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Assessing Dengue Vector Seasonality and Insecticide Susceptibility: Findings from an Ovitrap Survey in Colombo District, Sri Lanka

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Key words: **Dengue, Aedes, Seasonality, Susceptibility**

Abstract

Introduction

With a high incidence of dengue in the Colombo District of Sri Lanka, recorded as 17,456 cases in 2022 and 18,242 cases in 2023. Maharagama recorded 1,095 dengue cases in 2018, 2,292 cases in 2019, and 1,453 cases in 2022.

Objectives

The study aimed to investigate the seasonal patterns and insecticide susceptibility of *Aedes aegypti* and *Aedes albopictus.*

Methodology

An ovitrap-based survey was conducted in Maharagama MOH area from October 2022 to September 2023. Conventional ovitraps were deployed inside and outside 50 randomly selected houses to capture *Aedes* mosquitoes breeding in both environments, with collections made after five nights. Counted eggs were allowed to hatch and after the identification of larvae were reared under the laboratory conditions. Adults aged 2–3 days were exposed to common insecticides according to the WHO protocol.

Results

Outdoor ovitraps collected a total of 459 *Ae. aegypti* and 6,610 *Ae. albopictus*, while indoor ovitraps collected 566 *Ae. aegypti* and 4,521 *Ae. albopictus*. The *Aedes* ovitrap index was highest in December (62%) and April (55%). *Ae. aegypti and Ae. albopictus* populations fluctuated with the changing seasons. The density of *Ae. aegypti* peaked in March and May, whereas *Ae. albopictus* reached its highest levels in January and April. *Ae. albopictus* showed resistance to 0.8% Malathion, (mortality = 5%), but was susceptible to 4% Malathion, (mortality = 99%). *Ae. aegypti* was fully susceptible to both 5% Malathion and 0.25% Deltamethrin, with a mortality rate of 100% for each.

Conclusion

The persistent occurrence of the primary dengue vector may have contributed to the high prevalence of dengue in the district. Since *Ae. aegypti* is more prone to outbreaks and breeds more prevalently indoors (55%) than outdoors, this should be considered when formulating dengue vector control programs

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Introduction

Dengue is a major mosquito-borne disease prevalent in tropical and subtropical regions worldwide. *Aedes* mosquitoes (Diptera: Culicidae) serve as primary vectors for various arboviral diseases, including dengue, yellow fever, chikungunya, and Zika virus infections. The infection is caused by any of four closely related dengue virus serotypes: DENV-1 to DENV-4. Nearly half of the global population is currently at risk of dengue, with an estimated 100 to 400 million infections reported annually [1]. Dengue cases in Sri Lanka have risen at an alarming rate in recent years and the Western Province faces the highest dengue threat, with frequent outbreaks being reported (NDCU, 2022, Epidemiology Unit, 2012). Dengue was first serologically confirmed in Sri Lanka in 1962, and the first island-wide outbreak occurred in 1965 [2]. With a high incidence of dengue in the Colombo District of Sri Lanka, recorded as 17,456 cases in 2022 and 18,242 cases in 2023. Maharagama recorded 1,095 dengue cases in 2018, 2,292 cases in 2019, and 1,453 cases in 2022.

In Sri Lanka, *Aedes aegypti* Linnaeus and *Ae. albopictus* Skuse have been incriminated as vectors of dengue [3,4]. Vector control plays a key role in the prevention and management of dengue, particularly in the absence of an effective vaccine or treatment. Although eliminating vector breeding sites is a key strategy for vector control, its success is often hindered by limited resources and insufficient community support. As a result, insecticides play an important role in dengue prevention, with the four major classes being organochlorines, carbamates, organophosphates, and pyrethroids. [5,6].

The widespread use of insecticide-based control methods has led to the development of insecticide resistance, resulting in greater challenges in eliminating *Aedes* mosquitoes and, consequently, an increased risk of dengue transmission [7]. Monitoring vector bionomics is vital for understanding transmission dynamics, informing targeted control strategies, optimizing resource allocation, enhancing surveillance systems, and engaging communities in preventive measures.

