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Co-occurrence of *Lutzia fuscana* and *Aedes albopictus* (Diptera: Culicidae) in Native Habitats: as an Implication for Biological Control

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ABSTRACT

The larval stages of *Lutzia fuscana* are predators and feed on larvae of other mosquito species. The present study aimed to investigate the co-occurrence of *L. fuscana* and *Aedes albopictus* in Thitthagalla in the Imaduwa Pradeshiyasaba area which is in the Galle District of the Southern Province of Sri Lanka. As the mean of number *L. fuscana* larvae per cement container increased, there was a significant reduction in the mean number of *Ae. albopictus* larvae per cement container (-0.619, P<0.01). There was no significant correlation between the mean numbers of *Ae. albopictus* pupae per cement container per week and the mean number of *L. fuscana* larvae per cement per week. *L. fuscana* larvae per cement container per week showed a positive linear correlation with temperature (Pearson correlation= 0.713, P<0.01), a negative linear correlation with relative humidity (Pearson correlation = -0.419, P<0.01) and no linear correlation with the rainfall (Pearson correlation=-0.200, P>0.05). Container indices (% of positive containers) of 50% and 32% for *L. fuscana* were recorded for water storage cement tanks and tyres respectively, and for *Ae. Albopictus* larvae 50% and 72% were respective container index values.

1. Introduction

Biological control of mosquitoes using natural predators is presently getting promoted due to its least effect to the ecosystem function and species assemblages. Vector borne disease transmission can be reduced successfully by measures against the vectors, especially in mosquitoes [1]. Interaction between the natural predators and mosquitoes is a part of relationship among the community members at large [2]. Predators of mosquito are found in diverse numbers in large mosquito larval habitats [3] in contrast to the smaller larval habitats.

As one of the few predators [3] that are common in the smaller mosquito larval habitats, the larval stages of the mosquito *Lutzia fuscana* (Diptera: Culicidae) [2, 4, 5] are common to abundant in a variety of mosquito larval habitats in India, Sri Lanka, Thailand and Pakistan. Recent taxonomic studies [6] have led to the elevation of subgenus *Lutzia* to the genus level; hence the erstwhile name of *Culex fuscanus* is presently replaced as *L. fuscana*.

The larval stages of *L. fuscana* are aquatic predators that feed on the larvae of other mosquito species [5, 7, 8] as well as the chironomid larvae and tubificid worms [9], making them a potential as biological control agent of different mosquito species [4, 5, 10, 11,12]. However, little effort by Singh et al. [5] has been made to evaluate their predatory potential to promote them as biological control agent against the vector mosquitoes that thrive in small mosquito larval habitats. In view of this, an attempt was made to assess the potential of *L. fuscana* larvae as a biological control measure against the dengue vector *Ae. albopictus* (Diptera: Culicidae) under natural field conditions.

In view of the expansion of geographic range of the vector, it is possible that the role of *Ae. albopictus* in the transmission of Dengue and chikungunya will increase [13]. Previous studies have indicated that *Ae. albopictus* occur in greater abundance than *Ae. aegypti* in several areas of Sri Lanka that have experienced outbreaks of Dengue in the past [14].

The present study was carried out to determine the co-occurrence of *L. fuscana* and *Ae. albopictus* in their native habitats, the breeding site of *L. fuscana* and *Ae. albopictus* and the correlations of *L. fuscana* population with climatic factors.

2. Material and Methods

A preliminary study was conducted in December 2016 prior to the present study. Factors such as past entomological data, environmental conditions, availability of breeding sites, and feasibility of field operations to collect relevant data were considered in selecting study areas.

The present study was carried out at Thitthagalla (Longitudes $80^0.22'$ and Latitudes $6^0.03'$) in the Imaduwa Pradeshiyasaba area which is in the Galle District of the Southern Province of Sri Lanka mainly termed as a rural setting with large trees and other vegetation surrounding human habitations. The selected site is approximately 2km in an area bounded by Paddy fields. The study was conducted regularly throughout January 2017 to December 2017.

A total of 10 cement containers sized with 25 cm ×25 cm × 5 cm filled with 1000 ml of water were placed in ten selected outdoor stations 100 m apart. The number of *Aedes* and *Lutzia* larvae were recorded at the end of each week, after which the water was discarded and the containers were refilled with water. Preliminary identification in the field was done by visually examining the larvae during collection; species identification was confirmed in the laboratory, using standard keys [15, 16].

A preliminary survey identifying all potential breeding habitats such as domestic and peri-domestic water storage containers, cement tanks, ponds, ditches and overhead tanks, in 100 premises was conducted for a period of one month prior to the research study. *Ae. albopictus* and *L. fuscana* species were examined for their larval breeding and 10 mature 4^{th} instars larvae from each container were randomly collected in separate containers and identified in the laboratory using standard keys [15, 16].If a container had less than 10 larvae, all larvae were collected [17]. Pipetting, siphoning and dipping techniques were used for collecting larvae depending on the nature of the breeding habitat. The percentage of positive containers for *Ae. albopictus* and *L. fuscana* is termed as Container Index (CI).

