DEVELOPMENT OF LARVAL STAGES OF *Aedes aegypti* IN POLLUTED WATER HABITATS IN COLOMBO DISTRICT AND THEIR SUSCEPTIBILITY TO TEMEPHOS INSECTICIDE

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Dengue fever, primarily transmitted by *Aedes aegypti* and *Aedes albopictus,* affects approximately 400 million people worldwide annually. In Sri Lanka, recurring outbreaks have occurred since the 1960s, with severe epidemics in Colombo, especially in 2017. Recent findings indicate that *Aedes aegypti* can breed in polluted water and develop resistance to insecticides such as temephos. This study investigated the developmental characteristics and temephos susceptibility of *Aedes aegypti* larvae reared in polluted water (PW) and freshwater (FW) under laboratory conditions to assess the role of polluted habitats in mosquito larval development. Field-collected eggs were reared to the fourth (F4) laboratory generation, with water quality (temperature, dissolved oxygen, pH, conductivity, total dissolved solids, salinity) assessed for both PW and FW. Morphometric analysis of all larval instars included head, thorax, antenna, siphon, anal papillae, abdominal features, and total body length using binocular microscopy. Temephos susceptibility was evaluated using five concentrations, ranging from 0.0125 mg/L to 0.625 mg/L, on third- and early fourth-instar larvae. Testing involved 20 larvae per concentration across four replicates, with mortality assessed after a 24-hour exposure period. Statistical analysis employed log-probit methods to determine lethal concentration values. Results revealed that both populations exhibited temephos resistance, with PW larvae showing significantly higher resistance levels. LC_{50} values were 0.019 mg/L (95% CI: 0.014-0.027) for FW versus 0.046 mg/L (95% CI: 0.00-0.109) for PW populations. LC_{99} values reached 0.102 mg/L (95% CI: 0.055-0.660) for FW and 0.602 mg/L (95% CI: 0.306-23.933) for PW populations. Morphometric analysis revealed significant differences in PW larval siphon and anal papillae development, showing faster growth and distinct developmental patterns compared to the consistent progression of FW larvae. These results indicate that polluted habitats foster temephos resistance and morphological adaptations in *Aedes aegypti*, highlighting the need for revised vector control and integrated management strategies to prevent dengue transmission.

Keywords: *Aedes aegypti*, development, morphometry, polluted water, susceptibility, temephos, vectors.

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INTRODUCTION

Dengue fever is a mosquito-borne viral disease caused by four dengue virus serotypes (DENV 1-4) transmitted primarily by *Aedes aegypti* and *Aedes albopictus* mosquitoes (Palanichamy et al., 2023). The disease affects approximately 3.6 billion people in high-risk areas globally, with 400 million annual infections and 10,000 deaths. Dengue ranges from asymptomatic to severe hemorrhagic fever and shock syndrome (Wang et al., 2020). Originally confined to small areas, now endemic in over 125 countries across tropical and subtropical regions. Current control relies on vector management through source reduction, environmental management, chemical control, and biological methods, as effective vaccines and treatments remain limited (Palanichamy et al., 2023). Recent studies show *Aedes aegypti* adapting to polluted and saline water, expanding potential breeding sites and complicating control efforts (Fernando et al., 2020; Chandrasiri et al., 2019; Surendran et al., 2019).

OBJECTIVES

To study the effects of polluted water on *Aedes aegypti* larval development through quantitative assessment of morphometric analysis, and to examine the susceptibility of *Aedes aegypti* larvae to temephos by comparing those raised in polluted water (PW) with those reared in freshwater (FW).

METHODOLOGY

This study used ovitraps to collect *Aedes aegypti* eggs from 20 houses in Orugodawatta, Colombo, with four traps per house containing fresh and polluted water. Traps were placed in shaded locations and eggs collected fortnightly for three months. Larvae hatching out from collected eggs were reared in laboratory conditions using polluted canal water and dechlorinated tap water. Larvae were fed with fish meal powder daily at densities of 200 per tray. Water quality parameters, including temperature, dissolved oxygen, pH, conductivity, total dissolved solids, and salinity, were monitored throughout the process.

Pupae were transferred to two separate adult cages to establish laboratory populations from both polluted and freshwater larvae. Adults were fed 5%–10% glucose solutions and blood using Balb/c mice to obtain four generations (F4). Larval development was compared between FW and PW environments across all four larval instars. Ten same-age larvae per stage were examined using a microscope at 40 and 100 magnifications. Thirteen morphometric parameters, including head, thorax, antenna, siphon, anal papillae, and body dimensions were measured, with Dyar's ratio (Hernández et al., 2023) calculated for developmental assessment. Temephos susceptibility was assessed in *Aedes aegypti* larvae from FW and PW using five concentrations (0.0125 mg/L, 0.025 mg/L, 0.125 mg/L, 0.25 mg/L, and 0.625 mg/L) of temephos dissolved in ethanol. Dechlorinated tap water and canal water were used as controls. Third and early 4th instar larvae were tested in quadruplicate (n=80) with 24-hour mortality assessment. Testing followed the Sri Lankan National Dengue Control Unit and WHO standard protocols for *Aedes* insecticide resistance monitoring.