The Colombo district, located in the wet zone of the Western province, is a significant region in the country, particularly in terms of its economic contribution. However, its high level of urbanization has made it vulnerable to urban diseases like dengue, which have persisted for many years. With a high incidence of dengue in the Colombo District of Sri Lanka, recorded as 17,456 cases in 2022 and 18,242 cases in 2023. Maharagama Medical Officer of Health area (MOH) recorded 1,095 dengue cases in 2018, 2,292 cases in 2019, and 1,453 cases in 2022. Within the district, the Maharagama MOH area stands out as a major hotspot for dengue transmission, with a significant level of insecticide usage. Consequently, the current study aimed to assess the seasonality of dengue vectors and evaluate their susceptibility to insecticides.

Methods

Study Site

Maharagama (6° 50′ N: 79° 55′ E) is a highly populated urban residential area. The study site, Pamunuwa was situated in close proximity to Maharagama town, which served as the adjacent environment.

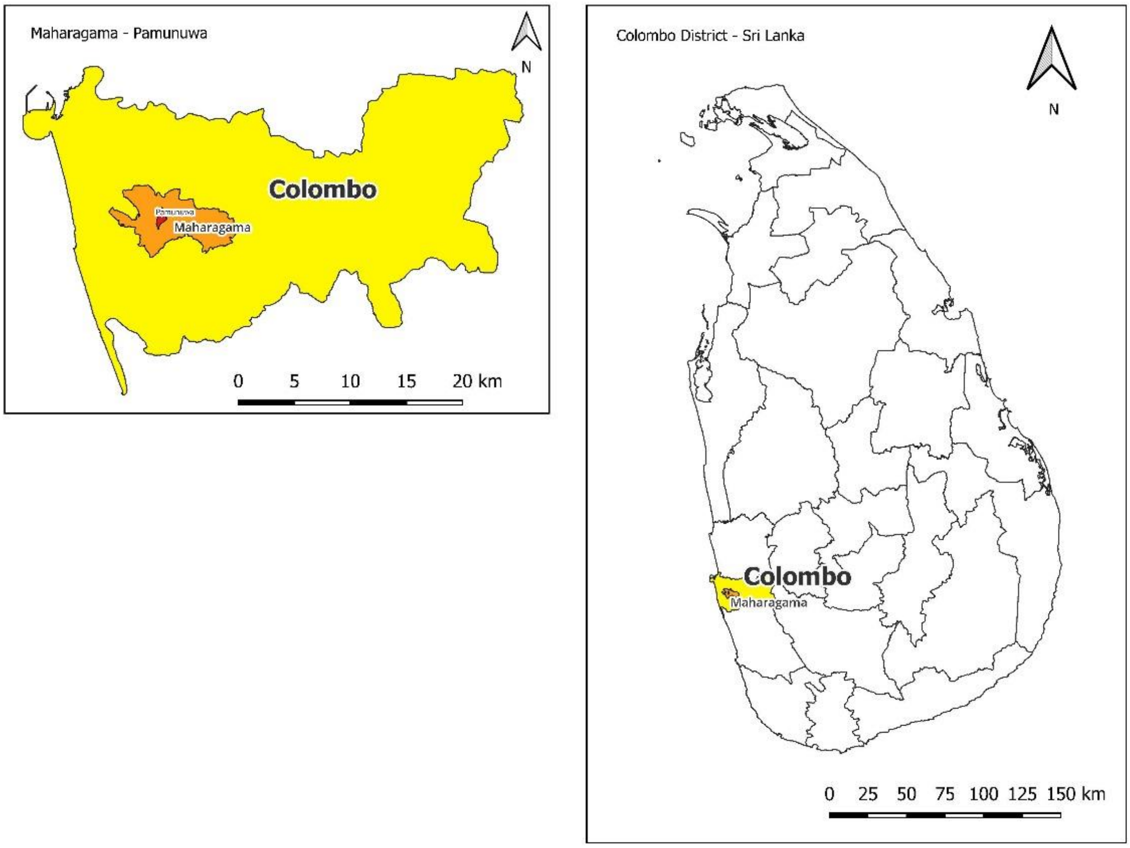

Figure 1. GPS map of the study sites (red colored area), in Maharagama, Colombo district, Sri Lanka.

