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An entomological analysis on the prevalence of dengue vectors in urban areas of Ernakulam district, Kerala, India

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Abstract
Dengue is one of the major mosquito-borne diseases in Kerala, posing a threat to human health. *Stegomyia aegypti* and *Stegomyia albopicta* are serving as the primary and secondary vector of the dengue virus, respectively in Kerala. The present study was conducted to know the diversity and breeding sites along with the level of infestation of dengue vectors in the urban areas of the Ernakulam district of the Kerala state. The present study was carried out for a period of 11 months, i.e. from October 2017 to August 2018. A cross sectional survey entomological survey was carried out in the peridomestic areas for the breeding of mosquito immatures in the water holding containers in the study area. A total of 7018 water holding containers form 3025 houses were surveyed during the study period of which 801 containers from 508 houses were harboring mosquito immatures either larvae or pupae. *Stegomyia albopicta* was the dominant container breeding mosquito in the area and presence of *Stegomyia aegypti* was also identified in the area. Co-infestation of mosquito species in breeding habitats was reported in the study. The most frequently encountered breeding sites were plastic containers, earthen, glass containers, tyres and cement tanks etc. Twelve species were recorded in the present study including the major dengue vector, *Stegomyia aegypti* and the urban malaria vector, *Anopheles stephensi*. Stegomyia indices were high during the month of June and lowest in the month of February. This baseline data on the vector density can help the authorities in planning and evaluation of vector control activities specific to the local ecology of the vector.

Keywords: Entomological survey, breeding habitats, vector-borne diseases, vector surveillance, dengue

Introduction
Vector borne diseases such as dengue, malaria, chikungunya, and filarial are major threat to the public health. These diseases are caused by virus, bacteria and parasites and are transmitted by mosquitoes and other arthropod vectors. During the past few years there are reports of sudden eruption of these diseases suggesting the reemergence of these diseases in many parts of the world with multiple foci of outbreaks and continuous circulation of these pathogens in the nature [1]. During recent years the state of Kerala is fronting these vector borne diseases as a major public health concern. There are reports of increase in the incidence of these diseases due to many factors including unplanned urbanization and climate change which favors the vector proliferation [2]. The increase in vector density can be attributed to the heavy monsoons and hot humid weather supported by the developmental activities, urbanization, population growth, environmental and ecological changes in the area. The container, natural or artificial which can hold water for about a week can support the breeding of mosquitoes. Faunal diversity and breeding behavior of the mosquito vectors in the urban areas has to be studied for an effective and efficient mosquito control program by the local authorities. Dengue is one of the major public health problems challenging the health systems of Kerala. The rapid urbanization in Ernakulam district has altered the vector diversity and factors supporting the vector breeding. Dengue is one of the major arboviral diseases affecting the Kerala due to its increase in travel, population growth and urban living conditions. The vector density and transmission of dengue varies with seasons depending on various ecological and environmental factors. Vector surveillance is a routine practice by the local health departments as recommended by the world health organization which provides the baseline data on the vector density and distribution [3, 4]. The monitoring of the progress of vector control

activities and dengue vector density relies on survey of larvae and pupae. Three most commonly used Stegomyia indices were the House index (HI), the Container index (CI) and Breteau index (BI) which estimates the infestation level of the dengue vectors [5, 6]. Since these indices gives poor correlation with the abundance of adult vectors an alternative Pupal indices (PI) was developed in order to provide a reliable indices on the risk of transmission [7-10]. Ernakulam district is a modern metropolis of 3.2 million people, and has been afflicted by different dengue virus serotypes i.e. 1, 2 and 3 over time. Reports on high rates of co infection with more than one serotype have also been reported from the district [11]. The state is hyper endemic for dengue and the virus has been isolated from the Stegomyia vector mosquitoes from the state suggesting it to be the foci of dengue virus transmission [12].
During 2017 the district had an annual cases of 494 confirmed dengue cases (2.25% of total cases in Kerala), followed by 177 cases in the year 2018 (4.34% of total cases in the state) [13]. The district hosts the highest number of international and domestic tourist and being the economical capital of the state, the outbreak of vector borne diseases mainly dengue causes huge economic loss. The density of dengue vectors in the urban areas of the district would give and insight on extend of risk of the transmission potential of the diseases. This data could evaluate the present vector control strategies by the local health authorities and gives an insight for instigating better vector control strategies if necessary [15]. The cohabitation of *Stegomyia aegypti* with *Stegomyia albopicta* and the change in breeding pattern due to the influence of biotic and abiotic factors may lead to an expansion of these vectors and disease transmission. Recent study in the district had reported the presence of different vector species including dengue vectors *Stegomyia aegypti* and *Stegomyia albopicta* [15]. However the actual geographical distribution and breeding densities are not reported in this area. The present study was carried out in the corporation areas of Ernakulam district thru Stegomyia larval profiling in terms of larval diversity, density (as indices) and breeding source specificity. The data thus obtained will serve as the baseline data on the vector density and can be used for planning and evaluation of vector control activities specific to the local ecology of the vector.

