RESEARCH ARTICLE

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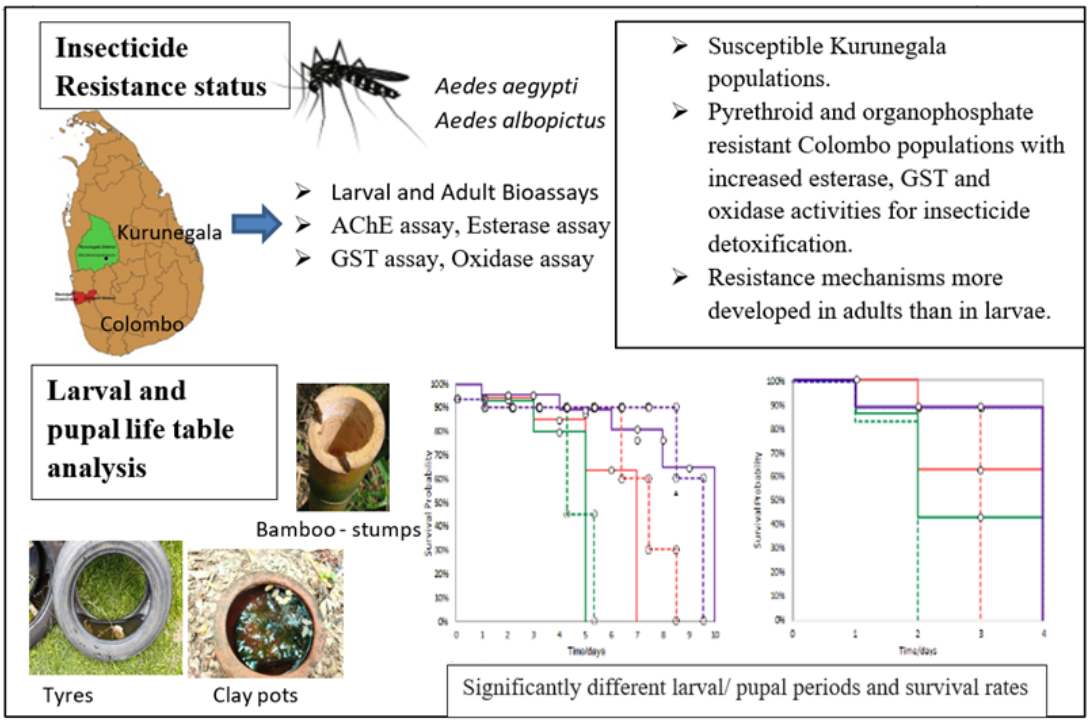

Highlights

- Dengue vector mosquitoes from Colombo, Sri Lanka are resistant to insecticides.
- *Aedes aegypti* is more resistant to insecticides than *Ae. albopictus.*
- Insecticide resistance mechanisms are more developed in adult mosquitoes compared to their larvae.
- Growth rate and survivorship of *Ae. aegypti* immature stages are higher compared to *Ae. albopictus* immature stages.

Ceylon Journal of Science 53 (2) 2024: 161-170
DOI: http://doi.org/10.4038/cjs.v53i2.8301

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Stage-specific insecticide susceptibility and life-table analysis of the dengue vector mosquitoes *Aedes aegypti* and *Ae. albopictus*

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Received: 24.12.2023; Accepted: 08. 03.2024

Abstract: Vector resistance to insecticides is a significant challenge in the control of mosquito-borne diseases such as dengue. Analysis of the growth rate and survivorship of the immature stages of larvae has been important in formulating effective vector control strategies. The present study aimed to assess insecticide resistance in both larvae and adults of *Aedes aegypti* and *Ae. albopictus,* and the life tables of their immature stages.

Mosquito eggs collected from Kurunegala and Colombo districts of Sri Lanka from January to March 2023 were reared under laboratory conditions. Larval bioassays were carried out with temephos (0.0125 ppm, 0.0625ppm and 0.125ppm) and, the adult bioassays were with pyrethroids (0.03% deltamethrin, 0.05% and 0.08% lambdacyhalothrin, 0.04% permethrin) and malathion (1%and 5%). Biochemical assays were used to evaluate the activity of insecticide-detoxifying enzymes and the insensitivity of the organophosphate target site acetylcholinesterase. Survivorship of immature stages under laboratory conditions and semi-field conditions *i.e.,* bamboo stumps, tyres and clay pots, were studied using Kaplan-Meier survival analysis.

Larvae and adults of both *species* from Kurunegala were susceptible to all the insecticides tested, whereas Colombo populations were resistant. Acetylcholinesterases were largely sensitive in all vector populations. Higher activities of esterases, glutathione s-transferases and monooxygenases were found in Colombo populations. Results indicated that *Ae. aegypti* larvae were more resistant to insecticides than *Ae. albopictus* larvae. Also, the mechanisms of insecticide resistance were well developed in adults than in larvae.

Aedes aegypti larval development time (5 days) was significantly shorter than that of *Ae. albopictus* (9 days) under laboratory conditions. Survival probabilities of them were 76-80%. Egg hatching to adult emergence period was 8 days for *Ae. aegypti* and 13 days for *Ae. albopictus*. This period, under semi-field conditions were; bamboo stumps 7-7.2 days, tyres 10-11.3 days, and clay pots 13.8-14 days with survival rates varied from 28.8 to 63% for both species. This study highlights stage specific response of dengue vectors to insecticides and survivorship of their immature stages in different habitats. This knowledge can be effectively used in future dengue vector control programmes.

***Keywords*:** *Aedes aegypti, Aedes albopictus*, insecticide resistance, stage-specific enzyme expression, life table analysis

INTRODUCTION

Dengue is a major mosquito-borne arboviral disease transmitted to humans by *Aedes* mosquitoes (WHO, 2005). More than 80% of the world's population lives in areas with the transmission of this disease. Vector control plays a key role in reducing the dengue disease burden in the absence of proper vaccines or treatments. Neuroinhibitory insecticides, delivered as larvicides or space spraying adulticides are widely used to control dengue vectors, especially during disease outbreaks. Organophosphates are used for larviciding and, both pyrethroids and organophosphates are used for space spraying. As a result of extensive exposure to insecticides, resistance to insecticides has emerged in vector mosquitoes and spread across all regions of the world (WHO, 2018a, Moyes et al., 2017, Karunaratne et al., 2018).

In Sri Lanka, reported cases of dengue have increased at an alarming rate recently (NDCU, 2022). Reduction in disease transmission is primarily attempted through the control of the vectors *Aedes aegypti* and *Ae. albopictus*. Although the elimination of vector breeding places is a major vector control strategy, lack of resources and poor community support hinder its success. Therefore, chemical control methods are used extensively for vector management (Sirisena et al., 2016). In Sri Lanka, health authorities use the organophosphate temephos (Abate®) as a larvicide and pyrethroids (PestGuard® 161: d-tetramethrin 4% + cyphenothrin 12%, and Gokilaht 10MC: cyphenothrin 10.4%) as adulticides (Fernando et al., 2020). Organophosphate malathion was previously used as an adulticide until 2009 (NDCU, 2022, Fernando et al., 2020). Resistance has been reported to neuroinhibitory insecticides in both *Ae. aegypti* and *Ae. albopictus* from different regions of the country (Nugapola et al., 2020, Fernando et al., 2018, Fernando et al., 2020, Hegoda et al., 2017).