Collection of Immature *Aedes* mosquitoes

Immature stages (F_0 generation) of *Ae. albopictus* and *Ae. aegypti* mosquitoes were sampled from the Pamunuwa PHI area in Maharagama (Figure 1). Standard black plastic ovitraps, as described by Fay [8]) and Service [9], were deployed at residents' premises along with contact details and instructions, with a request not to remove the cups. A total of 100 ovitraps were deployed to capture *Aedes* mosquitoes across 50 randomly selected households, with each household receiving two traps: one placed indoors and the other outdoors. Outdoor ovitraps were positioned in shaded areas with vegetation, while indoor traps were placed in the kitchen (beneath the sink), bedrooms (under the bed) or living areas (under the furniture). Each coded ovitrap, with a capacity of 250 ml, contained 100 ml of water and cloths. The rough cloth surface along the cup wall was used as an oviposition substrate to facilitate mosquito egg-laying. After collection, the cups were wiped with a damp paper towel to remove any eggs from the interior. Collections were carried out on a monthly basis from October 2022 to September 2023. The collected ovitraps were transported to the laboratory.

Egg Counting and Rearing

After checking all the ovitraps, the positive ones were collected and left to dry. The eggs in each trap were counted under a dissecting microscope (Labomed CZM4 Binocular Zoom Microscope) and then returned to the same ovitrap. 250 ml of Dechlorinated water were added to each positive ovitrap, and the eggs were allowed to hatch. Larvae were fed fish food granules daily, and third and fourth-instar larvae were observed under a light microscope (Olympus CX31, Japan) and identified [10]. The total number of larvae in each ovitrap was recorded and separated into *Ae. aegypti* and *Ae. albopictus*. The larvae were then placed in two labeled enamel trays (30 x 45 x 6.5 cm) with 2 L of dechlorinated tap water, kept under laboratory conditions. Feeding continued until pupation. The pupae were transferred to adult emergence cages (30 cm × 30 cm × 30 cm), and 3-5-day-old female mosquitoes were used for experiments [1].

Adult Insecticide Susceptibility Test

Adult bioassays were conducted using 3- to 5-day-old female *Ae. aegypti* and *Ae. albopictus* mosquitoes, exposed to insecticide-impregnated papers in standard test kits, following WHO guidelines [11]. The insecticide-impregnated papers used in this study were obtained from the National Dengue Control Unit, Sri Lanka. 20 female mosquitoes were placed in a holding tube in an upright position for one hour. Mosquitoes were blown through the open slit to the exposure tubes and kept for one hour. When testing for pyrethroid chemicals, the number of knocked-down mosquitoes was recorded at 10, 15, 20, 30, 40, 50, and 60 minutes. After one hour of exposure, the mosquitoes were carefully transferred into holding tubes. A 10% sugar solution was provided as food on top of the mesh in each tube. The tubes containing mosquitoes were maintained in the laboratory at 27 ± 2 °C and 70 ± 10% relative humidity for 24 hours. For each chemical, 100 mosquitoes were exposed to the chemical, while 40 mosquitoes were used as controls. Control experiments were conducted using both untreated papers and solvent-only papers without insecticides [11]. Susceptibility tests were conducted using 0.4%, 4%, and 5% Malathion and 0.25% Deltamethrin.

Meteorological Data

Rainfall, temperature, and relative humidity data were obtained from the Meteorological Department in Colombo.

Analysis

A t-test was conducted to assess differences in the breeding preferences of *Aedes* mosquitoes between indoor and outdoor environments. Regression analysis was performed to examine the correlation between *Aedes* mosquito density and rainfall. Additionally, the percentage of positive ovitraps was calculated. Insecticide susceptibility results were interpreted according to WHO guidelines [11]. Mosquitoes were classified as resistant (R) if the mortality was less than 90%, suspected resistant if the mortality rate was between 90–97%, and susceptible (S) if the mortality rate was greater than 98%.

Results

Seasonality and Breeding Preferences of *Ae. aegypti* and *Ae. albopictus*

A total of 12,156 *Aedes* mosquito larvae were emerged from the collected ovitraps during the study period. Outdoor ovitraps collected a total of 459 *Ae. aegypti* and 6,610 *Ae. albopictus*, while indoor ovitraps collected 566 *Ae. aegypti* and 4,521 *Ae. albopictus* larvae.

