SHORT COMMUNICATION

ASSESS THE PREVALENCE AND INSECTICIDE RESISTANCE OF MAJOR DENGUE VECTORS *AEDES SP.* IN SELECTED LOCATIONS OF BATTICALOA DISTRICT

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ABSTRACT

Dengue has become the major vector borne disease in Sri Lanka. Control of the vectors *Ae.aegypti* and *Ae.albopictus* through elimination of breeding habitats and application of insecticides are considered as the most effective ways to suppress the dengue epidemic. The mosquito larval survey was undertaken in three MOH areas such as Oddamavadi, Eravur, and Vantharumoolai, during the period in between January to March and September to October, 2020 in Batticaloa district. Five areas in each MOH area were selected randomly for the survey. Larval indices such as House index (HI), container index (CI) and Breteau index (BI) were calculated by using standard WHO guidelines in order to find the dengue epidemic risk. Resistance status of dengue vectors to four commonly used insecticides such as DDT, malathion, deltamethrin and carbamate were also assessed by using standard World Health Organization (WHO) procedures. Findings of the container survey and larval survey revealed that the three study locations are abundant with wet containers that are potential for the dengue mosquito breeding. There was a significant difference (p = 0.001) in the abundance of indoor containers in all three locations whilst there is no significant difference (p = 0.697) in the abundance of outdoor containers in all three locations. The results also indicated that both species can breed in urban, suburban, and rural areas. But *Ae.albopictus* prefers mostly rural and outdoor premises where there is dense vegetation. *Ae.aegypti* was the dominant species in all three locations. The larval indices have showed that Oddamavadi and Eravur were at high dengue epidemic risk and Vantharumoolai was at moderate risk. Results of insecticide bioassay revealed that both of the species were susceptible to deltamethrin and both were resistance to DDT and carbamate. It has also been found that both of the species were possibly resistance to malathion. From this result we can conclude that deltamethrin can be effectively used for space spraying programmes during the outbreak of Dengue in Sri Lanka.

Keywords: *Bioassay, resistance, larval indices, dengue vectors*

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1. INTRODUCTION

Dengue fever (DF) and dengue haemorrhagic fever (DHF) is a vector born viral disease which is a major public health concern and endemic in several countries located in both tropical and subtropical regions around the world [1]. *Aedes aegypti* (Linnaeus) and *Aedes albopictus* Skuse are the major vectors of dengue [2]. Dengue is an acute febrile disease caused by *Flavivirus*, which exists in four different viral serotypes, namely DENV-1, DENV-2, DENV-3, and DENV-4 [1]. Dengue fever can also transform into dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS), which may result in the fatal condition causing hemorrhages and leakage of plasma [3]. Any licensed vaccines have not yet been introduced for dengue fever, therefore effective vector control is necessary to defeat the spread of dengue disease.

A rapid increase in dengue cases has been reported in the Batticaloa district since 2016 [4]. In the year 2020, a total of 3367 dengue cases were reported in Batticaloa district [4]. Generally, mosquito vector control can be classified as Source reduction and environmental management, biological control, genetic control, and chemical control [5]. The most effective ways to control Aedes population is the removal of the larval breeding sites and the application of insecticides. Insecticide application has been used to control *Aedes* population by spraying or fogging methods [6]. Major groups of insecticides used are; Organochlorines, organophosphates, carbamates, and pyrethroids. Due to the application of insecticide to control *Aedes* mosquito for several years, resistance has now evolved to all four classes of insecticides [7]. The ability of mosquitoes to tolerate to a standard dosage of insecticide is known as insecticide resistance [6]. Resistant individuals will have selective advantage over susceptible individuals, which result in the spread of resistance genes in the population [6]. So the insecticide resistance will affect the successful control of the vectors.

Though Dharshini et al., (2011), reported the prevalence and susceptibility of dengue vectors in Batticaloa district, not much recent reports are available regarding the insecticide tolerance test for dengue vectors in the Batticaloa district [8]. Therefore, the study was carried out to report the prevalence of larval habitats and the insecticide tolerance for different insecticide which are used in these areas for adulticidal application.

2. MATERIAL AND METHODS

2.1 Study area

The Batticaloa district is situated in the dry zone of Sri Lanka with a hot and humid tropical climate. The elevation of the district is about 8 meters above the mean sea level. The temperature ranges from 25^{o}C to 35.4^{o}C. The annual rainfall varies from 864mm to

3081mm and most of the rain is being received from October to January by inter monsoon and North-East Monsoon types [9].