Insecticide resistance is achieved by mosquitoes mainly through two major mechanisms; target site insensitivity and increased detoxification (Karunaratne et al., 2018).

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Target sites become insensitive due to gene mutations which prevent the target site from binding to insecticides. Increased detoxification is achieved through increased activity of insect metabolic enzymes. Quantitative or qualitative changes of these enzymes in resistant mosquitoes cause insecticide detoxification at a much faster rate than done by their counterparts in susceptible mosquitoes. There are three groups of such enzymes; monooxygenases (also known as oxidases or cytochrome P450s), esterases and glutathione S-transferases (GSTs) (Hemingway et al., 2004, Karunaratne et al., 2018).

Vector resistance to insecticides and the prevalence of resistance mechanisms can vary markedly across vector populations in different regions, even within a country, reflecting the levels of selection pressure exerted by insecticide usage. Local vector populations can become resistant to several insecticides at any given time and they will not revert to susceptibility, at least not at a rate that is of immediate relevance to programmatic decision-making. Understanding the type and level of resistance of local vector populations to insecticides in current use, or planned for use, is crucial to ensure the efficacy of the interventions (WHO, 2018b).

Survivorship analysis of the target species provides important data on the mortality, survival, and life expectancy of a vector species and has been important in formulating effective mosquito control strategies (Carey, 2001; Aida et al., 2011; Afrane et al., 2007; Carron et al., 2008).

The present study aimed to determine the survivorship of dengue vector immature stages and to compare insecticide resistance status between immature stages and adults collected from two different regions of Sri Lanka. Stage-specific expression of insecticide metabolizing enzymes and the presence of insensitive target site acetylcholinesterases were also examined in these two populations.

METHOD

Mosquito collection

Aedes aegypti and *Ae. albopictus* eggs were collected weekly using 50 ovitraps, placed indoors in randomly selected 50 houses from Bandaranayakapura in Kurunegala district and from the Municipal Council area of Colombo district from January, 2023 to March, 2023 (Fig. 01). Study sites were residential areas where dengue incidence occurs year-round with monsoonal peaks. Larviciding using temephos sand granules, and fogging with pyrethroids have been in practice to control dengue transmission over the last 10 years in both these areas. However, exposure of mosquito populations to insecticides can differ probably due to the frequency of application, land-use pattens and other human activities.

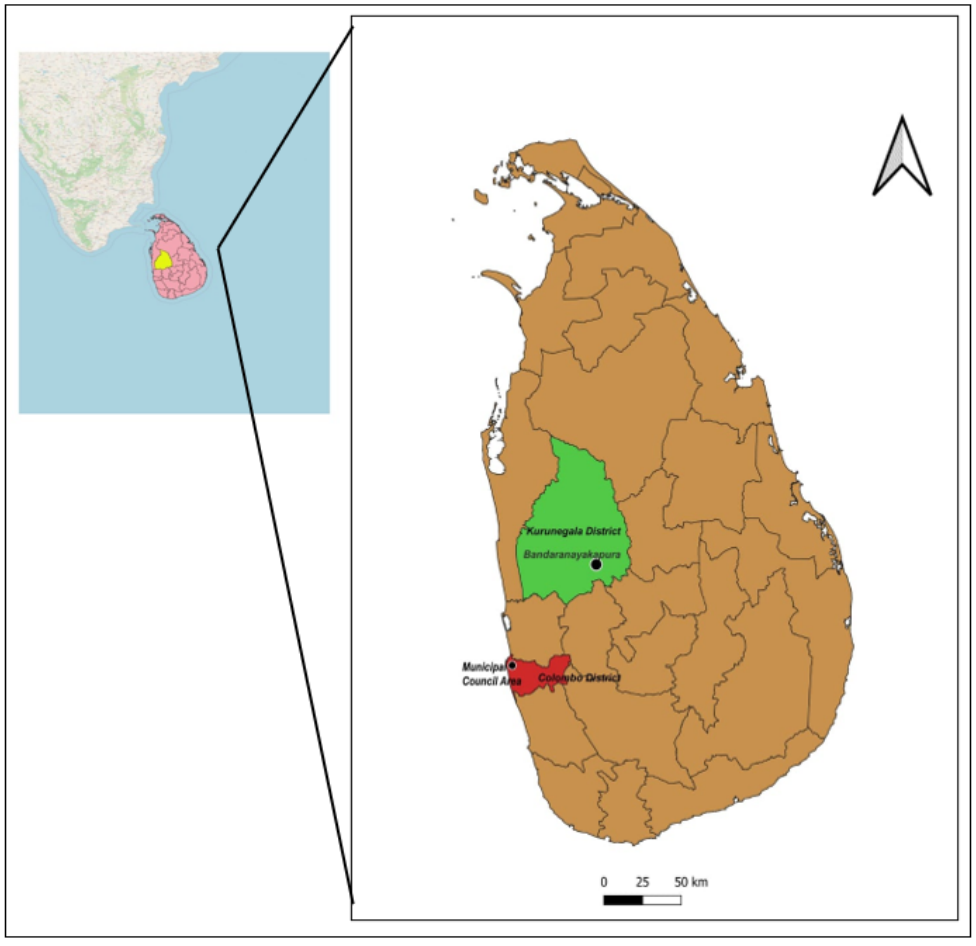

Figure 1: Map of Sri Lanka showing the study sites

Mosquito Rearing and survivorship experiments

Field-collected eggs were placed in separate white colour trays (32.5 x 26.5 x 6.5 cm) with 2 L of dechlorinated tap water under laboratory conditions (temperature = 25.6°C ±1.9; relative humidity = 69.2± 2.1; 12L:12D photoperiod). Once hatched, larvae were transferred into fresh trays with a 1.5 cm depth of water and fed with fish food (Prima fish food with 39% protein, Ceylon Grain Elevator, Sri Lanka) at a rate of approximately 0.1 g per 100 larvae per day. Pupae were transferred into adult emergence cages (30 cm × 30 cm × 30 cm), and 3-5-day-old adult female mosquitoes were used for experiments after species identification (WHO, 2022b). Daily observations were recorded from the larval emergence to pupal emergence. Larval life-table experiments were repeated three times for each species. For the pupal life-table tests, 50 newly emerged pupae were placed in mosquito cages and daily observations were made. Pupal experiments were also repeated three times per each species.