Materials and Methods

Study area

The present study was carried out at Cochin Corporation area of Ernakulam district of Kerala state, India situated in the central part of that state. The entomological survey was conducted from October 2017 to August 2018 extensively in the corporation areas (Figure 1). The study area lies at the heart of the district and is an important cultural, educational and commercial centre comprised of various types of residential and commercial constructions. The survey sites were selected with an intention to cover entire Corporation area of the district.

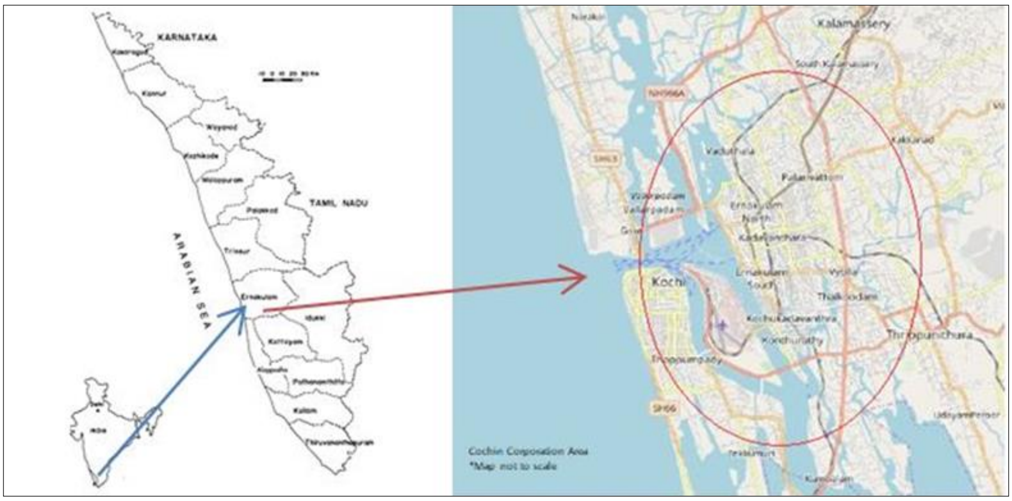

Fig 1: Study Area (Cochin corporation-ernakulam)

Entomological surveillance

The study was conducted as a cross sectional survey and houses were selected randomly for the survey in a way to cover residential areas in the corporation area of the district. During the larval survey, every accessible water-holding container in and around the house was inspected for the presence of mosquito immatures. A container containing any amount of water was considered as wet container and the wet container containing any number of immatures (larvae, pupae or both) was considered as positive container. Habitat evaluation method as described by Service M W (1993) was adopted in collecting the larvae from different habitats using appropriate sampling method (e.g. dipping, pipetting, etc.) [16]. Larvae and pupae were collected from all the positive breeding habitats and were transferred to the laboratory in plastic vials for rearing and identification with containers labeled with the code of breeding source, place of collection, house number and date of collection. The data on larval collections were recorded in the pre-designed survey forms of the Zonal Entomology Unit.