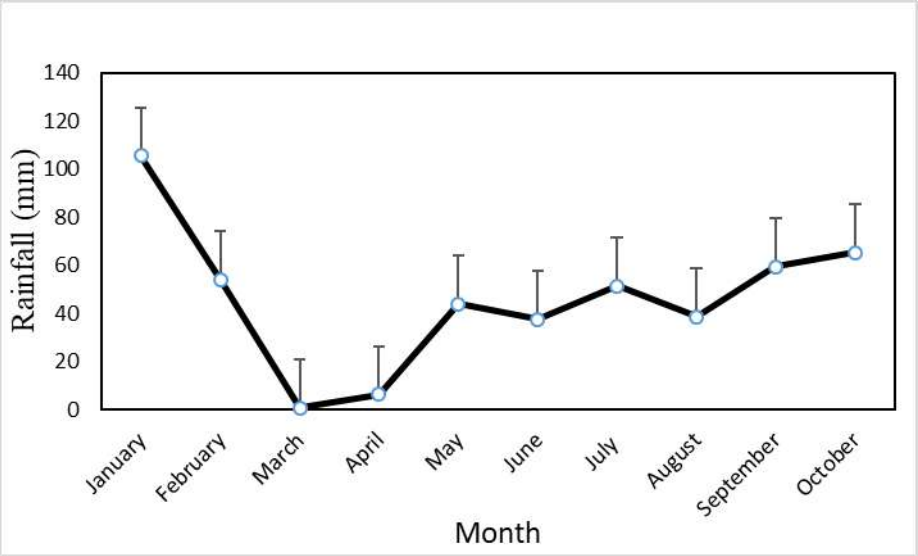

Figure 1: Average monthly rainfall in Batticaloa district, 2020
(Source: Department of meteorology, Sri Lanka)

The mosquito larval survey was undertaken in three MOH areas such as Oddamavadi, Eravur, and Vantharumoolai, during the period in between January to March and September to October, 2020. The study sites Oddamavadi (Location-1), Eravur (Location-2), and Vantharumoolai (Location-3) were classified into urban, suburban and rural respectively based on population density and available facilities for the inhabitants.

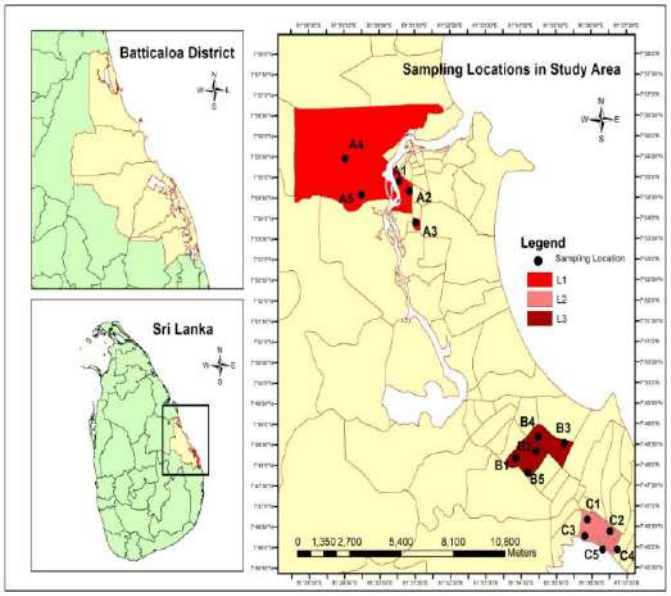

Figure 2: Study location with sampling areas

2.2 Sampling design

Larval surveys were conducted monthly. Five areas in each locality were selected randomly for the survey. In Oddamavadi location (L1); Oddamavadi 2 (A1), Meeravodai muslim east (A2), Meeravodai muslim west (A3), Maancholai (A4), and Oddamavadi 3 (A5) were selected. In Eravur location (L2); Eravur 3 (B1), Eravur 2 (B2), Eravur 5 (B3), Eravur 6 (B4) and Eravur 1 (B5) were selected. In Vantharumoolai location (L3); Ufpoadai area (C1), Bakehouse area (C2), Ambalathadi area (C3), Sankar mill area (C4), and Aalayadi area (C5) were selected. During each visit, randomly selected 30 house premises in each study site were surveyed for the presence of containers with water and for *Aedes* larvae within the containers. In each houses, indoor and outdoor premises were inspected for potential wet containers. All the larvae were collected into a labelled water bottles with small holes on the top for ventilation. The containers were filled with water from the same habitat. Date, location, container type and salinity of the water were recorded on the bottle and transported to the laboratory, Department of Zoology, Eastern University, Sri Lanka and reared separately until it reaches its adult stage.

