance comprises of surveying of *Aedes* breeding sites (detection of the presence of vector) and vector abundance^{11, 14}. The surveillance, behaviour of *Aedes* mosquitoes is well studied using several indices⁵ comprising both adult and larval indices^{5, 11}. The most commonly applied *Stegomyia* indices include the premise/house index (percentage of houses infested with larvae and/or pupae), container index (percentage of water-holding containers infested with larvae and/or pupae), Breteau vector dynamics index (number of positive containers per 100 houses) or oviposition trap (ovitrap) data, all of which were intended to detect the presence or absence of *Ae. aegypti*¹¹. Out of these, ovitrap index (oviposition trap data) provides temporal and spatial information about *Aedes* larval densities proving that it would be a promising tool for studying the behaviour of *Aedes* mosquitoes¹⁵.

Knowledge about flight range and dispersal of mosquito vectors is essential for understanding vector-borne disease transmission dynamics among human populations, since flight range and dispersal influence mosquito population dynamics, patterns of gene and pathogen transfer through vector populations. In an applied sense, monitoring *Aedes* populations and dispersal are important factors for determining appropriate control limits necessary to interrupt pathogen transmission¹⁶⁻¹⁸. Several studies on *Aedes* mosquito distribution and surveillance have been conducted worldwide focusing mainly on the horizontal dispersal of the mosquito¹⁹. However, there is a paucity of information, especially on the adaptation of *Ae. aegypti* within and outdoors of houses at different elevations^{15, 19-21}.

Several environmental and anthropogenic changes such as housing types (design of the houses) including increase in the number of high rise apartments have placed mosquitoes on strange behavioural adaptations such as the vertical dispersal in such buildings. Since, dengue victims can be found in houses at ground level and in high rise buildings, and as researchers have failed to show a clear picture about the behaviour of *Aedes* on different

elevations, the necessity of studies on vertical dispersal of *Aedes* species has emerged.

Though, few research studies have been carried out on the vertical dispersal of *Aedes* mosquitoes in multiple storey buildings in other countries^{15, 19-22}, similar investigations have not been carried out in Sri Lanka, so far. Since, there is a huge increase in the number of high rise buildings with the rapid development taking place recently, it is important to survey the vertical dispersal of *Aedes* mosquitoes. This difference in housing types, in Sri Lanka might have altered the flight and breeding behaviour of *Aedes* mosquito population. Hence, this research study has become highly important.

The present study was conducted to determine the vertical dispersal and abundance of *Aedes* mosquitoes in multiple storey buildings in the Colombo district and the effect of the meteorological parameters (wind speed, rainfall, temperature and relative humidity) and coastal/non-coastal nature of the environment in the vertical dispersal of *Aedes* mosquito population with respect to the enclosed and open nature of the buildings.

MATERIAL & METHODS

Study sites

The study was implemented from 17 August to 10 November 2013 in four selected urban sites, located in four residential areas in the Colombo district. According to the Urban Development Authority (UDA), all selected study sites were defined to be urban sites. The study sites included one coastal site and three non-coastal sites (Table 1). Geographical position of each site was obtained from the geographical positioning system (GPS) (*etrex VISTA GARMIN*, 26549203, Taiwan). The geographical, physical and the ecological description of the sites are mentioned in Table 1.

Ovitrap surveillance

Continuous ovitrap surveillance was carried out once a week in all four study sites. "Oviposition trap (ovitrap)" as described in previous studies²³ was used in this ovitrap surveillance. Five ovitraps were placed randomly in each floor of the apartment from ground level to the highest level. Ovitrap were placed indoors along the corridors near stairways, open verandas, nearby ornamental plants, behind doors, near windows and inside rooms. Dechlorinated water which was used in every ovitrap was refreshed and the paddles were replaced weekly. Plastic cups were rubbed and washed thoroughly to remove mosquito eggs. A set of new cups was used as ovitraps every two weeks.

Larval identification

Larvae in the collected sample were examined using the binocular light microscope (MC30, Austria) under 10×40 magnification, and identified up to species level (*Ae. aegypti* or *Ae. albopictus* *etc.*) based on one (number and shape of comb teeth) of the three main characteristics; comb scales, pecten teeth and ventral brush²⁴. Paddles were individually packed into plastic bags and brought back to the laboratory. They were dried for at least 24 h and the eggs were counted with the use of a Stereo microscope (Leica MZ6, Germany) under 10×1.6 magnification. The ovitrap paddles were then submerged in water to facilitate hatching of larvae and were identified and counted again.