Identification of immatures

The immatures (larvae and pupae) collected were reared in white plastic bowls and fed with larval feed for their emergence into adults. The emerged adults were morphologically identified to species level by the Entomology Consultant in Zonal Entomology Unit using standard key and catalogues [17, 18].

Data analysis

Descriptive statistics, i.e., frequency and percentage distributions, were utilized to describe the containers identified in this survey. The entomological indices like Breteau index (BI), container index (CI), pupal index (PI) and house index (HI) were calculated to understand the risk of infestation of mosquito vectors transmitting diseases such as dengue, chikungunya and Zika. The average CI, HI, BI, and PI for each month was computed using the following formulas:

$$\text{Container Index (CI)} = \frac{\text{Number of positive containers}}{\text{Total number of container inspected}} \text{X } 100$$

$$\text{House Index (HI)} = \frac{\text{Number of positive houses}}{\text{Total number of houses inspected}} \text{X } 100$$

$$\text{Breteau Index (BI)} = \frac{\text{Number of positive containers}}{\text{Total number of houses inspected}} \text{X } 100$$

$$\text{Pupal Index (BI)} = \frac{\text{Number of pupae}}{\text{Total number of houses inspected}} \text{X } 100$$

Ethics statement

The investigators obtained informed consent from a member of each household prior to the conduct of the larval survey in and around the house. All our work during this study was on biological and geographical material and did not involve any human subjects.

Results

A total of 3025 houses were surveyed of which, 16.8% (508/3025) houses had mosquito breeding sites in their premises. Overall, 7018 water holding containers were identified during the survey period, of which 801 (11.4%) were positive for mosquito immatures (Table 1). Stegomyia indices were high during the month of June followed by July and August with Breteau index 67.1, 36.6, and 32.9 respectively (Figure 2). Container index and house index showed similar trends with Breteau index with highest value during the month of June. A low infestation rate was shown in the month of February with BI and HI (4.26) and CI (2.84). The present study could identify various breeding habitat and water holding containers in the urban setting of Ernakulam district which ranged from discarded plastic to overhead tank (Table 2). Of the 7018 containers surveyed during the study, plastic containers (69.7%) were the major container with the potential of mosquito breeding followed by discarded metal container (8.6%). Among the *Stegomyia aegypti* positive containers, plastic container were the major breeding habitat with (2.75%), followed by Flower pots (1.87%) (Figure 3). In the present study *St. aegypti* immatures were not found to breed in Coconut Shell and Cement tanks even though it accounts for about 8.11% and 16.67% respectively among the positive containers (Table 2). *St. albopicta* was the most dominant mosquito breeding in the water holding container habitats with about 626 containers which accounts about 78.2% among the mosquito breeding containers surveyed, followed by *Ar. subalbatus* (8.36%), and *Cx. quinquefasciatus* (6.49%) (Figure 4) (Table 3). Whereas the major dengue vector in the area *St. aegypti* was present in 64 (7.99 %) containers. Other major mosquito species breeding in the container habitat in the study area were *Fredwardsius vittatus*, *Anopheles stephensi*, *Culex quinquefasciatus* and *Armigeres subalbatus*. Twelve species were recorded in the present study including the major dengue vector, *St. aegypti* and the urban malaria vector, *An. stephensi*. Of the 801 water holding containers positive for mosquito immature breeding, 702 were positive for dengue vector breeding ie. *St. aegypti* and *St. albopicta*. Plastic containers were the major breeding habitat for Stegomyia species with 71% positive for breeding followed by metal (9.4%) and Earthen (7.7%) whereas the breeding preference ratio was higher for tarpaulin (2.37) followed by Earthen (1.95) and metal (1.76). The breeding preference of plastic container was 0.96 and tyre was 1.58 in the study area (Table 4).