2.3 Rearing and feeding methods

Both larvae were identified using standard keys [10, 11] and reared in separate 50 ml yoghurt cups. The cups were covered with double layered net tied with a rubber band to prevent the escape of adult. The colony was maintained in laboratory conditions, temperature 25^{o}C- 35^{o}C and the relative humidity 60-80%. The photoperiod was in the natural day and night conditions. Powdered fish meal pellets were given as larval food [12].

The enclosed adults were transferred to separate adult mosquito cages (30cm × 30cm × 30cm) and each species were reared separately. The adults were reared in the same climatic condition as mentioned above in the larval rearing. To maintain the humidity, a wet towel was placed over the cage. Adult mosquitoes were fed with 10% glucose solution [12].

2.4 Larval indices survey

Larval indices, House index (HI), container index (CI) and Breteau index (BI) were computed from the collected data by using standard WHO guidelines, to determine the vector density level of the respective study sites [13].

$$HI = \frac{\text{Number of houses positive for } \textit{Aedes} \text{ larvae or pupa}}{\text{Total number of houses searched}} \times 100$$

$$CI = \frac{\text{Number of wet containers found positive with } \textit{Aedes} \text{ larvae}}{\text{Total number of wet containers searched}} \times 100$$

$$BI = \frac{\text{Total number of containers positive for } \textit{Aedes} \text{ larvae/pupae}}{\text{Number of houses inspected}} \times 100$$

Udayanga et al., (2020) have stated that chemical fogging is the routine vector reduction strategy which is practiced when the BI value exceeds 5%, with reported dengue cases in a considering area or when BI > 20% even without reported cases [14]. According to Jesha et al., (2015), the values of BI>5%, CI>3%, and HI>10% are considered to be the epidemic risk [15].

2.5 Bioassay test

The standard World Health Organization (WHO) procedures were followed to determine the susceptibility/resistance status of adult mosquitoes with WHO standard tubes and insecticide impregnated papers containing diagnostic dosages (4%, DDT, 0.8% malathion, 0.1% carbamate, and 0.25% deltamethrin) prepared in University Sains, Malaysia [16]. Batches of 15-20 mosquitoes (2-3 day old) in each species were exposed to the insecticide impregnated papers for 1h at 25◦C- 35◦C and the 60-80% relative humidity. Mortality counts were made after 24 h and affected mosquitoes that were knockdown also counted as dead. Four replicates were carried out per insecticide/species. Control experiment was carried out by exposing the mosquitoes to papers impregnated with carrier oil alone.

2.6 Statistical analysis

The statistical significance of abundance of containers in both indoor and outdoor among the locations and sites were compared by one-way ANOVA test using Minitab version 17.

3. RESULTS AND DISCUSSION

From the survey conducted in all three locations, a total of 435 indoor containers and 3933 outdoor containers were observed. Among the different breeding habitats

examined, 3.91% of indoor containers and 1.04% of outdoor containers were infested with *Aedes* larvae. *Aedes aegypti* was the dominant species contributing 81.03% of the total positive wet containers in both indoor and outdoor and the rest was *Aedes albopictus.*

Table 2: Percentage of wet containers presented in each study sites

	Percentage (%) of wet containers in each study sites														
	Oddamavadi					Eravur					Vantharumoolai				
	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	C1	C2	C3	C4	C5
Indoor	24.31	22.1	17.13	18.78	17.68	20	26	18.67	18.67	16.67	24.04	21.15	17.31	15.38	22.12
Outdoor	29.59	24.9	13.37	12.06	20.03	29.3	28.78	14.35	12.6	14.96	29.07	24.04	14.66	13.55	18.67

3.1 Container survey in Oddamavadi

In the Oddamavadi location, a total of 181 indoor wet containers and 1443 outdoor wet containers were observed. Highest percentage of wet containers in both indoor (24.31%) and outdoor (29.59%) were noted in A1 area (table 1). The least number of outdoor wet containers were noted in A4 and indoor wet containers in A3 (table 1). Flowerpots, cans and bottles, buckets, and discarded electrical appliances were the most recorded wet containers in Oddamavadi. The most common types of indoor wet containers observed were fridge drip pan, toilet flush tank, and buckets.

Highest percentage of positive wet containers (6.82%) were noted in the A1 area (table 2). In all five areas, nine indoor containers and 17 outdoor containers were found positive for *Aedes* larvae. Most of the larvae obtained belong to the species *Aedes aegypti*.