Tyres, clay pots and bamboo stumps were used as breeding habitats for semi-field experiments. They were placed in three separate sets, each set comprising one from each type. They were filled with dechlorinated water and left in the field for three days to simulate natural conditions. Laboratory-reared 1st instar larvae from each species (50 larvae per container) were carefully placed in each habitat type and covered with a nylon mesh to prevent mosquito egg laying. All experimental settings were kept in semi-open locations under natural temperature and humidity, and half-shed lighting. Larval development was recorded daily and the larvae were not fed artificially. After the pupal emergence, observations were continued for the pupal study.

Larval Bioassays

To evaluate the efficacy of temephos (organophosphate), larval bioassays were carried out for three concentrations i.e., diagnostic dosage for temephos = 0.0125 ppm, x5 diagnostic dosage = 0.0625ppm, and x10 diagnostic dosage = 0.125ppm, following WHO recommendations and protocols (WHO, 2005; 2016). Different concentrations were prepared by diluting the 3.125 ppm WHO stock solution (provided by the National Dengue Control Unit, Sri Lanka) with dechlorinated tap water. In each bioassay experiment, twenty 3rd instar larvae were placed in 100 ml insecticide solution in 250 ml bioassay cups and each concentration was repeated five times. Control experiments were carried out using dechlorinated tap water alone. After a 1hr exposure period, dead larvae were counted and recorded. Mortality values were adjusted according to control mortalities using Abbott's formula (WHO, 2016; 2022a). Test results were rejected if the control mortality exceeded 20%.

Adult Bioassays

Adult bioassays were performed using three- to five- day old female mosquitoes of *Ae. aegypti* and *Ae. albopictus,* by tarsal contact exposure to insecticide-impregnated papers in standard test kits according to WHO guidelines (WHO, 2016; 2022c). Insecticide-impregnated papers and control papers were obtained from the Vector Control Research Unit, University of Sains Malaysia. Susceptibility levels to 0.03% deltamethrin (discriminating dosage of deltamethrine for both species), 0.05% lambdacyhalothrin (discriminating dosage for *Ae. aegypti*), 0.08% lambdacyhalothrin (discriminating dosage for *Ae. albopictus*), 0.04% permethrine (discriminate dosage for both species) and 1% malathion (discriminating dosage for *Ae. aegypti*), 5% malathion (discriminating dosage for *Ae. albopictus*) were evaluated. For each bioassay, a batch of 20 mosquitoes was exposed to the insecticide-treated paper for one hour and the mortalities were recorded after a 24-hour recovery period. Each experiment was repeated five times (n=100). Control experiments were carried out using solvent alone control papers without insecticides. Mortality values were adjusted according to control mortalities using Abbott's formula (WHO, 2016; 2022c). Test results were rejected if the control mortality exceeded 20%.

Biochemical assays

All the biochemical experiments were carried out according to the procedures outlined by WHO (1998). From each study site, 50 healthy 3rd instar larvae and 50 healthy adult mosquitoes from each species were individually subjected to total protein, esterase, GST, monooxygenase and acetylcholinesterase (AChE) assays. Each mosquito sample was homogenized in 200µl ice-cold distilled water. An aliquot of 100 µl was taken for AChE assay and the rest was centrifuged at 13,000 rpm for two minutes. The supernatant was used for esterase, GST, monooxygenase and protein assays.

Acetylcholinesterase (AChE) assay

Two, 25 µl replicates from each mosquito homogenate were placed in adjacent wells of a microtitre plate. The membrane-bound AChE in the mosquito homogenate was solubilized by adding 145 µl of Triton phosphate buffer [1% (v/v) Triton X-100 in 0.1 M phosphate buffer pH 7.8] to each. To one set of homogenates, 25 µl of 0.01 M acetylthiocholine iodide (ASChI) and 10 µl of 0.1 M propoxur solution (2.5 ml 0.1 M ASChI + 10 µl of 0.1 M propoxur in acetone) were added. To the other replicate, 25 µl of ASChI alone was added. The kinetics of the enzyme reaction were continuously monitored at 405 nm for five minutes using a microplate reader (BioTek-800 TS Microplate reader). Results were expressed as a percentage of remaining activity in the inhibited fraction compared to the control (uninhibited) activity.

Esterase assay

Ten (10) µl of each homogenate was mixed with 200 µl of 1 mM p-nitrophenyl acetate (pNPA) working solution (100 mM pNPA in acetonitrile: 50 mM sodium phosphate buffer pH 7.4, 1:99) in a microtitre plate well. The reaction was read immediately at 405 nm for two minutes as a kinetic assay at 21°C. An extinction co-efficient of 6.53 mM-1 (corrected for a path length of 0.6 cm) was used to convert the absorbance values to moles of product. Esterase specific activity per individual was reported as µmol product min-1 mg-1 protein.

Glutathione S-transferase assay

Ten (10) µl of each homogenate was mixed with 200 µl of reduced glutathione (GSH)/1-chloro-2,4 dinitrobenzene (CDNB) working solution [95 parts of 10 mM reduced glutathione (GSH) in 100 mM phosphate buffer pH 6.5 + 5 parts of 63 mM CDNB diluted in methanol] in a microtitre plate well. The reaction was read at 340 nm immediately as a kinetic assay for 5 minutes. An extinction coefficient of 5.76 mM^{-1} (corrected for a path length of 0.6 cm) was used to convert absorbance values to moles of product. GST specific activity was reported as CDNB conjugated µmol product min-1 mg-1 protein.

Monooxygenase assay

Twenty (20) µl of homogenate was mixed with 80 µl of potassium phosphate buffer pH 7.2 + 200 µl of 6.3 mM tetramethyl benzidine (TMBZ) working solution [(0.01 g TMBZ dissolved in 5 ml methanol and then in 15 ml of sodium acetate buffer pH 5.0) + 25 µl of 3% (v/v) H2O2 solution] in a microtitre plate well. After a two-hour incubation at room temperature, the plate was read at 630 nm as an end-point assay. This assay does not measure the monooxygenase activity, but titrates the amount of bound haem in the mosquito homogenate. Since haem is present in the active site of monooxygenases, major changes in the amount of monooxygenases produce a measurable increase in haem. By using a standard curve of cytochrome C (which also contains bound haem) a crude estimate of the amount of the monooxygenases present was obtained and expressed as equivalent units of cytochrome P450 per mg protein.

Protein assay

To obtain specific activities of enzymes, protein concentrations of the individual homogenates were determined by Bio-Rad protein determination. In a microtitre plate well, 10 µl of each homogenate was mixed with 300 µl of BIO-RAD working solution (prepared according to the manufacturer's instructions) and absorbance was read at 630 nm after a five-minute incubation at room temperature.

Statistical analysis

Mortality levels were defined as susceptible (>97% mortality), possible resistance (90-97% mortality), and resistant (<90% mortality) according to the WHO susceptibility criteria (WHO, 2016). Significance in the spatial variations in corrected percentage mortalities of *Ae. aegypti* and *Ae. albopictus* larvae and adults was evaluated using one-way ANOVA by Tukey's test for mean separation. Enzyme activity/quantity values of *Ae. aegypti* and *Ae. albopictus* populations were compared using one-way ANOVA. Differences were considered significant for $P < 0.05$. Tukey's *posteriori* test was applied to determine significant differences between individual means. All statistical tests were performed, using Minitab 20.0 program.