Meteorological parameters

Wind speed at each study site was measured using the digital Anemometer (AM-4201, Taiwan). Other meteorological data, such as daily rainfall, maximum and minimum temperature, and relative humidity, were obtained from the Colombo and Ratmalana Weather Stations of the Meteorology Department, Sri Lanka. The data

were collected within the same period of the larval collection, which started from the August to November 2013.

Larval indices

All the data obtained from the study were analyzed with four indices mentioned in the previous research of Lau *et al*²⁰.

Statistical analysis

Data analysis was carried out using the statistical software MINITAB 14; One-way ANOVA and Tukey’s test to compare indices with height, general linear model (GLM), for the analysis of abiotic factors with height and indices. For all statistical programmes mentioned, statistical significance was determined at $p \leq 0.05$.

RESULTS

Almost, all (99.99%) of the total observed larvae were *Ae. aegypti* with two observations from water-submerged ovipaddles, distinguished as *Ae. albopictus* larvae.

Table 2 shows the indices: Ovitrap index (OI), mean

Table 1. Geographical, physical and ecological description of study sites

Study site	Category	Geographical description	Physical description of buildings	Ecological description
1.	Non-coastal	Sri Jayewardenepura Kotte N 06°51'59.5" E 079°55'43.4"	13 floors, each having 3 m (10 ft) height.	Shrubs were planted in the garden. Vegetation around—High. Environment—Clean. Mosquito control—Done.
2.	Non-coastal	Sri Jayewardenepura Kotte N 06°53'10.6" E 079°53'10.8"	Six floors, each having 3 m (10 ft) height.	Scattered vegetation. Environment around—Not very clean. No mosquito control.
3.	Non-coastal	Attidiya-Dehiwala N 06°50'16.2" E 079°53'00.3"	Six floors, each having 3 m (10 ft) height.	Scattered vegetation. Environment around the building—Clean.
4.	Coastal	Colpity-Colombo N 06°55'11.3" E 079°50'52.6"	31 floors, each having 3 m (10 ft) height.	Nearly no vegetation around. Environment—Very clean. Mosquito control measures are highly concerned.

Table 2. Larval indices at seven height categories from 17 August to 10 November 2013

Height level	Height (ft)	Ovitrap index (%)	Mean number of larvae (Per ovitrap)	MED (Ovipaddle)	Mean number of larvae (Per ovipaddle)
1.	0–10	18.40 ± 3.40	1.761 ± 0.593	1.788 ± 0.400	1.309 ± 0.272
2.	11–20	23.88 ± 4.10	1.308 ± 0.252	3.580 ± 1.030	3.165 ± 0.705
3.	21–30	12.24 ± 3.13	1.179 ± 0.424	3.138 ± 0.887	1.345 ± 0.474
4.	31–40	8.85 ± 2.21	1.036 ± 0.483	1.757 ± 0.450	1.168 ± 0.328
5.	41–50	6.83 ± 2.07	0.714 ± 0.307	1.763 ± 0.787	1.712 ± 0.671
6.	51–60	13.19 ± 2.98	1.366 ± 0.527	3.492 ± 0.655	2.070 ± 0.421
7.	<60	4.87 ± 1.37	0.625 ± 0.272	1.378 ± 0.516	1.134 ± 0.361
One-way ANOVA	<i>p</i> -value	0.0*	0.536	0.104	0.041*

Results given for seven height categories (Height) in mean ± standard error of mean (SEM) and the statistical analysis carried out under the 95% CI (95% CI) ($p < 0.05$); by One-way ANOVA; *Significantly different under 95% CI ($p < 0.05$); MED—Mean egg density.

number of larvae (per ovitrap), mean egg density (MED) and the mean number of larvae-hatched (per ovipaddle) of *Ae. aegypti*, obtained from ovitrap surveillance conducted in four multiple storey buildings in Colombo and Attidiya. Mean values for each height category (height level) of the indices are shown comparing the four study sites.

Ovitrap were positive at all height levels up to the highest elevation (above 60 ft) with the highest OI of 23.88% observed in height level 2 (11–20 ft). OI showed a significant effect on height ($p < 0.05$) in all study sites (Fig. 1). According to the Tukey's test, OI at height level 1 was significantly different from height level No. 3, 4, 5 and 6 except level 2. At level 2, OI was significantly different from all other height levels except level 1.