Table 3: Percentage of wet containers found positive for Aedes larvae in five selected areas in Oddamavadi

Study areas	Percentage (%) of wet containers positive for *Aedes* larvae in			
	Indoor		Outdoor	
	Ae.aegypti	*Ae.albopictus*	*Ae.aegypti*	*Ae.albopictus*
A1	6.82	0.00	1.41	0.23
A2	5.00	0.00	1.11	0.00
A3	6.45	0.00	1.04	0.52
A4	0.00	0.00	0.57	0.00
A5	6.25	0.00	1.04	0.35

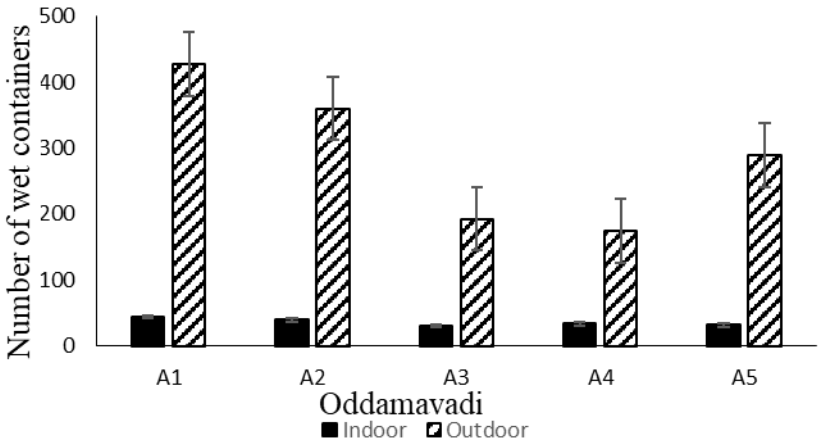

Figure 3: Variation in the number of inspected indoor and outdoor wet containers in Oddamavadi

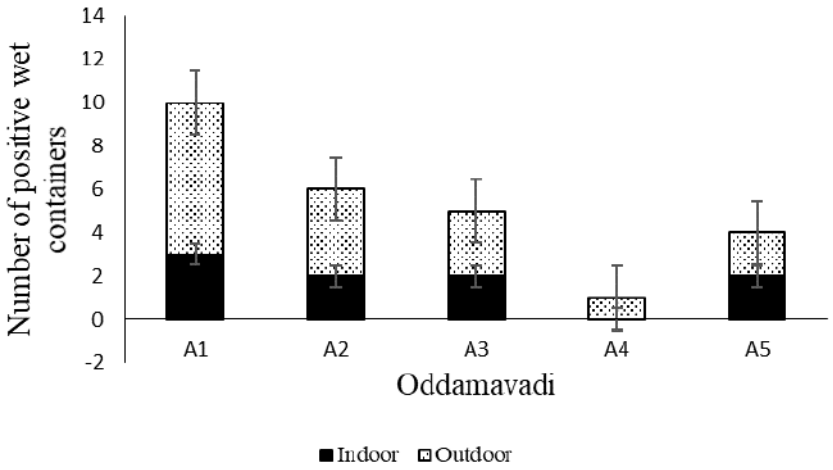

Figure 4: Number of wet containers found positive for Aedes larvae in both indoor and outdoor premises of the houses in Oddamavadi

3.2 Container survey at Eravur

In Eravur, a total of 150 indoor wet containers and 1317 outdoor wet containers were noted in all five sites. Highest percentage of indoor containers (26%) are found in B2 and the lowest (16.67%) in B5 (table 1). The highest percentage of outdoor wet containers can be seen in B1 and lowest in B4 (table 1). The most common types of outdoor wet containers observed in all five areas of Eravur location were, clay pots, cans and bottles, wells, flower pots and animal feeding tray. Refrigerator drip pan and clay pots were the major indoor wet containers. Here a total of six indoor and 13 outdoor wet containers were found positive for *Aedes* larvae. Most of the larvae belong to the species

Aedesaegypti. The highest percentage of positive wet containers were observed in the area B1 (Table 3).