In life table experiments, larval and pupal survivorship was evaluated using Kaplan–-Meier survival analysis (Goel et al., 2010) Minitab 20 version. Log-rank test was used to compare the survival curves between different breeding habitats and the comparisons were made using one-way ANOVA and Tukey's pair-wise tests.

RESULTS

Larval Bioassay

Larvae of both *Ae. aegypti* and *Ae. albopictus* from Kurunegala were completely susceptible (100% mortality) to all tested temephos concentrations (Table 1). Temephos discriminating concentration 0.0125 ppm (WHO, 2005) could kill only 34% *Ae. aegypti* and 54% *Ae. albopictus* larvae of Colombo populations. Even the 10x discriminating concentration could only kill 84% of *Ae. aegypti* and 91% *Ae. albopictus* showing that Colombo dengue vector larvae are highly resistant to the organophosphate temephos (Table 01). Results also indicate that *Ae. aegypti* larvae are more resistant to temephos than *Ae. albopictus* larvae.

Adult Bioassays

Adult bioassays were conducted by exposing a total of 1800 adult female mosquitoes (*Ae. aegypti* = 900 and *Ae. albopictus* = 900) from each site to six insecticide-discriminating dosages along with control experiments. Figure 02 displays the resistance profiles of both vector species from the two study areas. Both *Ae. aegypti* and *Ae. albopictus* from Kurunegala showed >98% mortality for all the insecticides tested and therefore, susceptible to deltamethrin, lambdacyhalothrin, permethrin and malathion, according to WHO classification (WHO, 2022).

However, both species from Colombo showed less than 90% mortality rate for all pyrethroids tested indicating that they are resistant to pyrethroids. *Aedes aegypti* adults from Colombo showed less than 90% mortality for both the 1% (*Ae. aegypti* diagnostic concentration) and 5% (*Ae. albopictus* diagnostic concentration) malathion (Fig: 02) showing that they are resistant to malathion. *Aedes albopictus* adults were possible resistance to the 5% malathion diagnostic dosage specified for it and resistant to the 1% *Ae. aegypti* discriminating dosage (Fig: 02).

Table 01: Percentage mortality of *Aedes aegypti* and *Ae. albopictus* larvae exposed to different concentrations of temephos. Five batches of 20 third instar larvae of each species from each location were tested for each concentration.

Concentration (ppm)	Percentage Mortality (%)			
	Colombo		Kurunegala	
	Ae. aegypti	Ae. albopictus	*Ae. aegypti*	Ae. albopictus
0.0125	34 ±1.23	54±1.7	100±0.00	100±0.00
0.0625	52±2.8	68±1.02	100±0.00	100±0.00
0.125	84±1.46	91±1.9	100±0.00	100±0.00

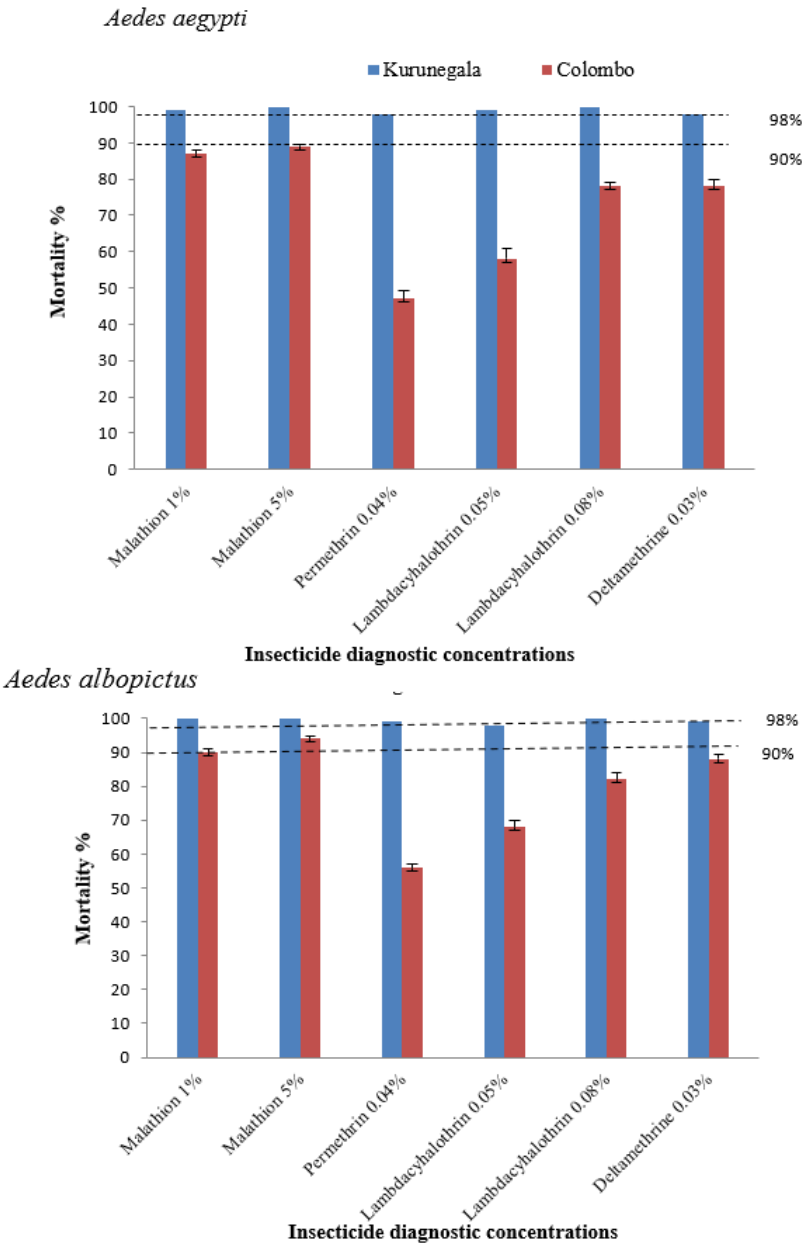

Figure 02: Susceptibility of *Aedes aegypti* and *Ae. albopictus* from two different study sites to the discriminating dosages of three different pyrethroids and the organophosphate malathion (discriminating dosages 1% malathion and 0.05% lambda-cyhalothrin are for *Ae. aegypti*; 5% malathion and 0.08% lambdacyhalothrin are for *Ae. albopictus*). Susceptible (≥98%. mortality), possibly resistant (90–97% mortality) and resistant (<90% mortality) (WHO 2018).

Biochemical assays

Activity profiles of AChEs, esterases, GSTs and the amounts of monooxygenases of larvae and adult of both vector species from two different locations are shown in Fig. 03.