Data analysis was conducted using IBM SPSS Statistics Version 29.0.2.0. Temephos susceptibility data were analyzed using log-probit regression to determine LC_{50} and LC_{99} values with 95% confidence intervals (Kokila et al., 2023). Morphometric measurements were obtained using ImageJ software and assessed for normality with the Shapiro-Wilk test. Non-parametric Kruskal-Wallis ANOVA was applied to compare morphometric differences between freshwater and polluted water populations (Ananya et al., 2013; Heng et al., 2013).

RESULTS AND DISCUSSION

Morphometric analysis revealed limited significant differences between freshwater (FW) and polluted water (PW) larvae populations. Shapiro-Wilk normality tests identified significant variations only in specific parameters: anal papillae (length and width), head length, and thorax length between PW and FW colonies. Most other morphometric measurements showed minimal variance between populations.

Dyar's ratio analysis demonstrated consistently higher values in PW populations compared to FW, except for siphon length (Figure 1). The most pronounced differences occurred in anal papillae length and width, head capsule width, and total body length, indicating enhanced growth rates in PW larvae, particularly for anal papillae development.

Water quality assessment revealed distinct environmental conditions between breeding habitats. FW parameters included: temperature 32.2 ± 0.9°C, dissolved oxygen 5.42 ± 0.59 ppm, pH 7.32 ± 0.28, conductivity 109
± 8 µS/cm, TDS 75.6 ± 5.3 ppm, and salinity 0.05 ± 0.01 ppt. PW samples showed: temperature 32.9 ± 0.7°C, dissolved oxygen 2.75 ± 0.38 ppm, pH
7.91 ± 0.54, conductivity 673 ± 199 µS/cm, TDS 459.5 ± 149.1 ppm, and salinity 0.33 ± 0.10 ppt. Significant differences ($p < 0.001$) were observed in dissolved oxygen, conductivity, TDS, and salinity between water types,

confirming distinct environmental stress conditions in polluted habitats that may influence larval development and growth patterns.

Bioassay testing demonstrated varying mortality rates between fresh water (FW) and polluted water (PW) *Aedes aegypti* larvae across temephos concentrations ranging from 0.0125 to 0.625 mg/L. Control groups maintained zero mortality with pupation rates below 6%, confirming test validity.

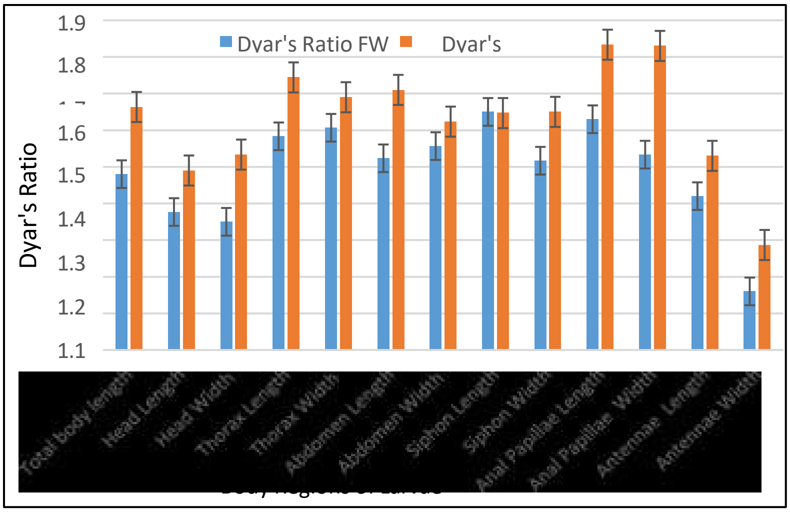

Figure 1: Dyar's ratio of Aedes aegypti FW and PW colonies

Table 1: Susceptibility of L3 and early L4 larvae from FW and PW colonies over exposure of 24 hours

Colony	After 24h exposure to temephos	
	LC_{50} (95% CI) mg/L	LC_{99} (95% CI) mg/L
FW	0.019 (0.014-0.027)	0.102 (0.055-0.660)
PW	0.046 (0.00-0.109)	0.602 (0.306-23.933)

According to the discriminative dose of temephos recommended by WHO, which is 0.0125mg/L, *Aedes aegypti* larvae in polluted water show extreme temephos resistance. Pollution-adapted larvae are smaller but develop faster with enlarged detoxification structures like anal papillae. Environmental stress from contaminated urban breeding sites might trigger physiological adaptations, including enhanced enzymes and modified sodium channels. This resistance evolution severely compromises dengue vector control efforts.

CONCLUSION AND RECOMMENDATIONS

Water quality analysis revealed that polluted water (PW) had significantly reduced dissolved oxygen, and 6-7 times higher conductivity, total dissolved solids, and salinity compared to freshwater (FW). *Aedes aegypti* larvae showed remarkable developmental plasticity in PW, with higher Dyar's ratios across all morphometric parameters, particularly in respiratory and osmoregulatory structures like anal papillae and antennae. PW larvae demonstrated dramatically increased temephos resistance, requiring nearly six times higher concentrations at LC_{99} levels (0.602 vs 0.102 mg/L). These systemic adaptations enable mosquito persistence in polluted urban habitats and highlight the need for alternative vector control strategies beyond conventional insecticide approaches.

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