Rainfall, temperature and relative humidity data for the study area were obtained from the Meteorological Department, Sri Lanka.

The data was analyzed by using SPSS statistical software version 20. Pearson correlation was applied to test for significant correlation between mean *Ae. albopictus* and *L. fuscana* larvae per cement container. Pearson correlation was applied to test for significant correlation between the mean number of *L. fuscana* larvae per cement container per week with the temperature, relative humidity and rainfall.

3. Results

3.1 Container index values

The types of containers surveyed and number of positive containers for *Ae. albopictus* and for *L. fuscana* larvae are shown in Table 1. In addition, the results of container index are mentioned. The main inspected container type was discarded receptacles. As such the most positive container type was also discarded receptacles. Among other types of containers, clay pots and tires were commonly inspected and also highly positive. Discarded receptacles and clay pots were highly positive for *Ae. albopictus* larvae, whereas tires were commonly positive for *L. fuscana* larvae (Table 1).

In the collection of outdoor potential breeding sites all the collected *Aedes* larvae were *Ae. albopictus* and all the collected *Lutzia* larvae were *L. fuscana*.

Discarded receptacles (93%), overhead tanks (75%) and Tires (72%) showed high CI value for *Ae. albopictus* larvae. The CI values of *L. fuscana* in the above containers were 5%,25% and 32% respectively. The CI values of water storage cement tanks were 50% for both larval species. Whereas, in the view of biological control, it could be clearly seen that container indices for *Ae. albopictus* got increased comparatively with the decreasing of CI values for *L. fuscana* (Table 01).

3.2 The occurrence of *Ae. albopictus* larvae,pupae and *L. fuscana* larvae

The mean number of *Ae. albopictus* larvae in cement containers fluctuated significantly week to week. In the same cement tank, the mean number of *L. fuscana* larvae did not vary much in general, but the mean number of *L. fuscana* larvae increased during the weeks 2, 13 and 17, and interestingly, the mean number of *Ae. albopictus* larvae reduced significantly. However, the mean number of *Ae. albopictus* pupae did not vary significantly (Figure 1).

Table 1: Breeding site preferences and container Index values of *Ae. albopictus* larvae and *L. fuscana* larvae in Thittagalla area in Galle district

Container type	No. of inspected containers	No. of positive containers	Positive for *Ae. albopictus* larvae	Positive for *L. fuscana* larvae	CI for *Ae. albopictus* larvae	CI for *L. fuscana* larvae
Water storage cement tanks(already within the area)	8	5	4	4	50%	50%
Clay pots	29	20	20	4	68.96%	13.79%
Ponds	19	8	7	4	36.84%	21.05%
Overhead tanks	12	9	9	3	75%	25%
Tires	25	22	18	8	72%	32%
Natural habitats	15	9	9	1	60%	6.67%
Discarded receptacles	43	40	40	2	93%	5%