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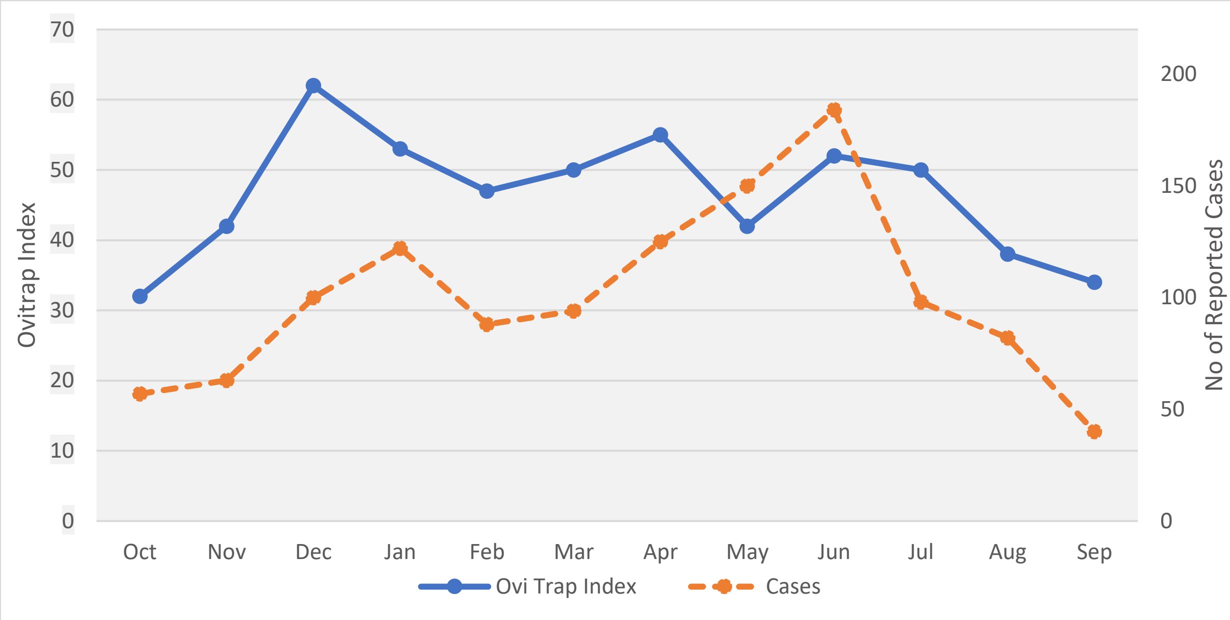

Figure 2: Ovitrap Index and the reported Dengue cases in Maharagama.

The *Aedes* ovitrap index was highest in December (62%) and April (55%). No of reported dengue cases were highest in January (122) and June (184).