Table 1: Detail of monthly *Aedes* immature survey and their indices (October 2017- August 2018)

Date of survey	No. of houses examined	No. of houses positive for immatures	No. of containers observed	No. of immature positive containers	Total No. of pupae seen	HI	CI	BI	PI
October	273	38	626	68	29	13.92	10.86	24.91	10.62
November	198	29	524	46	35	14.65	8.78	23.23	17.68
December	160	13	366	15	0	8.13	4.10	9.38	0.00
January	342	33	591	41	4	9.65	6.94	11.99	1.17
February	141	6	211	6	0	4.26	2.84	4.26	0.00
March	66	3	52	3	0	4.55	5.77	4.55	0.00
April	395	54	776	72	30	13.67	9.28	18.23	7.59
May	242	38	642	57	21	15.70	8.88	23.55	8.68
June	216	75	732	145	22	34.72	19.81	67.13	10.19
July	579	132	1383	212	83	22.80	15.33	36.61	14.34
August	413	87	1115	136	7	21.07	12.20	32.93	1.69
Total	3025	508	7018	801	231	-	-	-	-

Table 2: Different breeding habitats of collected mosquito species in the study area

Species	Type of containers (%)									
	Flower Pot	*Plastic*	*Metal*	*Mud/ Earthen*	*Glass*	*Coconut Shell*	*Tarpaulin*	*Tyre*	*Cement Tank*	*Overhead Tank*
Stegomyia aegypti	78.95	3.67	5.88	2.30	23.53	0.00	6.25	50.00	0.00	11.11
Stegomyia albopicta	21.05	75.00	71.76	67.82	76.47	64.71	81.25	32.14	17.65	33.33
Fredwardsius vittatus	0.00	4.50	0.00	3.45	0.00	0.00	0.00	0.00	5.88	0.00
Anopheles stephensi	0.00	3.17	0.00	0.00	0.00	0.00	12.50	0.00	23.53	44.44
Culex quinquefasciatus	0.00	4.67	15.29	12.64	0.00	0.00	0.00	0.00	0.00	0.00
Culex tritaeniorhynchus	0.00	1.33	0.00	0.00	0.00	0.00	0.00	0.00	17.65	11.11
Culex vishnui	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00	5.88	0.00
Culex pallidothorax	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.00	5.88	0.00

Lutzia fuscana	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.76	0.00
Armigeres subalbatus	0.00	7.17	5.88	11.49	0.00	35.29	0.00	7.14	5.88	0.00
Toxorhynchites splendens	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.71	0.00	0.00
Culex fuscocephala	0.00	0.00	1.18	2.30	0.00	0.00	0.00	0.00	5.88	0.00
Total No. of Container (%)	2.4	69.7	8.6	7.9	1.9	2.2	1.9	2.7	2.0	0.9
Total Breeding (%)	7.09	10.79	18.45	22.74	5.86	8.11	25.42	15.83	16.67	3.9

Table 3: Container positive of mosquito species

Species	Container positivity (%)
Stegomyia aegypti	7.99
Stegomyia albopicta	78.15
Fredwardsius vittatus	3.87
Anopheles stephensi	3.62
Culex quinquefasciatus	6.49
Culex tritaeniorhynchus	1.50
Culex vishnui	0.37
Culex pallidothorax	0.25
Lutzia fuscana	0.25
Armigeres subalbatus	8.36
Toxorhynchites splendens	0.37
Culex fuscocephala	0.50

Table 4: Infestation and breeding preference ratio of stegomyia species in the study area

Type of container	Water holding containers		Immature positive containers		Containers positive for stegomyia breeding		Breeding preference ratio (y%/x %)
	n= 7018	*%(95%CI) (x %)*	*n= 801*	*% (95% CI)*	*n=702*	*% (95% CI) (y %)*	
Flower Pot	268	3.82 (3.67-3.97)	19	2.37 (1.62-3.12)	19	2.71 (1.96-3.46)	0.71
Plastic	5173	73.71 (73.57-73.85)	558	69.66 (69.03-70.29)	499	71.08 (70.32-71.84)	0.96
Metal	374	5.33 (5.19-5.47)	69	8.61 (8.01-9.21)	66	9.40 (8.78-10.02)	1.76
Mud/ Earthen	277	3.95 (3.8-4.1)	63	7.87 (7.35-8.39)	54	7.69 (7.15-8.23)	1.95
Glass	256	3.65 (3.39-3.91)	15	1.87 (1.15-2.59)	9	1.28 (0.47-2.09)	0.35
Coconut Shell	222	3.16 (3.0-3.32)	18	2.25 (1.09-3.41)	11	1.57 (0.41-2.73)	0.50
Tarpaulin	59	0.84 (0.65-1.03)	15	1.87 (1.18-2.56)	14	1.99 (1.28-2.70)	2.37
Tyre	139	1.98 (1.81-2.15)	22	2.75 (1.91-3.59)	22	3.13 (2.23-4.03)	1.58
Cement Tank	96	1.37 (1.22-1.52)	16	2.00 (1.22-2.78)	4	0.57 (0.34-0.80)	0.42
Overhead Tank	154	2.2 (2.09-2.29)	6	0.75 (0.15-1.35)	4	0.57 (0.36-1.50)	0.26