The mean number of larvae per recovered ovitrap was the highest (1.761) at the first height level (0–10 ft) with successive reduction from lowest to the highest height level (Fig. 2). The height level had no significant effect towards the mean number of larvae ($p > 0.05$).

Height (increasing height) did not have any significant effect ($p > 0.05$) for mean egg density and the mean number of larvae hatched from submerged ovipaddles showed a significant effect on height ($p < 0.05$). The highest mean number of eggs per ovipaddle and the highest mean number of larvae per ovipaddle (the larvae emerged when the field-collected paddles were submerged in water) were 3.58 ± 1.03 and 3.165 ± 0.705 respectively and both observed at the second height category (11–20 ft). Second highest values for the above indices were recorded at height level 6 (51–60 ft) (Fig. 2). Height level 1 did not show any significant effect ($p > 0.05$) for mean number of

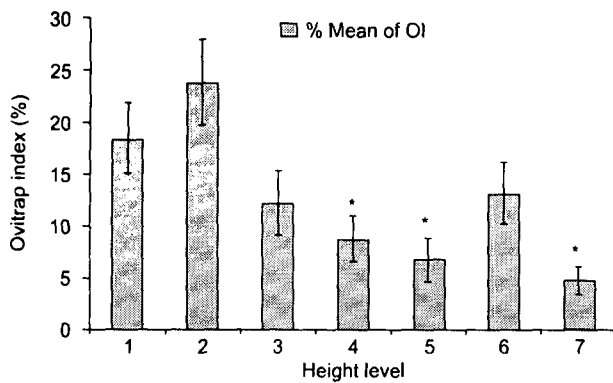


Fig. 1: Mean ovitrap index (OI) of all study sites to each height elevation from 17 August to 10 November; Height levels are: 1 (0–10 ft), 2 (11–20 ft), 3 (21–30 ft), 4 (31–40 ft), 5 (41–50 ft), 6 (51–60 ft) and 7 (> 60 ft). Mean values of OI at each height level are represented by the bars and the error bars are marked with standard error of mean (SEM); *Significant difference at 95% CI ($p < 0.05$).

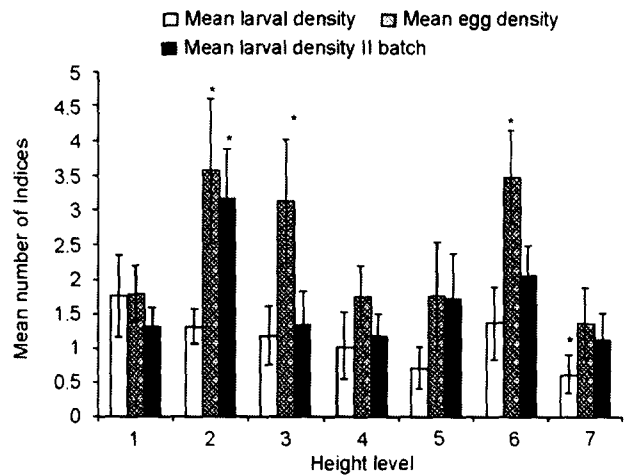


Fig. 2: Mean larval density, mean egg density and mean larval density II batch (Per recovered ovipaddle) from 17 August to 10 November with height levels; Results were given as Mean \pm SEM (SE of Mean). Height level 1 (0–10 ft), 2 (11–20 ft), 3 (21–30 ft), 4 (31–40 ft), 5 (41–50 ft), 6 (51–60 ft) and 7 (> 60 ft). Error bars are included with the SE; *Significant difference at 95% CI ($p < 0.05$).

larvae per recovered ovitrap to other levels except level 7, and the level 2 had no significant difference of the same index with all other height levels.

Correlation between larval indices and height

Applying Pearson's correlation coefficient, the relationship between larval indices and the height level was calculated and are shown in Table 3. As $p < 0.05$ there is no linear relationship between ovitrap index (OI) and height while Pearson's correlation ($r \pm < 0.5$ (negative), there was a weak negative relationship between OI and height. All other indices also showed no linear relationship ($r \pm < 0.5$) with height.