Propoxur inhibited larval and adult AChEs from both vector species showed remaining activities of less than 33% indicating that the target site AChEs are largely sensitive. However, insensitivity was emerging in both vectors from Colombo. *Aedes aegypti* larvae from Kurunegala showed a significantly higher sensitivity for inhibition (lower remaining activity) than that of adults (Fig. 03). Esterase activities from Colombo *Ae. aegypti* were significantly higher than that of Kurunegala *Ae. aegypti* and, were even greater than the discriminating activity of 0.25 µmol/mg/min specified for resistant *Anopheles* mosquitoes (Perera et al., 2008). *Aedes albopictus* adults from Colombo showed a significantly higher (p=0.021) esterase activity than that of their larvae (fig.03). Adults of both species had significantly higher GST activities than that of their larvae and these values were greater than the GST discriminating value of 0.40 µmol/mg/min given for resistant anophelines (Perera et al., 2008). Significantly higher amounts of monooxygenases were observed in Colombo *Ae. aegypti* and these values were above the monooxygenase discriminating amount of 0.35 equivalent units of cytochrome P450 per mg protein specified for resistant anophelines (Perera et al., 2008). Also, the adult monooxygenase levels were significantly higher than that of larvae in this population.

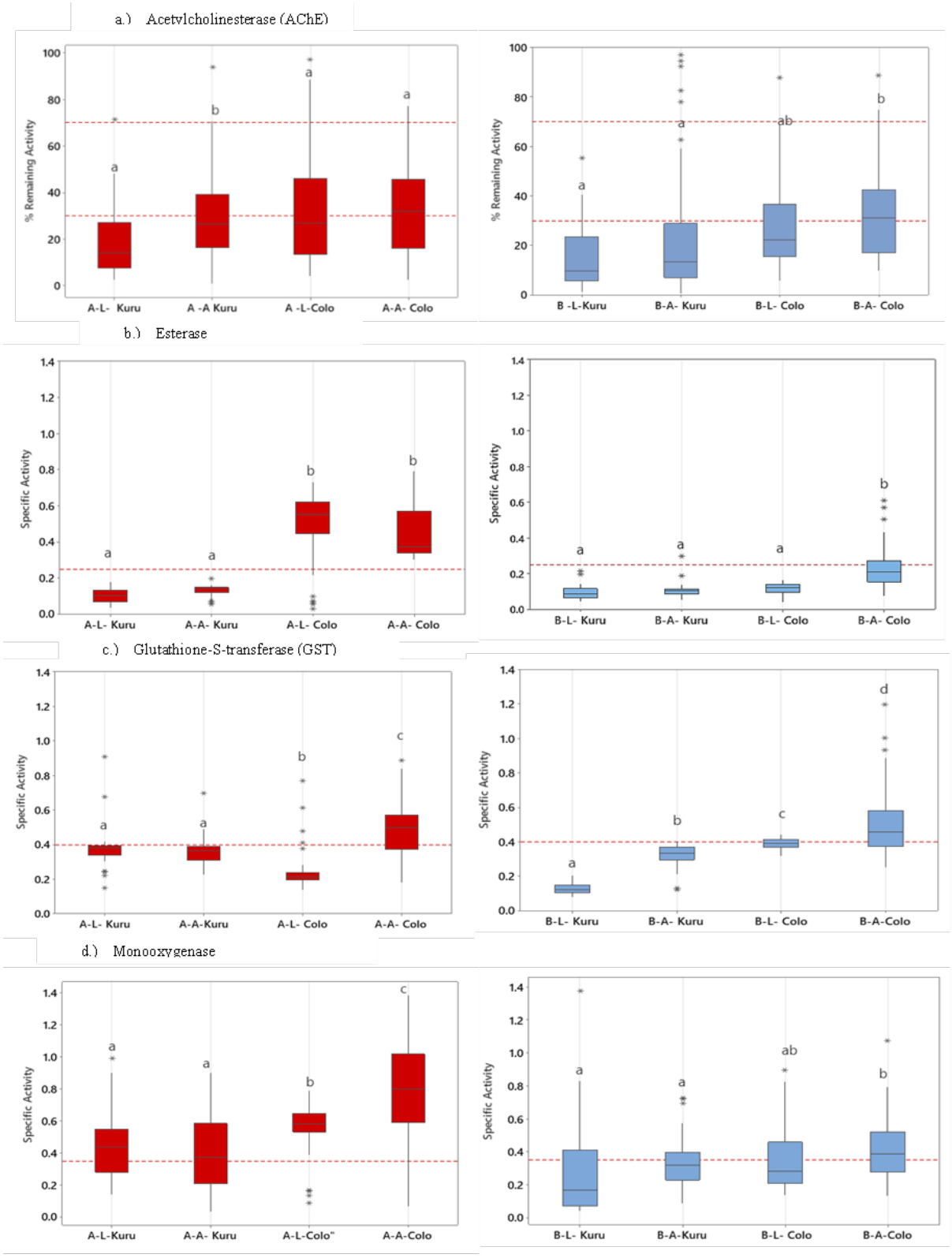

Figure 03: Enzyme activity profiles for *Aedes aegypti* (A-L-Kuru- larvae from Kurunegala; A-A-Kuru- adults from Kurunegala; A-L-Colo- larvae from Colombo; A-A-Colo- adults from Colombo) and *Ae. albopictus* (A-L-Kuru- larvae from Kurunegala; A-A-Kuru- adults from Kurunegala; A-L-Colo- larvae from Colombo; A-A-Colo- adult from Colombo) populations. a) acetylcholinesterase (AChE) remaining activity (%), b) esterase specific activity (µmol/mg/min), c) glutathione-S-transferase (GST) specific activity (µmol/mg/min) and d) monooxygenase amounts (equivalent units of cytochrome P450/mg). For AChE, <30% = homozygous sensitive (SS), 30-70% = heterozygous (RS), and > 70% = homozygous insensitive (RR). Discriminating activity for resistant *Anopheles* mosquitoes are shown in dotted lines for esterases (0.25 µmol/mg/min), GSTs (0.40 µmol/mg/min) and monooxygenases (0.35 equivalent units of cytochrome P450/mg) (Perera et al., 2008). Different letters indicate a significant difference ($p<0.05$) between the profiles within each group (t-test pairwise comparison).

Life table analysis of dengue vector species

Survivorship of larval and pupal immature stages of both vector species under laboratory and semi-field conditions, obtained using Kaplan-Meier survival analysis, are shown in the Fig. 04. *Aedes aegypti* larval development time (5 ± 0.14 days) was significantly shorter than that of *Ae. albopictus* (9 ± 0.31 days) under laboratory conditions. Their respective survival probabilities were 80% and 76%. Although the larval periods were significantly different among the different semi-field breeding habitats for *Ae. aegypti* (F=11.16, p=0.023) and *Ae. albopictus* (F=10.6, p=0.03), both species had comparable development periods in each semi-field type. Shortest larval periods were observed in bamboo stumps (5-5.2 days) and the highest were observed in clay pots (9.8-10 days) for both species. Survival probabilities varied from 64-80% for *Ae. aegypti* and from 32-64% for *Ae. albopictus*.