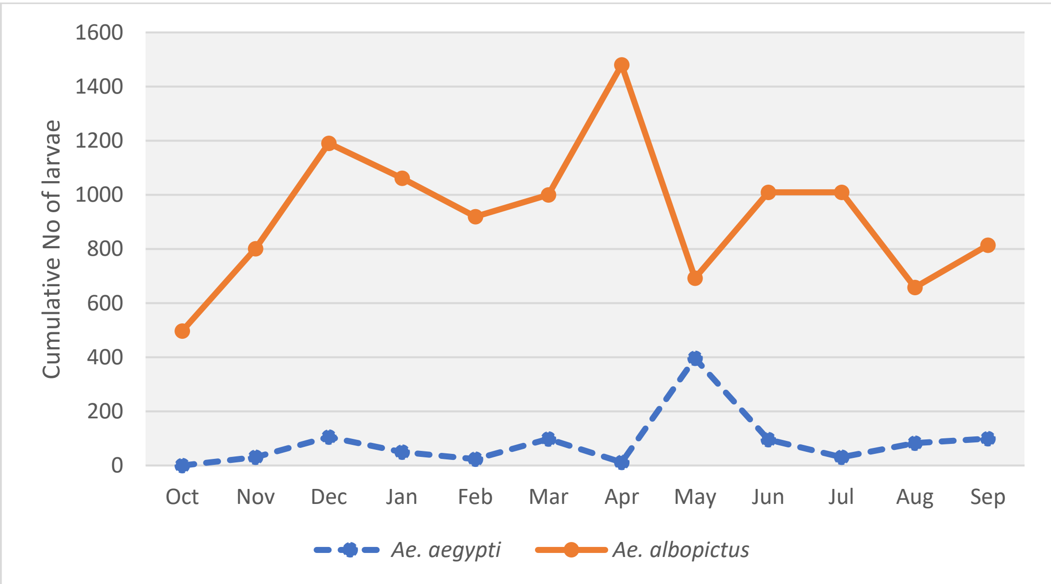

Figure 3: Temporal distribution of *Ae. aegypti* and *Ae. Albopictus*

Ae. aegypti and Ae. albopictus populations fluctuated with the changing seasons. The density of *Ae. albopictus* was comparatively higher than that of *Ae. aegypti*. However, the density of *Ae. aegypti* peaked in May, whereas *Ae. albopictus* reached its highest levels in April.

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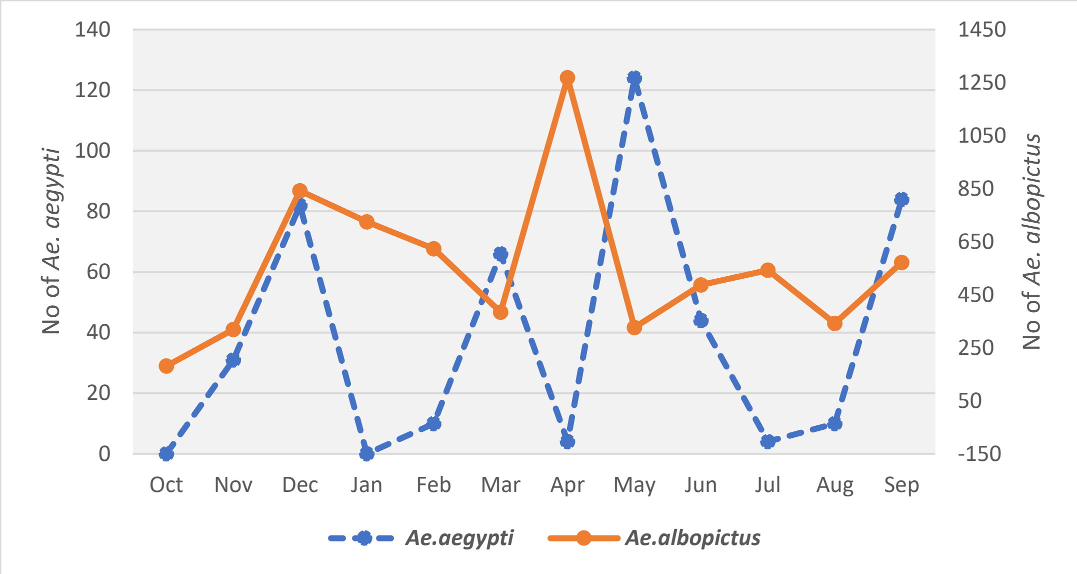
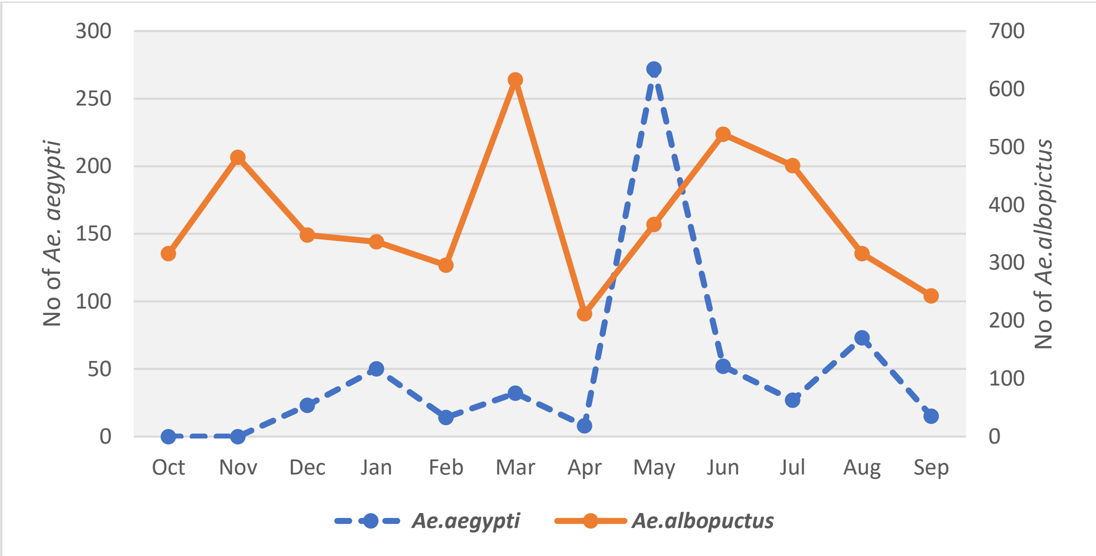