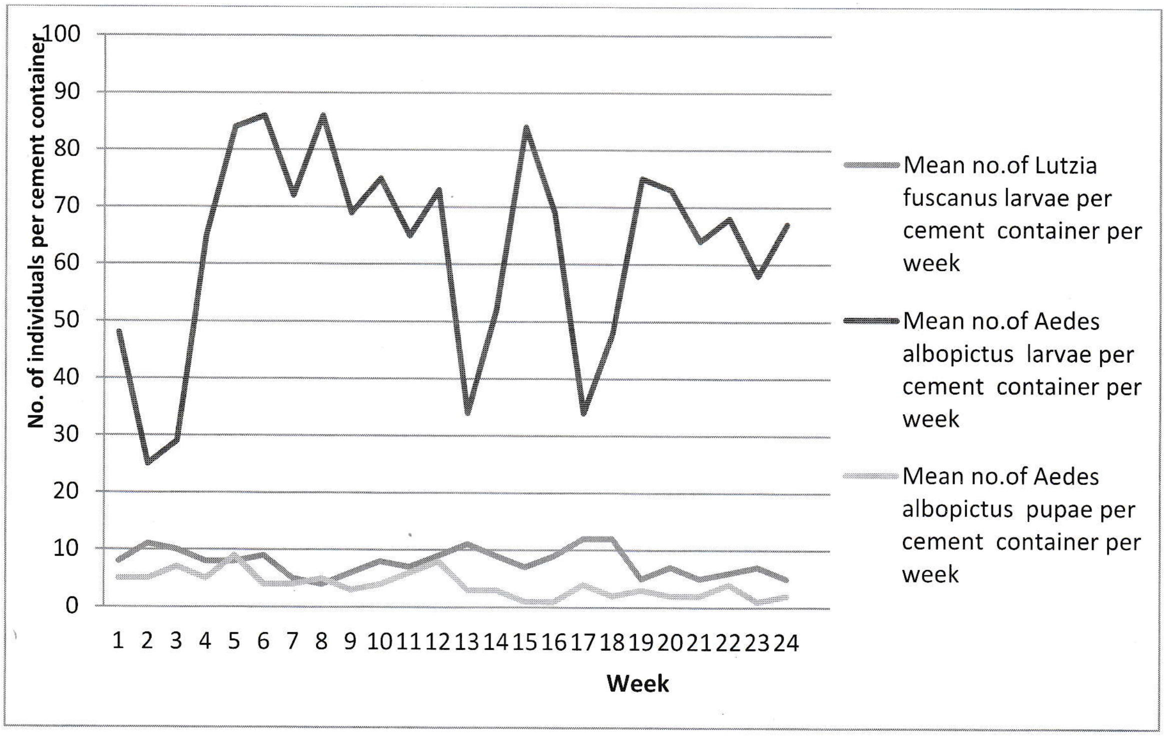

Figure 1: Mean number of *Ae. albopictus* larvae, pupae and *L. fuscana* larvae per cement container per week

3.3 The relationship *of L. fuscana* larvae with *Ae. albopictus* larvae, and pupae and with environmental factors

The number of *Ae. albopictus* larvae reduced significantly with increasing number of *L. fuscana* larvae per cement container. (Pearson correlation of mean *Ae. albopictus* larvae per cement container and mean *L. fuscana* larvae per cement container were -0.619, P<0.01 (Figure 2).

The number of *Ae, albopictus* pupae per cement container per week with the number of *L. fuscana* larvae per cement container per week was not significantly correlated (Figure 3).

The mean number of *L. fuscana* larvae per cement container showed a significant positive linear correlation with the temperature (Pearson correlation= 0.713, P<0.01) (Figure 4).

Mean number of *L. fuscana* larvae per cement container showed a significantly negative linear correlation with relative humidity (Pearson correlation = -0.419, P<0.01) (Figure 5).

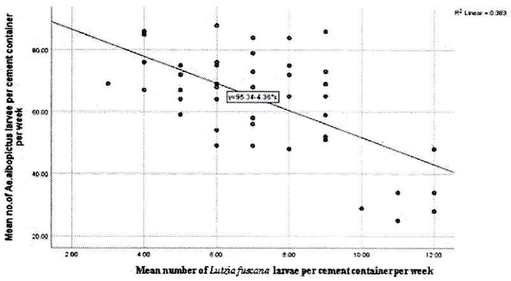

Figure 2: Mean number of *L. fuscana* larvae per cement container per week with the mean number of *Ae. albopictus* larvae per cement container per week

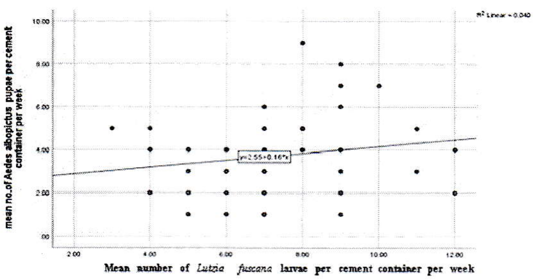

Figure 3: Mean number of *L. fuscana* larvae per cement container per week with the mean number of *Ae. albopictus* pupae per cement container per week

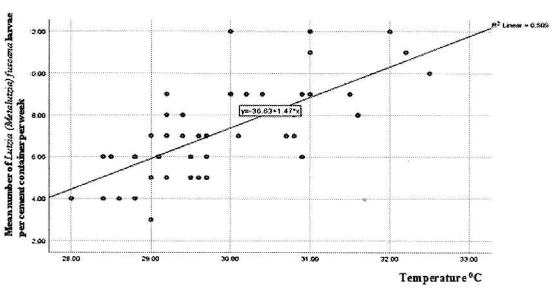

Figure 4: Mean number of *L. fuscana* larvae per cement container per week with the temperature

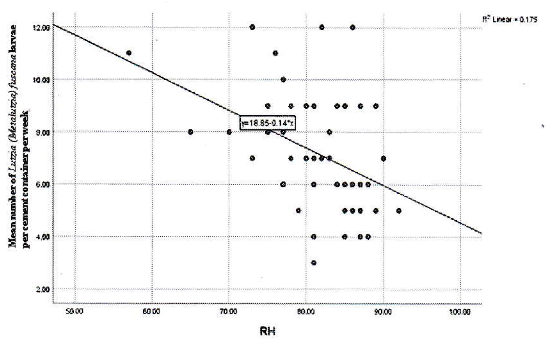

Figure 5: Mean number of *L. fuscana* larvae per cement container per week with the relative humidity