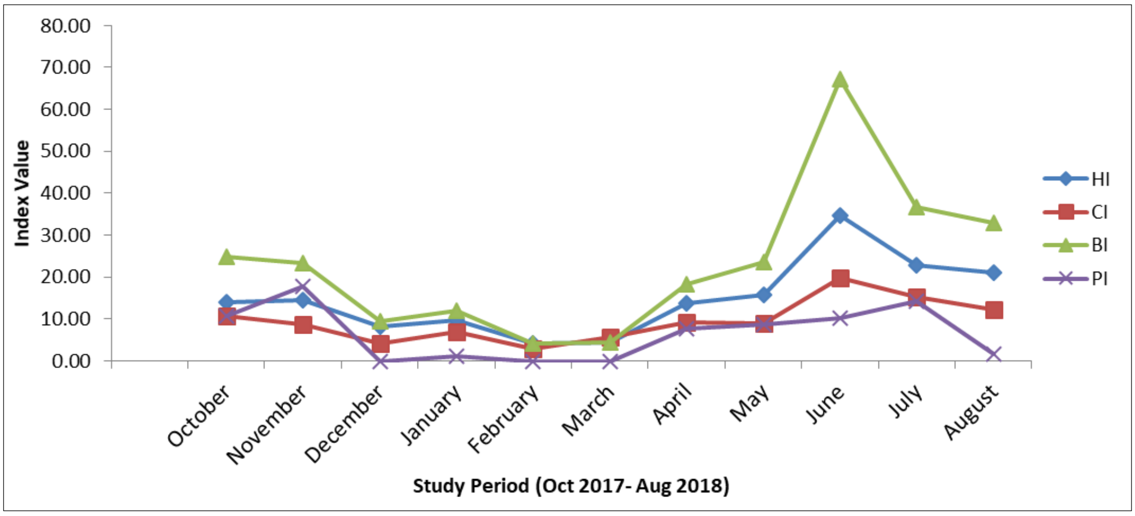

Fig 2: Monthly trend in stegomyia indices during the study period

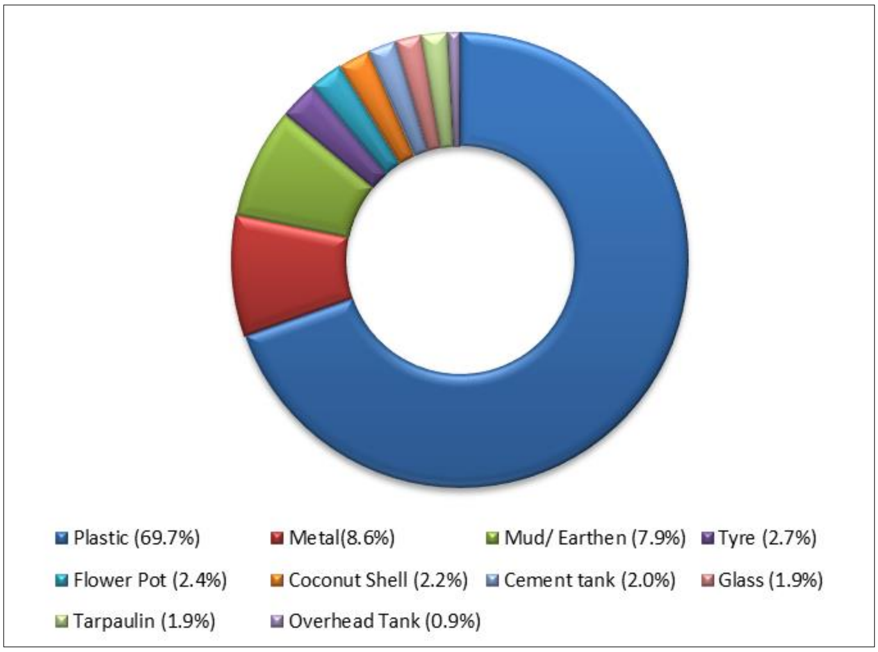

Fig 3: Major breeding habitats of mosquito immatures in the study area

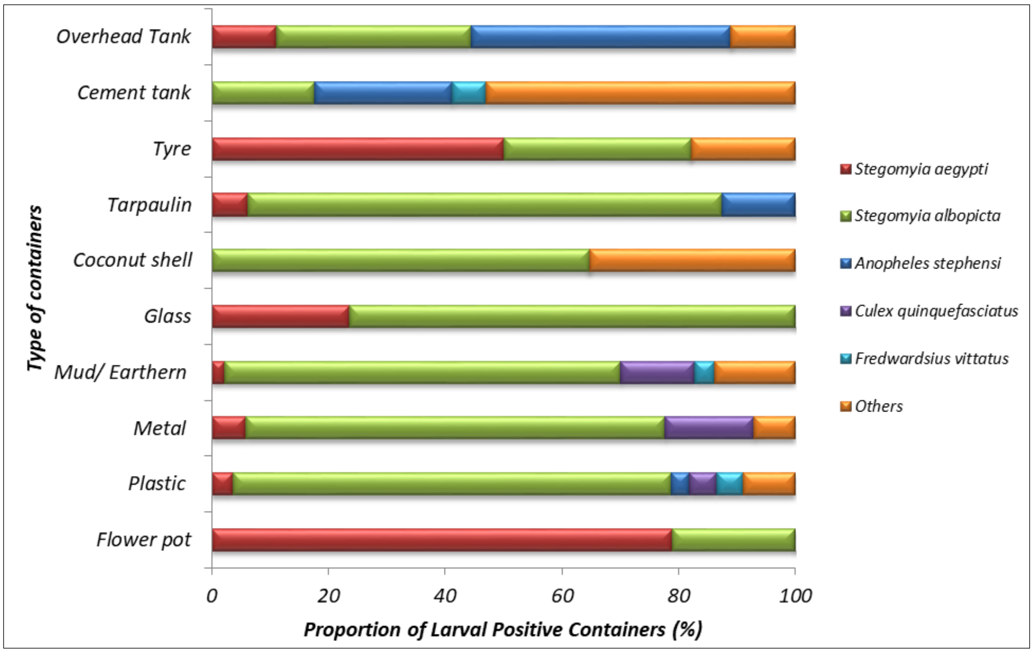

Fig 4: Proportion of containers supporting breeding of major mosquito species in the study area

Discussion

Vector borne diseases are still a major cause of morbidity in the population of Kerala for decades. Various programmes for the control and prevention of vector borne diseases including source reduction activities, health education on vector control strategies are being carried out by the state health department. The increase in distribution and density of dengue vectors are greatly influenced by anthropogenic activities along with environmental factors. The results of this study suggest that the container habitats in the urban areas are utilized highly by several mosquito species. Discarded plastic containers were the major breeding habitat in the area. The plastic containers and other discarded water holding containers were left upright and are filled with rainwater.

Stegomyia albopicta was found to be the most dominant species distributed throughout the entire study area in all types of breeding habitats, while *Stegomyia aegypti* was less dominant and was found to be breeding predominantly in flower pots, tyres and open overhead tanks, but their prevalence differed with containers and relatively to seasons. *St. aegypti* preferred to breed in containers that contain less amount of organic matter whereas St. albopicta was reported to breed in all types of containers irrespective to the concentration of organic matter [19]. The vector population and distribution fluctuate with rainfall and other water storage practices in the urban area [20]. No breeding were found in the fridge tray and 90% of the households had newer version of refrigerator without water holding tray and the surveyed fridge trays were dry/ without breeding.