Effect of wind speed, coastal environment and environmental contact factor (ECF) towards the larval indices

Table 4 describes how the wind speed, height level, coastal nature (whether a particular site is coastal or not)

Table 3. Correlation between larval indices and height

Index	P-value	Linear relationship	Pearson's correlation
OI and height	0.0	Not linear	-0.224
Mean number of larvae and height	0.0960	Not linear	-0.087
Mean number of eggs and height	0.4640	Not linear	-0.039
Mean number of larvae hatched from paddles and height	0.368	Not linear	0.047

Results were analyzed by Pearson's correlation coefficient (r); Statistical software—MINITAB 14.

Table 4. Effect of wind speed, coastal environment and environmental contact factor towards the larval indices

Variable	Source	P-value
Ovitrap index (%)	Wind speed	0.022
	Height level	0.001
	ECF*	0.0
	Coastal/Non-coastal	0.0
	Rainfall	0.237
	Temperature	0.008
	Relative humidity (%)	0.07
Mean larval density	Wind speed	0.0
	Height level	0.7
	ECF*	0.007
	Coastal/Non-coastal	0.0
	Rainfall	0.007
	Temperature	0.591
	Relative humidity (%)	0.051
Mean egg density	Wind speed	0.036
	Height level	0.173
	ECF*	0.003
	Coastal/Non-coastal	0.0
	Rainfall	0.063
	Temperature	0.032
	Relative humidity (%)	0.007
Mean larval density (II batch)	Wind speed	0.102
	Height level	0.153
	ECF*	0.005
	Coastal/Non-coastal	0.0
	Rainfall	0.058
	Temperature	0.021
	Relative humidity (%)	0.031

*ECF—Environmental contact factor: Based on whether the indoor ovitraps were in contact with the outer/ambient environment; Statistical analysis: General linear model (GLM) under 95% CI.

and the environment contact of ovitraps, affected each larval index. Wind speed at each site revealed that the wind speed had a significant effect ($p < 0.05$) to the larval indices, *i.e.* the ovitrap index, mean number of larvae per ovitrap and the mean number of eggs in the multiple storey buildings; proving its impact towards the vertical dispersal of *Aedes* mosquitoes. Further, coastal nature and environmental contact with ovitraps (ECF) caused a significant effect towards all indices while rainfall showed no significant effect as evidenced from the Table 4.

Effect of wind speed towards Aedes dispersal (Larval indices)

For ovitrap index, mean number of larvae per recovered ovitrap and mean number of eggs per ovipaddle, there was a significant effect of wind speed since for these in-

dices, the p -value was < 0.05 , but for the mean number of larvae in second generation (hatched) there was no effect from the wind speed ($p > 0.05$).

Effect of coastal environment towards larval indices (Aedes dispersal)

P -values (mentioned in Table 4 as $p = 0.0$) obtained from the statistical analysis (General linear model), revealed that the coastal environment produced significant effect to all the indices with $p < 0.05$.

Effect of environmental contact (ECF) of ovitrap to larval indices

P -values for the indices; ovitrap index, mean number of larvae per recovered ovitrap, mean number of eggs per ovipaddle and mean number of larvae emerged from ovipaddles due to the ECF were 0.0, 0.007, 0.003 and 0.005 respectively ($p < 0.05$), confirming its effect on all the considered larval indices.

DISCUSSION

Almost all the counted larvae in study sites were *Ae. aegypti* (L.), reconfirming the efficient role of the major vector of the disease in urban settings²⁵. The presence, and the dominant nature of *Ae. aegypti* in all sites were expected observations, since all selected sites were urban sites (according to the classifications of the Urban Development Authority). *Ae. albopictus* was recorded in the second batch of larvae emerged from the eggs of the paddles which were submerged in water and no larva was recorded from the collected water samples of ovitraps themselves. Maximum height observed for *Ae. albopictus* was ≥ 11 ft (11–20 ft category) and hence the elevations closer to ground floor with scattered vegetation around seems to be suitable for *Ae. albopictus* to breed. In contrast to the finding of a previous research study²¹ which stated that both *Ae. aegypti* and *Ae. albopictus* were found even at the highest elevation (height) of their study sites [Vista Angkasa (VA) and Indera Loka (IL) apartments located in Kuala Lumpur, Malaysia], the current study revealed the presence of *Ae. albopictus* only at first two floors from the ground level.