Table 4: Percentage of wet containers found positive for *Aedes* larvae in five selected areas in Eravur

Study areas	Percentage (%) of wet containers positive for *Aedes* larvae in			
	Indoor		Outdoor	
	Ae.aegypti	*Ae.albopictus*	*Ae.aegypti*	*Ae.albopictus*
B1	6.67	0.00	1.04	0.26
B2	5.13	0.00	0.53	0.00
B3	0.00	0.00	1.06	0.00
B4	0.00	0.00	0.00	0.00
B5	4.00	4.00	2.03	0.00

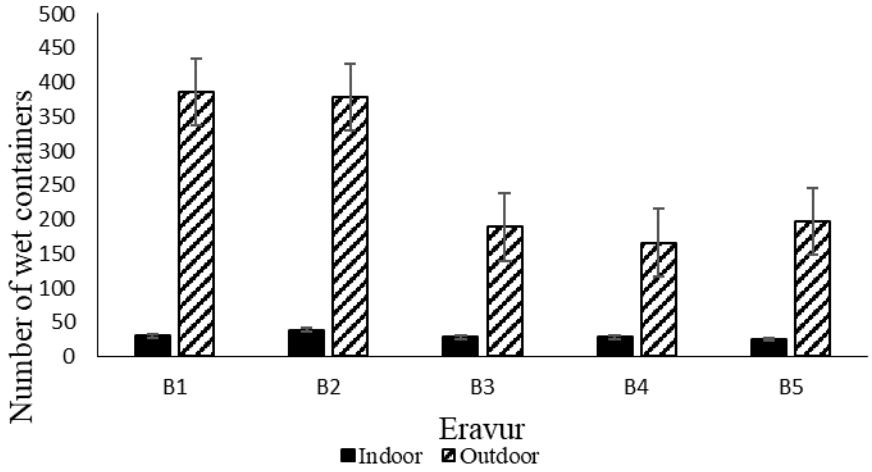

Figure 5: Variation in the number of inspected indoor and outdoor wet containers in Eravur

Figure 6: Number of wet containers found positive for Aedes larvae in both indoor and outdoor premises of the houses in Eravur

3.3 Container survey in Vantharumoolai

In the Vantharumoolai, a total of 150 indoor wet containers and 1317 outdoor wet containers were found. Figure 7 shows that the highest and lowest number of wet containers were observed in the C1 and C4 respectively. The most common outdoor containers observed in were buckets, coconut shells/husks, flower pots, wells, and clay pots. Clay pots and buckets were the most common indoor wet containers. Among the total wet containers, two indoor and 11 outdoor containers were found positive for *Aedes* larvae. *Aedes aegypti* larvae were found in all the areas except C4. The highest percentage of positive wet containers were observed in C1 (Table 4).

Table 5: Percentage of wet containers found positive for *Aedes* larvae in five selected areas in Vantharumoolai.

Study areas	Percentage (%) of wet containers positive for *Aedes* larvae in			
	Indoor		Outdoor	
	Ae.aegypti	*Ae.albopictus*	*Ae.aegypti*	*Ae.albopictus*
C1	4.00	0.00	0.59	0.88
C2	4.55	0.00	0.00	0.35
C3	0.00	0.00	0.00	0.58
C4	0.00	0.00	0.63	0.00
C5	0.00	0.00	0.91	0.46

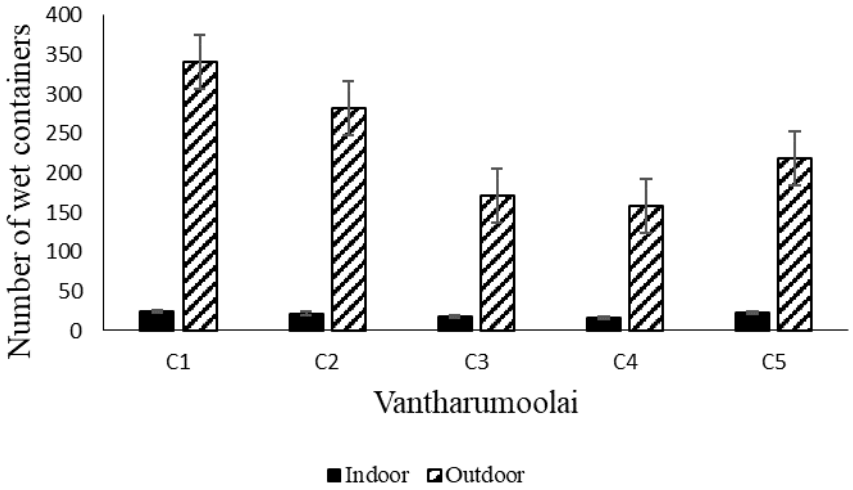

Figure 7: Variation in the number of inspected indoor and outdoor wet containers in Vantharumoolai.