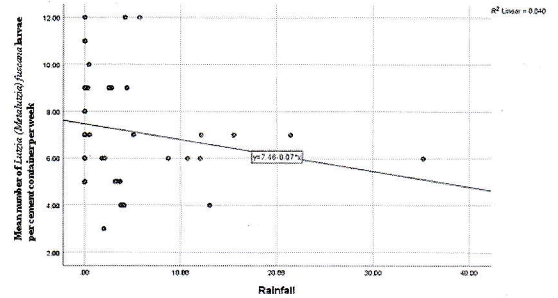

Figure 6: Mean number of *L. fuscana* larvae per cement container per week with the Rainfall

There was no linear correlation between the mean number of *L. fuscana* larvae per cement container per week with the rainfall (Pearson correlation=-0.200, P>0.05 (Figure 6).

4. Discussion

There have been several reports on predatory and feeding behavior of *L. fuscana* [5, 9]. These behaviours were studied in laboratory conditions. Currently, there is a lack of studies conducted in the natural environment. This study attempted to investigate the breeding potential of *L. fuscana* on *Ae. albopictus* in outdoor natural environment.

According to the present study, all *Aedes* larvae found in the study site, including the outdoor potential breeding habitats were *Ae. albopictus* and all *Lutzia* larvae were *L. fuscana*. This is mainly due to the environmental factors. The study area was in a rural setting of large trees with tree holes, covered leaf axils and other vegetation surrounding the area, which are known natural breeding sites of *Ae. albopictus* and *L. fuscana.* The number of *Ae. albopictus* larvae per cement container per week were significantly reduced with the increasing number of predatory larvae *L. fuscana.* This is probably due to the prey-predator relationship of the two species. However, as there was no significant correlation between the *Ae. albopictus* pupae and the *L. fuscana* larvae, it may be the *L. fuscana are* able to fulfill the food requirement with the higher abundance of *Ae. albopictus* larvae.

According to the Singh *et al* [4], the breeding temperature of the predatory mosquito *L. fuscana* were at 18 ^{0}C–34 ^{o}C and contained some decaying leaf debris from the trees. Our study also showed that the mean number of *L. fuscana* increased with the temperature between 28 ^{0}C-33 ^{0}C. Singh *et al* [4], reported that the optimum relative humidity for breeding in the laboratory condition is about 60-70 RH. In this study the number of *L. fuscana* increased with relative humidity and the range between 70-90 RH at the field. Low rainfall has previously been stated to reduce the breeding in *L. fuscana* [4]. Within our study, there was no correlation between the number of *L. fuscana* and the rainfall. The result may be due to the fact that the area has no specific rainfall fluctuations throughout the year.

This study further reveals that, *L. fuscana* co-occurs with *Ae. albopictus* larvae in outdoor breeding containers such as water storage cement tanks, clay pots, ponds, overhead tanks, natural habitats, and discarded receptacles. According to Jin et al. [9], *L. fuscana* co-occurred with chironomid larvae, *Cx. sitiens*, *Cx. quenquifasciatus* and *Ae. albopictus*. This co-occurrence is useful to implement the biological control of *Ae. albopictus* (Dengue vector) through *L. fuscana* as a natural enemy. A study on surveillance of *Ae. aegypti* in Jodhpur showed cement tanks as the most important containers for *Aedes* breeding [18]. Our study revealed that *L. fuscana* also prefers to breed in the same types of cement containers which are peri-domestic and placed under shades of big trees. *L. fuscana* is a container and tree hole breeder [6, 19, 20], and will oviposit in the containers where other mosquitoes are breeding as the other larvae serve as a food source for this mosquito larvae [21, 22].

Introduction of a predatory species to public tanks that cannot be contaminated with insecticides, such as those filled for cattle and other animals for drinking water and community use can prove to be very useful for vector control.

Environmental methods and biological control are alternatives to chemical control and are key components of the integrated strategy which may go hand in hand to the National Vector Control Program. The approach is cost-effective, ecologically balanced and sustainable for vector control if used in this type of climatic condition in areas where mostly outdoor breeder larvae are present.

5. Conclusions

Discarded receptacles are the most common positive container type for *Ae. albopictus*. *Lutzia. fuscana* shows the highest larval productivity in water storage cement tanks and tires respectively under natural conditions. The mean number of *Ae. albopictus* larvae shows a significantly negative correlation with the mean number of *L. fuscana* larvae. The *L. fuscana* larvae positively correlated with temperature and relative humidity. The CI values of containers for *Ae. albopictus* rising with the CI values of the containers for *L. fuscana*.

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