Pupae to adult mean development times for both vector species were 3 days under laboratory conditions. It was significantly different (p=0.03) from 4 days observed for *Ae. aegypti*, under semi-field conditions. *Aedes albopictus* pupal development times were significantly different (p=0.023) among semi-field habitats i.e., bamboo stumps -2 days, tyres - 3 days and clay pots – 4 days. Pupal survival probabilities were 72% under laboratory conditions for both species. Under semi-field conditions, *Ae. aegypti* pupae had a 90% survival probability in clay pots, 68.4% in tyres and 45.2% in bamboo stumps whereas *Ae. albopictus* pupae had a survival probability of 90% for all three different habitats (Fig.04).

The period from the first instar larva (egg hatching) to adult emergence is 8 days for *Ae. aegypti* and 13 days for *Ae. albopictus* under laboratory conditions. These figures for semi-field habitats were; bamboo stumps 7 and 7.2 days, tyres 10 and 11.3 days, and clay pots 14 and 13.8 days respectively for *Ae. aegypti* and *Ae. albopictus*. The survival rate from egg hatch to adult was 57.6% for *Ae. aegypti* and 54.72% for *Ae. albopictus* under laboratory conditions. These values were 32% for bamboo stumps, 38.4% and 28.8% for tyres, and 63% and 57.6% for clay pots respectively, for the two species.

DISCUSSION

Vector-borne diseases are a major cause of sickness worldwide and their prevention heavily depends on vector control (WHO, 2022a). Insecticides are the widely used effective tool in insect vector control and as a result of frequent and indiscriminate use of insecticides, resistance to insecticides has emerged among insect populations creating a major threat to insect control programmes worldwide (WHO, 2022a). Understanding the status of resistance in local vector populations to commonly used insecticides is crucial to ensure the success of insect vector control programmes. In Sri Lanka, organochlorine DDT was introduced in malaria control programmes in the late 1940s. Due to resistance development in mosquito vectors and environmental concerns, DDT was replaced with organophosphates for mosquito control in the health sector and with carbamates for insect pest control in the agricultural sector in 1975/77. Pyrethroids were introduced to mosquito control programmes in 1994 (Karunaratne, 1999). Pyrethroids and organophosphates are still widely used in mosquito control programmes (NDCU, 2022).

Both the bioassay and biochemical assays conducted during the present study revealed that Kurunegala dengue vector populations are susceptible to all tested pyrethroids and organophosphates. Colombo vector populations are resistant to both these groups of insecticides and have increased activities of esterases, GSTs and monooxygenases for insecticide detoxification. All these three enzyme groups are capable of detoxifying pyrethroids and organophosphates effectively (Karunaratne et al., 2018). Elevation of the activities of these enzymes above the discriminating levels given for *Anopheles* mosquitoes (Perera et al. 2008), indicates their significant contribution to organophosphates and pyrethroids. It is evident from our study that altered target site mechanism, with insensitive organophosphate target site AChE, is emerging in both *Ae. aegypti* and *Ae. albopictus* populations from Colombo. Fernando et al. (2018), Nugapola et al. (2021) and Dalpadado et al. (2021) have reported the presence of *kdr*-type mutations in the voltage-gated sodium channel genes, producing insensitive target sites for pyrethroids. During the present study, it was noted that both the larvae and adults of *Ae. aegypti* populations are more resistant to insecticides than those of *Ae. albopictus* both in Kurunegala and Colombo. Therefore, the reason behind recommending higher discriminating dosages for *Ae. albopictus* than those for *Ae. aegypti* by the WHO (2016; 2022c) is questionable.

It is clear from our study that the activities of esterases, GSTs and monooxygenases found in adults of both species were significantly higher than those found in their larvae. To our knowledge, this has not been reported in mosquitoes previously. However, a similar scenario has been reported from the tea defoliator *Hyposidra talaca* (Geometridae: Lepidoptera). Expression of the same three detoxifying enzymes varied greatly in all five larval stages of *Hyposidra talaca* and increased with each successive stage suggesting that these enzymes are serving as a defensive mechanism to increasing insecticidal stress (Roy et al. 2021). When dengue vectors are considered, insecticidal pressure is higher on adults than on larvae, since insecticides are the major tool used for adult control. Larval control is largely attempted by clearing breeding habitats. Although temephos has been recommended as a larvicide for larval control, practically it is impossible to apply larvicides to all possible larval breeding habitats and the insecticidal pressure on larvae is comparatively low. Also, there can be an evolutionary significance as adults are more vulnerable to xenobiotic exposure since, they feed on plant juice and animal blood.

Development time and survivorship of various stages of mosquitoes under different environmental conditions are of particular importance, as they affect the vectorial capacity, which is tightly linked to mosquito-borne disease transmission (Garrett-Jone et al., 1964). In this study, we observed that the micro-environmental conditions had a significant effect on the larval growth period and survival