Statistical analysis shows that there is a significant difference (p value = 0.001) in the abundance of indoor containers in all three locations (Oddamavadi, Eravur, and

Vantharumoolai) and there is no significant difference (*p* value = 0.697) in the abundance of outdoor containers in all three locations.

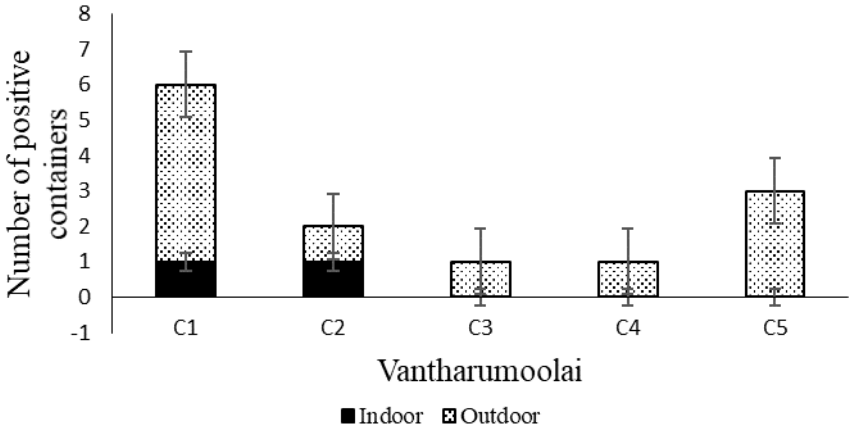

Figure 8: Number of wet containers found positive for *Aedes* larvae in both indoor and outdoor premises of the houses in Vantharumoolai

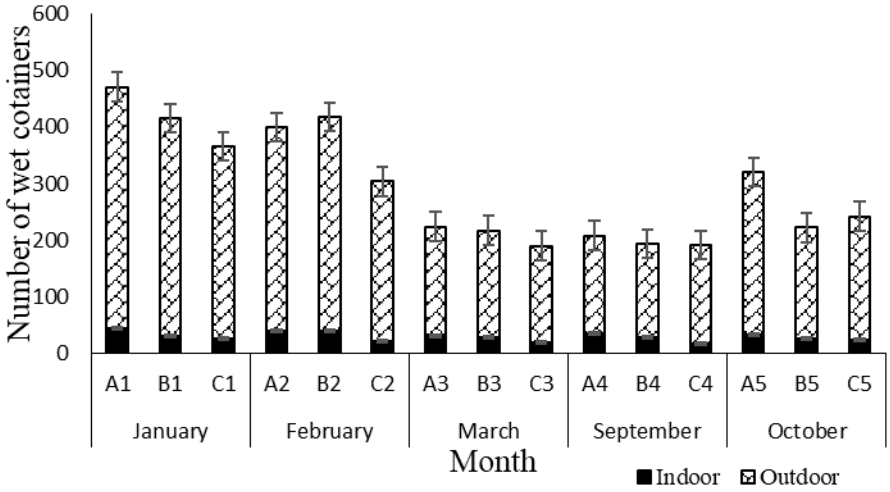

Figure 9: Number of wet containers found in indoor and outdoor premises in all three locations during the study period

3.4 Larval indices survey

When considering the average larval indices for the three locations, Oddamavadi and Eravur are at high risk for the dengue epidemic due to the abundance of dengue primary vector *Aedes aegypti* (figure 9). But there is no epidemic risk in these two areas due to dengue secondary vector *Aedes albopictus.* In Vantharumoolai, there is a moderate risk due to both *Aedes albopictus* and *Aedes aegypti* (figures 10 and 11). The results of the larval indices survey indicated that there is a serious need for dengue vector control in these areas to prevent the epidemic risk.