A



B

Figure 4: Monthly variation of collected larvae in outdoor (A) and indoor (B) ovitraps

The outdoor density of *Ae. aegypti* peaked in May and September, while that of *Ae. albopictus* was highest in April and December. During dry periods (March, June, and July), a high density of *Ae. albopictus* is observed indoors due to the limited availability of outdoor breeding sites. (Figure 4 B).

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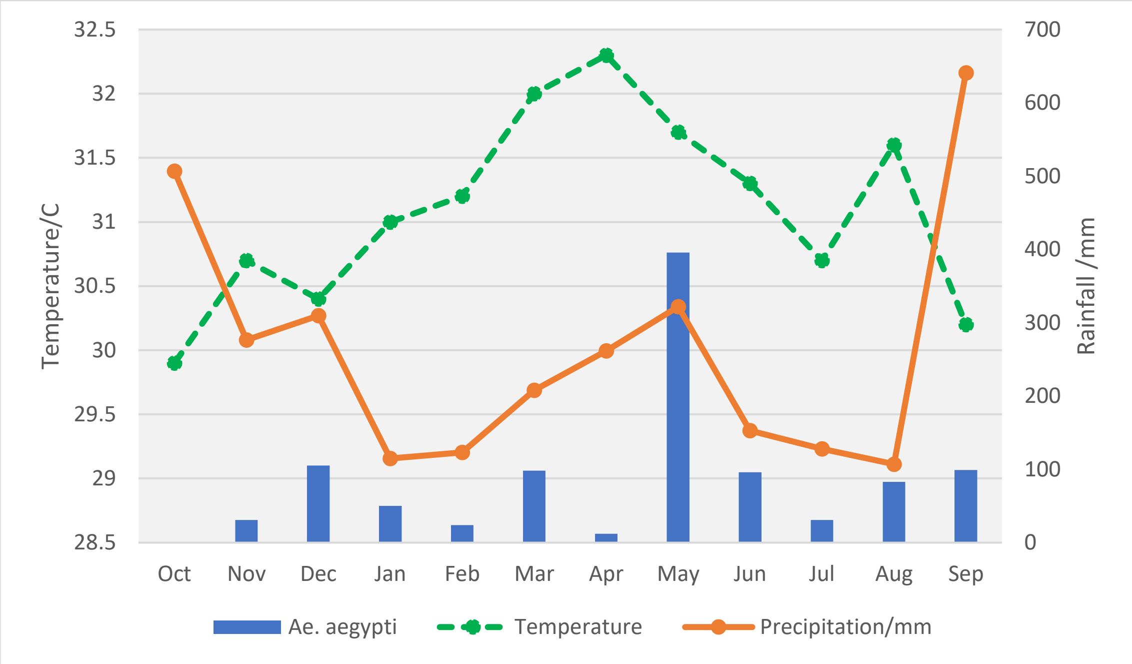

Figure 4: *Ae.aegypti* population density with the temperature and rainfall

The highest rainfall occurred in May and September, while the highest temperatures were recorded in March and April.

Table 1: Regression analysis (Pearson's correlation) of population density of *Ae.aegypti* mosquitoes with climatic variables in study site.