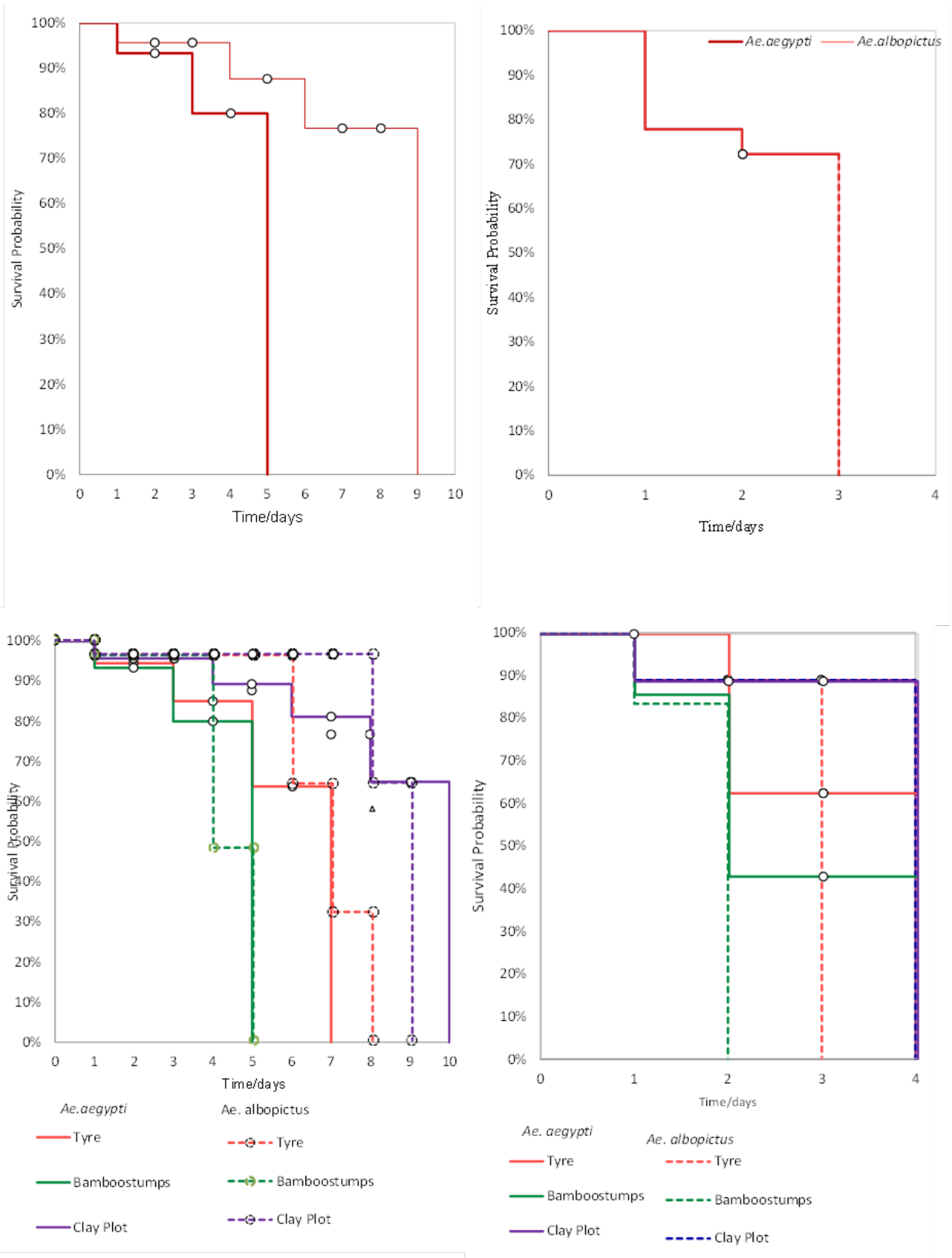

Figure 4. Survivorship of larval and pupal immature stages of *Aedes aegypti* and *Ae. albopictus* in laboratory and semi field condition. A- Larval survivorship curves under laboratory conditions, B- Pupal survivorship curves under laboratory conditions, C- Larval survivorship curves for different containers, D - Pupal survivorship curves for different containers.

rate of *Ae. albopictus* and *Ae. aegypti*. Under laboratory conditions, the larva to adult development period was 8 days, with a survival rate of 57% for *Ae. aegypti* and 13 days with 54% for *Ae. albopictus* indicating that, *Ae. aegypti* development is significantly faster. Similar to our results, Lopes et al. (2014) and Agnew et al. (2002) have shown that *Ae. aegypti* larval development period under laboratory conditions is 7 days. However, shorter periods (five days) of development had been achieved by rearing larvae individually in the laboratory, and longer periods (up to 10 days) had been observed with the larvae collectively reared outside the laboratory, a situation similar to our observation (Lopes et al., 2014; Almério et al., 1995; Yang et al., 2020). Our results show that the development period of dengue larvae from egg hatching to adult is 7 - 7.2 days for bamboo stumps, 10 - 11.3 days for tyres, and 13.8 – 14 days for clay pots. Survival rates were 32% for bamboo stumps, 28.8% - 38.4% for tyres, and 57.6% - 63% for clay pots. Almério et al. (1995) have shown longer periods for *Ae. albopictus* egg hatching to adult emergence as 19.6, 27.3 and 37.5 days, respectively for a tree hole, a bamboo stump and an auto tyre. Variation in the rate of larval growth of *Ae. aegypti* has occurred according to the food types given to them (Hotchnink, 1985). The faster growth rate in the laboratory may be due to the more nutritious larval food they received under laboratory conditions. Differences in the rate of development and survivorship under semi-field conditions can be due to the availability of food and various other biotic and abiotic factors.

CONCLUSIONS

Dengue vector mosquitoes from Kurunegala were susceptible to all insecticides tested, whereas those from Colombo were resistant to the tested insecticides. Resistance of the Colombo population was mainly due to increased activities of insecticide metabolising enzymes. Results indicated that, *Ae. aegypti* larvae were more resistant to insecticides than *Ae. albopictus* larvae. Also, adults were more resistant than larvae with well-developed resistant mechanisms. Development of *Ae. aegypti* is faster and their survival rate is also better than that of *Ae. albopictus.*

ACKNOWLEDGEMENT

This work was supported by the Office of Regional Director of Health Services, Kurunegala and the University of Peradeniya, Sri Lanka. Assistance given by Prof. W.A.P.P. De Silva of the Department of Zoology, University of Peradeniya, Sri Lanka and the staff of the Entomological Surveillance Unit, Office of Regional Director of Health Services, Kurunegala, Sri Lanka is acknowledged.

DATA AVAILABILITY

The data used to support the findings of this study are available from the corresponding author upon request.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

Afrane Y.A., Zhou G., Lawson B.W., Githeko A.K., & Yan G. (2007). Life-table analysis of *Anopheles arabiensis* in western Kenya highlands: effects of land covers on larval and adult survivorship. *Am J Trop Med Hyg*., 77, 660–6.

Agnew P., Hide M., Sidobre C., & Michalakis Y. (2002). A minimalist approach to the effects of density-dependent competition on insect life-history traits. *Ecol Entomol.,* 27, 396–402.

Aida, H.N., Dieng, H., Ahmad, A.H., Satho, T., Nurita, A.T., Che Salmah, M.R., Miake, F., & Norasmah, B. (2011). The biology and demographic parameters of *Aedes albopictus* in northern peninsular Malaysia. *Asian Pacific J Trop Biomed.,* 1(6), 472–477.

Almério de Castro Gomes, Gotlieb S.L., Marques C.C., de Paula M.B., & Marques G.R. (1995). Duration of larval and pupal development stages of *Aedes albopictus* in natural and artificial containers. Revista De Saúde Pública, 29(1), 15–19. doi.org/10.1590/S0034-89101995000100004

Carey J. R. (2001). Insect biodemography. *Annu. Rev. Entomol*., 46, 79–110.

Carron, A., Bichaud, L., Platz, N., & Bicout, D.J. (2008) Survivorship characteristics of the mosquito Aedes caspius adults from southern France under laboratory conditions. *Med. Vet. Entomol.*, 22(1), 70-73. doi: 10.1111/j.1365-2915.2008.00718.x. PMID: 18380656.

Coleman M., Hemingway J., Gleave K.A., Wiebe A., Gething P.W., & Moyes C.L. (2017). Developing global maps of insecticide resistance risk to improve vector control. *Malaria J*., 16, 86.

Dalpadado, R., Gunathilaka, N., Amarasinghe, D., & Udayanaga, L. (2021). A Challenge for a Unique Dengue Vector Control Programme: Assessment of the Spatial Variation of Insecticide Resistance Status amongst *Aedes aegypti* and *Aedes albopictus* Populations in Gampaha District, Sri Lanka. *BioMed Res Internat*, ID6619175, 8 pages.