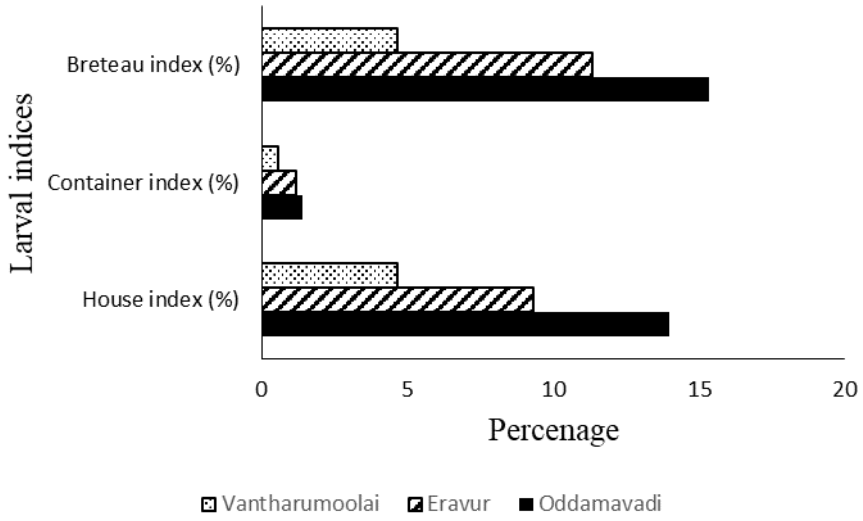

Figure 10: *Aedes aegypti* larval indices in three locations during the survey

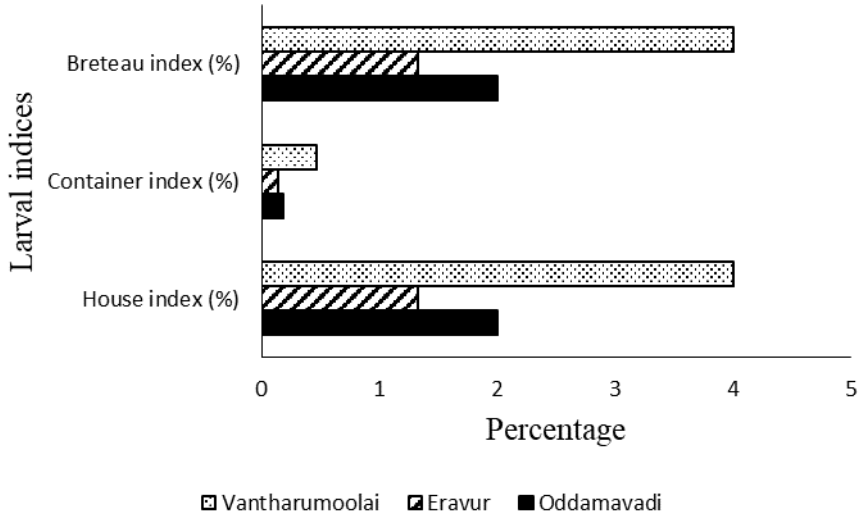

Figure 11: *Aedes albopictus* larval indices in three locations during the survey

3.5 Bioassay test

According to WHO, (2016), the results are grouped as susceptible (98-100% mortality), possibly resistant (90-97% mortality), and resistant (<90% mortality). Table 3.2 shows that populations of both species were possibly resistant to 0.8% Malathion and resistant to 0.1% carbamate and 4% DDT. But both of the species were susceptible to 5% deltamethrin.

Table 6: Percentage mortalities of *Aedes sp.* populations to the discriminating dosages of commonly used insecticides.

Insecticide	Percentage mortality of *Ae. aegypti*	Percentage mortality of *Ae.albopictus*
0.8% Malathion	91	85
5% Deltamethrin	97	99
0.1% Carbamate	4	5
4% DDT	1	3

During the survey period, the rainfall is higher in January and October and there was intermittent rain during the months February, March, and September (Figure 1). It was observed that the number of outdoor wet containers is greater than the indoor containers in all the areas. The number of outdoor wet containers were higher in January, followed by February and October. The number of wet containers observed during March and September was relatively low. This observation reveals that rainfall is primarily the source of water in these wet containers. But in indoor containers as well as in some of the outdoor containers like coconut shells, plastic cans, tins & bottles, residual water was already present.

When comparing the three locations, Oddamavadi is the place where the number of indoor containers is high. Here, buckets, abandoned toilet flush tanks, and fridge drip pan were the most common types of indoor wet containers observed. But in certain areas, the abundance of refrigerator drip pan is relatively low because new models of refrigerators were found in those areas where the drip pan is found inside the refrigerator. But in the Eravur, there were mostly old models of the refrigerator where the fridge drip pan is located outside of the refrigerator which favors larval breeding.

During the survey, it was observed that in Oddamavadi which is urban, and Eravur which is suburban, the numbers of houses are very high in the study areas and there were very crowded populations. There were also a high number of shop complexes which were very close to the human settlements. So these factors resulted in the generation of a higher number of waste containers which became the potential source for Aedes larval breeding. In Vantharumoolai which is rural, where the population is not crowded and there were enough spaces between the houses. During the survey, it was noted that in most of the houses both the outdoor and indoor premises were well cleaned. Here the

most abundant containers were coconut husks/shells which were preserved for cooking purposes, buckets for water storage purposes, and tube wells.