Ae. aegypti was found at each and every floor level (Levels 1–7) of four multiple storey buildings, indicating that the species was able to breed at any level of the building and not restricted by the height of the building. According to the statistical analysis (one-way ANOVA), the indices showed significant difference especially at height levels 2, 3 and 6 with respect to the ground floor of the building and, it can be suggested that they might have

shown a canopy inhabitation as it was also revealed in previous research studies²⁶.

This was proven by statistical analysis in which the majority of indices (mean number of larvae, mean egg density and the mean number of larvae hatched from submerged ovipaddles) had no significant difference between the height and the index, except the ovitrap index which showed a significant effect on height ($p < 0.05$) in all study sites (Fig. 1). This finding agreed with the study done by previous researchers²¹. In contrast to the findings of another group of scientists²² which emphasized the fact that the lower *Aedes* mosquito population to be found at higher level of multiple storey buildings, the present study revealed higher index values corresponding to higher mosquito population at the higher elevations (60 ft). The argument presented by Wan-Norafikah *et al*²², can not be proven without further research. Though the results of present study were contrasting to the findings of previous study²², it agreed with other recent studies^{15, 19-20}.

The environmental factors especially temperature, humidity and wind strongly influence mosquito behaviour²⁷. Since mosquitoes rely heavily on wind generated host-odor plumes to locate blood meals, wind should be given considerably more attention in any kind of a mosquito behavioural study. Hence, it should be given the same importance regarding the vertical dispersal of *Aedes* mosquitoes. There should be a suitable average wind speed, otherwise it is thought to reduce successful orientations due to mosquitoes' weak flight capabilities²⁷⁻²⁸. Wind speed of each site was studied over the study period and was found that the wind speed had a significant effect ($p < 0.05$) to the larval indices; the ovitrap index, mean number of larvae per ovitrap and the mean number of eggs in the multiple storey buildings proving that wind speed had an effect towards the vertical dispersal of *Aedes* mosquitoes. Not only the wind speed but also the other meteorological parameters considered in the present study were contrast to the finding of a study in Kuala Lumpur, Malaysia in 2013²¹.

According to the GLM, significant ($p < 0.05$) effect of temperature was observed for larval indices, but for rainfall it was not significant with most of the indices ($p > 0.05$). Although, there is a definite effect of meteorological parameters for *Aedes* behaviour in general, a significant difference of those parameters to the vertical distribution of *Aedes* mosquitoes could not be revealed in this study.

Statistical analysis confirmed that there was significant difference on the indices for all height levels between coastal and non-coastal sites. Coastal nature and the enclosed nature displayed a significant difference between larval indices and height ($p < 0.05$). Reasons for this

behaviour at coastal sites must be determined by further studies although they can be suggested to be the wind speed, the salinity of the atmospheric vapour, and the relative humidity.

It has been suggested that the vertical movement and high abundance of *Ae. aegypti* mosquitoes can be due to ecological niche with biotic and abiotic components providing blood meals, breeding sites and resting places in high rise buildings^{15, 20}. Structure of the building or the house design is one of the main abiotic elements^{15, 20} which was considered in this study. Although, this fact was emphasized by other studies none of them had carried out further investigations. Highest index values for all subjected indices were displayed at site 1. This site was a condominium site (a residency) which possessed a high human population density, thus, *Aedes* mosquitoes especially *Ae. aegypti* were provided with an ample source for blood meals. Open verandas and even the apartments were always in contact with the ambient environment through the windows and allowed the entry of *Ae. aegypti* into the building and indoors. Some floors in other three sites (Site Nos. 1, 2 and 3) were tightly enclosed and air conditioned (A/C), so that *Aedes* mosquitoes were deprived of breeding places and optimum temperature for hatching and development, hence low *Aedes* populations were observed. Apart from all other biotic and abiotic factors a general reason for this vertical dispersion of *Aedes* mosquitoes can be suggested as to be the abundance of the *Aedes* mosquitoes of each residential area. Abundance and extensive horizontal ovipositional dispersal of two *Aedes* mosquitoes which had been discovered¹⁹ may altogether construct a fine vertical dispersal in multiple storied buildings.

According to the findings, this study would be important in timely planning control measures against *Aedes* mosquitoes in high rise buildings. Vector control measures such as fumigation, spraying would not be effective in high rise buildings as in lower levels. Hence, more effective vector control methods should be used in high rise buildings, such as integrated traps with attractants and sticky surfaces (an ovitrap), *etc.*

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