Climatic variables	Pop. density of *Aedes* mosquitoes		95% CI	
	R	P- Value	Lower	Upper
Rain fall (mm)	0.117	0.358	-0.548	0.757
RH average (%)	0.222	0.244	-17.028	34.510
Temperature average (°C)	0.268	0.200	-50.410	193.486
No. of rainy days	0.183	0.285	-57.923	30.365

To assess the impact of weather variables on the density of *Ae.aegypti* mosquitoes a regression analysis was conducted. This analysis determines how various climatic factors such as temperature, rainfall and relative humidity influenced the population dynamics of dengue vectors. Statistical analysis revealed a positive, non-significant correlation ($p>0.05$) between *Ae.aegypti* density and rainfall, relative humidity, number of rainy days as well as temperature (Table 1). T- test was carried out and there was no statistically significant difference ($p > 0.05$) in the breeding preference of both species between indoor and outdoor environments.

Insecticide Susceptibility

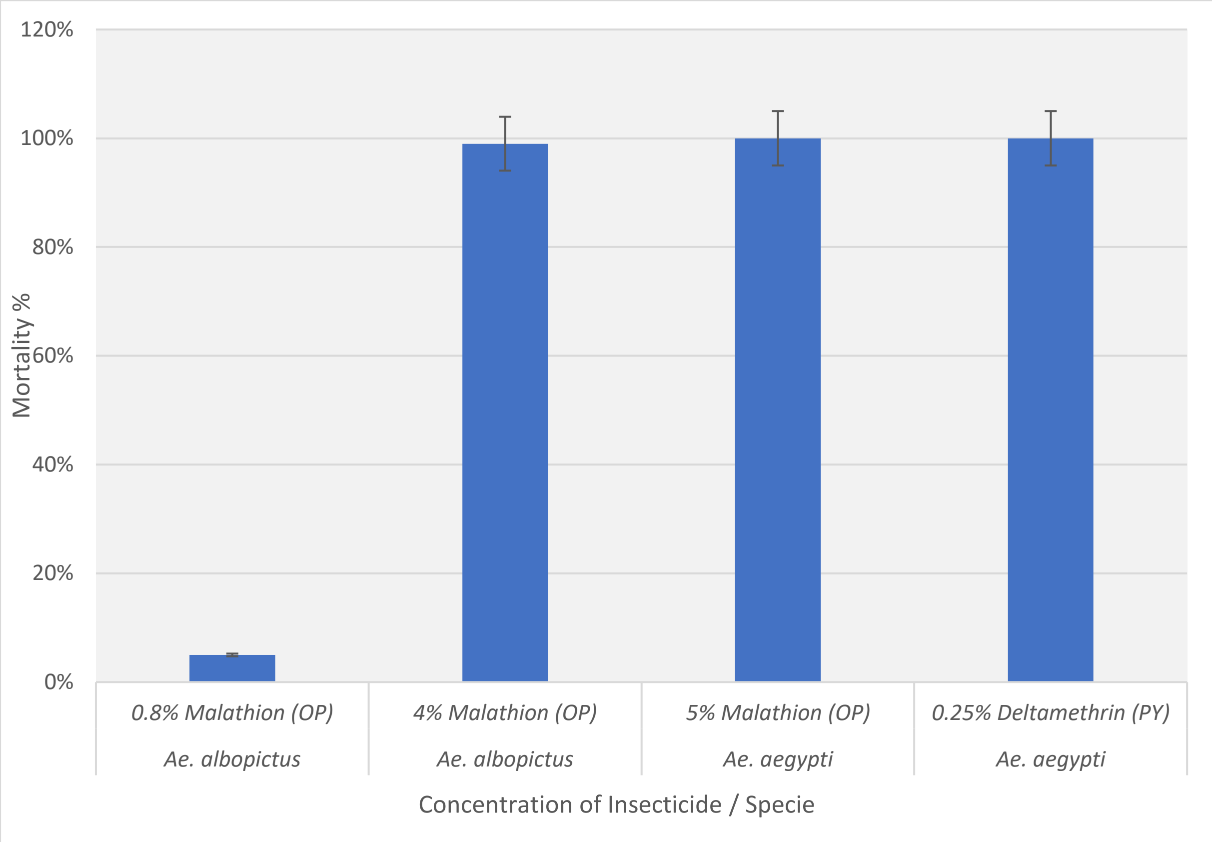

Figure 5: Susceptibility status of different chemicals of *Aedes* mosquitoes

Ae. albopictus exhibited resistance (5% mortality) to 0.8% Malathion, the lowest tested concentration, but was susceptible (99% mortality) at 4% Malathion. In contrast, *Ae. aegypti* was fully susceptible (100% mortality) to 5% Malathion. Furthermore, *Ae. aegypti* also demonstrated complete susceptibility (100% mortality) to 0.25% Deltamethrin.