This study has proofed that both *Ae. aegypti* and *Ae. albopictus* can able to breed in urban, suburban, and rural areas in variety of containers. Saleeza et al., (2013), have stated that gardening utensils such as flower pots, flower pot plates, and watering cans were the major breeding habitat for *Aedes* mosquitoes in Putrajaya, an urban area in Kuala Lampur, Malaysia [13]. Ferdousi et al., (2015), have mentioned that buckets, flower pots, cans, bottles, and earthen jars were the most common type of containers in Dhaka, Bangladesh. They have also stated that buckets, flower pots, and drums were the major types of indoor wet containers, while earthen jars, cans and bottles, and miscellaneous wet containers were most abundant in outdoor wet containers [17].

During the survey, it was also recorded that *Ae. aegypti* was the dominant species found in Oddamavadi and Eravur. In the Vantharumoolai, both species were found relatively equal. The reason may be that the locations Oddamavadi and Eravur were urban and suburban areas respectively where the human settlements are high and consist of more number of building. But Vantharumoolai is a rural area that has many paddy fields and much other vegetation around the human settlement areas. It was also observed that *Ae. aegypti* larvae were collected both indoor and outdoor. But *Ae. albopictus* larvae were collected only from outdoor premises. Ferdousi et al., (2015) have also mentioned that *Ae. aegypti* has the potential to breed in different urban habitats, especially indoor habitats because they human blood for feeding and prefer to 37 bite and rest indoor containers. But *Ae. albopictus* mainly found in rural areas and in outdoor containers because they feed on a variety of vertebrates outdoor [17]. These results agreed with those of Udayanga et al., (2020), who found that the presence of sufficient vegetation floured the breeding of *Ae. albopictus* in the areas Kolonnawa, Kandy Municipal Council (KMC), Piliyandala, and Gampola in Sri Lanka [14].

In addition, rainfall, dense vegetation, improper practices of garbage disposal, and lack of public awareness regarding the dengue epidemic were the reasons that resulted in the occurrence of variety of potential breeding habitats for *Aedes* in the three locations. Therefore, more efforts should be undertaken by the general public and government

organizations to minimize/eradicate the breeding habitats of *Aedes* mosquitoes in these areas.

The bioassay test revealed that deltamethrin and malathion can be used for the *Aedes* mosquito control in these three locations of Batticaloa, and among those deltamethrin is more effective. But anyhow further tests are required to confirm the results. Similar results were also observed by Dharshini et al., (2011) in the *Aedes* populations which were collected from Batticaloa municipal limit, where both *Ae. aegypti* and *Ae. albopictus* were highly resistant to 4% DDT and highly susceptible to 0.25% permethrin which is a pyrathroid. It was also mentioned that both species were susceptible to 8% malathion [8]. In the research conducted by Karunaratne et al., (2013) in six districts (Kandy, Kurunagale, Puttalam, Gampaha, Ratnapura, and Jaffna) of Sri Lanka, they have reported that populations of both species (*Ae.aegypti* and *Ae.albopictus*) from all the six districts were resistance to DDT and all the populations were more or less susceptible to malathion except for Jaffna *Ae.aegypti* population. All the populations were susceptible to propoxur (carbamate) except *Ae.aegypti* population in Kandy which showed possibly resistance and all the populations except Jaffna *Ae. aegypti* were resistant to permethrin [6].

Insecticide resistance to DDT may occur due to high exposure to the insecticide and also improper usage. From the results, we can found that the resistance to DDT in both dengue vectors is still prevailing even after three decades of cessation of DDT. High susceptibility to deltamethrin may be due to low exposure. After the cessation of DDT, malathion and other organophoshpates were used which are still being used to control the mosquito populations. That may be the reason for the decrease in the susceptibility to malathion. So it is presumed that the findings of the present study would be useful to the health authorities to use appropriate insecticides in these three study locations to delay the onset of resistance and to get the maximum effect in controlling the dengue mosquitoes.

CONCLUSION

This study reported that the three locations are abundant with a variety of potential containers that favors the breeding of *Aedes* sp. mosquitoes. The larval indices present that there is a high dengue epidemic risk in Oddamavadi and Eravur due to *Ae. aegypti*

and there is a moderate risk in Vantharumoolai due to both *Aedes* species. Therefore, more attention is required in these areas to suppress the emerging dengue risk. From the bioassay test, it can be concluded that deltamethrin could be more effectively used in dengue vector control in these study sites.

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