The distribution of *St. aegypti* is positively correlated with urbanization and *St. albopicta* distribution is associated with the vegetation cover in the urban areas [21-22]. In our study area, there is relatively thick vegetation in the urban areas which may contribute the higher distribution of *St. albopicta* in the area. The container breeding habitat also supported the breeding of potential vectors for urban malaria, Japanese encephalitis, filariasis and other nuisance mosquitoes. *Cx. vishnui*, *Cx. tritaeniorhynchus* and *Cx. quinquefasciatus* were also found to be breeding in container habitat in the study. *Anopheles stephensi* the urban malaria vector was also found to be breeding in the water holding container as reported in other studies [15, 23].

In this study the abundance, breeding habitat, mixed breeding and breeding preference ratio has been revealed. Co-Infestation of *St. albopicta* and *St. aegypti* was observed during our study similar to the observation of earlier reports (24). The most frequent co-infestation of container habitats was that of *St. albopicta* and *Cx. quinquefasciatus* followed by *St. albopicta* and *St. aegypti*. The flower pots and tyre were found to be mixed breeding site for *St. aegypti* and *St. albopicta* whereas cement tanks and earthen pots supported the mixed breeding of *St. albopicta* and *Cx. quinquefasciatus*. *Cx. fuscocephala* and *Fredwardsius vittatus* were also recorded from cement tanks.

The larval surveillance could enable us to identify the high risk areas and seasonal prevalence of the dengue vectors in the study area which can help the authority to initiate vector control strategies. The Stegomyia indices of the study area was high during the month of June followed by July and August reflecting the abundance of the dengue vectors during the monsoon and post monsoon season. This abundance of the vector mosquitoes increases the risk of virus transmission and increases the morbidity among the risk population. The abundance and density of the dengue vectors is reflected in the Stegomyia indices during the immature vector surveillance [25]. Even though the residents of the area are aware of the mosquito breeding habitats and physical control measures, aversion towards the reduction of breeding habitat supplies a large number of mosquito breeding habitats. Most of the water storing plastic containers and other metal containers were neither closed nor cleaned properly. The eradication of the water holding containers in the peridomestic areas is an effective mosquito control strategy at community level [26]. The Stegomyia indices are useful in determining the distribution, seasonal changes and preferential breeding habitats and also for evaluation of vector control strategies. These indices can also indicate the dynamics of the intensity of disease transmission. However the threshold level and indices at which the disease transmission occurs is influenced by many factors including environmental and climatic factors along with mosquito longevity, virus strain and immunity of the risk population [27].

Conclusion

The present study recorded the presence of dengue vectors *Stegomyia aegypti* and *Stegomyia albopicta* in the urban areas of Ernakulam district. Urban malaria vector *Anopheles stephensi* was recorded to be breeding in water holding containers. In our study the geographical distribution and density of *Stegomyia albopicta* was higher than that of *Stegomyia aegypti* in peridomestic areas of urban areas of the district. The epidemiological role of both dengue vector species needs to be studied to understand its role in the widespread disease transmission. The study revealed the different types of breeding habitats and the mixed breeding status of the vector mosquitoes. The discarded plastic containers were the most abundant potential breeding habitat followed by metal and earthen containers, where IEC activities will help in source reduction. The study shows high larval indices during monsoon and post monsoon months. Thus it is evident that there is a high risk of transmission and outbreak in these seasons. This baseline data on the container breeding mosquitoes gives an insight on the density of vector species and helps the authorities in instigating better prevention and control strategies to prevent the outbreak of diseases in the area. The community participation for mosquito control activities is not effective in survey sites which would result in repeated attacks of dengue and can even results in dengue hemorrhagic fever and dengue shock syndrome. The study also concludes that there is a need for community based educational intervention towards physical control of mosquito breeding.

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