Discussion

Continuous monitoring of vectors is essential for designing effective strategies to prevent and control dengue transmission. This study highlights the seasonal variations and insecticide susceptibility status of *Aedes* mosquitoes in Maharagama, Colombo district. Our findings revealed that an increase in the ovitrap index was associated with a rise in dengue cases within 6 to 8 weeks. Similar results were reported by De Melo *et al*. [12] who found that ovitrap surveillance is an effective method for predicting dengue incidence with high accuracy, up to two months in advance. Mosquito production is influenced by the availability of suitable and adequate habitats for the larval stages, which largely depends on rainfall [13].

The Colombo district experiences two main monsoon seasons: the Southwest monsoon from May to September and the Northeast monsoon from December to February. These seasonal rains create optimal conditions for *Aedes* mosquito breeding, leading to increased vector populations and higher dengue incidence during and shortly after these periods. Current study revealed that the species-wise distribution, the highest density of *Ae. albopictus* was recorded in April, whereas the highest density of *Ae. aegypti* was observed in May. Moore *et al*, [14]1978 and Aiken *et al,* [15] 1980 also suggest that the density of dengue vectors are correlated to the seasonal variability. Temperature can impact the survival of both adult and immature stages of the *Ae. albopictus* population [16]. Relative humidity affects mosquito longevity, mating, dispersal, feeding behavior, egg production, oviposition, and dengue virus transmission [17,18]. High humidity, particularly due to rainfall, creates favorable environmental conditions that promote mosquito development, survival, and enhance its viral capacity [19]. Rainfall followed by the formation of mosquito breeding habitats led to a significant increase in the emergence of *Aedes* population [20]. It is important to note that rainfall contributes to the creation of various secondary breeding sites, which are often overlooked and not included in routine intervention measures. These sites include discarded tires, blocked gutters, slabs, bottles, containers, and any water-holding surfaces that provide suitable oviposition habitats for *Ae. aegypti* and *Ae. albopictus.*

The study demonstrated distinct breeding preferences between *Ae. albopictus* and *Ae. aegypti*. *Ae. albopictus* exhibited a strong preference for outdoor habitats, with 6,610 larvae collected from outdoor ovitraps compared to 4,521 indoors. In contrast, *Ae. aegypti* displayed a more balanced distribution, with 459 larvae found in outdoor traps and 566 indoors. These findings suggest that *Ae. albopictus* predominantly utilizes outdoor breeding sites, while *Ae. aegypti* is more adaptable to urban environments, utilizing both indoor and outdoor habitats effectively. However, our findings revealed no statistically significant difference in the breeding preferences of both species between indoor and outdoor environments ($p > 0.05$). Similar results were reported in a study conducted in Jaffna, Sri Lanka [20].

In Sri Lanka, space spraying is predominantly carried out using pyrethroid insecticides, organophosphate to manage adult vector populations (NDCU, 2016, 2018). Recent studies indicate that *Aedes* mosquitoes in numerous countries worldwide are developing resistance or showing decreased susceptibility to synthetic insecticides [21,22]. The present study also identified insecticide resistance at the lowest concentration (0.8%) of Malathion (an organophosphate). Given that Maharagama is a dengue transmission hotspot, space spraying has been extensively employed in the area. Similar findings were observed in recent studies conducted in the Gampaha and Colombo districts [23,24]. Resistance in *Aedes* mosquitoes was not observed at other insecticide concentrations. The development of significant resistance to the currently used insecticide categories presents challenges for dengue control strategies. Therefore, further studies are essential to assess the susceptibility of *Aedes* mosquitoes to commonly used insecticides.

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