Fernando S. D., Hapugoda M., Perera R., Saavedra-Rodriguez K., Black W. C., & De Silva N. K. (2018). First report of V1016G and S989P knockdown resistant (Kdr) mutations in pyrethroid-resistant Sri Lankan *Aedes aegypti* mosquitoes. *Parasites & Vectors,* 11(1), 526-534.

Fernando, H.S.D., Saavedra-Rodriguez, K., Perera, R. *et al.* (2020). Resistance to commonly used insecticides and underlying mechanisms of resistance in *Aedes aegypti* (L.) from Sri Lanka. *Parasites & Vectors,* 13, 407. doi.org/10.1186/s13071-020-04284-y

Garrett-Jone C. (1964). Prognosis for interruption of malaria transmission through assessment of the mosquito's vectorial capacity. *Nature*, 204, 1173–5.

Goel M.K., Khanna, P., & Kishore J. (2010). Understanding survival analysis: Kaplan-Meier estimate. *Int J Ayurveda Res*., 1, 274–278. pmid:2145545

Hawaria, D., Kibret, S., Demissew, A. *et al.* (2021). Survivorship of *Anopheles gambiae* sensu lato in irrigated sugarcane plantation scheme in Ethiopia. *Parasites & Vectors,* 14, 142 https://doi.

org/10.1186/s13071-021-04630-8.

Hegoda W.K., Fernando H.S., De Silva B.G. (2017). Detecting of knock down resistance (*kdr*) F1534C allele in the dengue vector *Aedes aegypti* in peri-urban areas of Colombo South, *Sri Lanka. J Entomol Zool Stud*., 5, 1926–9.

Hemingway J., Hawkes N.J., McCarroll L., & Ranson H. (2004). The molecular basis of insecticide resistance in mosquitoes. *Ins Biochem Mol Biol.,* 34, 653–665.

Hotchkin, P.G. (1985). The duration of larval life of Ae. aegypti as affected by time of hatch. *Am. Mosq. Cont. Assoc.*, 1, 489-492.

Karunaratne, S.H.P.P. (1999) Insecticide cross-resistance spectra and underlying resistance mechanisms of Sri Lankan anopheline vectors of malaria. *Southeast Asian J Trop Med Pub Health,* 30(3), 460-469

Karunaratne, S.H.P.P., De Silva, W.A.P.P., Weeraratne, T.C. & Surendran, S.N., (2018). Insecticide resistance in mosquitoes: Development, mechanisms and monitoring. *Cey J Sci.*, 47(4), p.299-309. DOI: https://doi.org/10.4038/cjs.v47i4.7547

Lopes T.F., Holcman M.M., Barbosa G.L., Domingos M. de F., & Barreiros R.M. (2014). Laboratory evaluation of the development of *Aedes aegypti* in two seasons: influence of different places and different densities. *Rev Inst Med Trop Sao Paulo*., 56(5), 369-74.

Moyes C.L., Vontas J., Martins A.J., Ng L.C., Koou S.Y., Dusfour I, et al. (2017). Contemporary status of insecticide resistance in the major *Aedes* vectors of arboviruses infecting humans. *PLoS Negl Trop Dis.,* 11(7), e0005625. doi:10.1371/journal. pntd.0005625. Erratum in: PLoS Negl Trop Dis. 2021;15(1): e0009084.

National Dengue Control Unit Sri Lanka (2022). Guidelines for *Aedes* Vector Surveillance and Control, 2016,2018,2022, http://www.dengue.health.gov.lk/web/phocadownload/ guidlines_for_aedes_vector_surveillance_and_control_in_sri_lanka_new.pdf.

Nugapola, N.W.N.P., DeSilva, W.A.P.P., Weeraratne, T.C., & Karunaratne, S.H.P.P. (2020). kdr type mutations and enhanced GST based insecticide resistance in dengue vector mosquitoes *Aedes aegypti* and *Aedes albopictus*. *Int J Trop Insect Sci.*, 41, 409–417. doi.org/10.1007/s42690-020-00219-3.

Perera D.M.B., Hemingway J., & Karunaratne S.H.P.P. (2008). Multiple insecticide resistance mechanisms involving metabolic changes and insensitive target sites selected in anopheline vectors of malaria in Sri Lanka. *Malaria J.,* 7,168. doi: 10.1186/1475-2875-7-168.

Roy, S., Babu, A., Handique, G. *et al.* (2021). Stage specific differential expression of three detoxifying enzymes of larvae of tea defoliator, *Hyposidra talaca* Walker (Geometridae: Lepidoptera) and its bearing on their insecticide tolerance status. *Int J Trop Insect Sci.,* 41, 541–545. https://doi.org/10.1007/s42690-020-00238.

Sirisena P, Noordeen F. (2016). Dengue control in Sri Lanka–challenges and prospects for improving current strategies. *Sri Lankan J Infect Dis.*, 6, 2–16. doi: 10.4038/sljid.v6i1.8107.

World Health Organization (1998). Techniques to detect insecticide resistance mechanisms (field and laboratory manual). *World Health Organization,* https://apps.who.int/iris/handle/ 10665/83780.

World Health Organization (2022a). Manual for monitoring insecticide resistance in mosquito vectors and selecting appropriate interventions. *World Health Organization,* https://www.who.int/publications-detail-redirect/9789240051089.

World Health Organization (2022b). Report on insecticide resistance in *Aedes* mosquitoes (*Aedes aegypti, Ae. albopictus, Ae. vittatus*) in WHO South-East Asia Region countries. *World Health Organization,* https://www.who.int/publications/i/item/sea-cd-334.

World Health Organization (2022c). Standard operating procedure for testing insecticide susceptibility of adult mosquitoes in WHO tube bioassays. *World Health Organization,* https://www.who.int/publications/i/item/9789240043770.

World Health Organization (2005). Guidelines for Laboratory and Field Testing of Mosquito Larvicides. *World Health Organization*. https://apps.who.int/iris/handle/10665/69101.

World Health Organization. (2016). Monitoring and Managing Insecticide Resistance in *Aedes* Mosquito Populations: Interim Guidance for Entomologists 2016; *World Health Organization*, https://www.who.int/publications/i/item/WHO-ZIKV-VC-16.1.

World Health Organization (2018a). Global Malaria Programme: Insecticide resistance. *World Health Organization*, https://www.who.int/teams/global-malariaprogramme/ prevention/ vector-control/ insecticide-resistance.

World Health Organization. (2018b). Test Procedures for Insecticide Resistance Monitoring in Malaria Vector Mosquitoes. 2nd ed. *World Health Organization*, https://iris.who.int/handle/10665/250677

Yang D, He Y, Ni W, Lai Q, Yang Y, Xie J, *et al.* (2020) Semi-field life-table studies of *Aedes albopictus* (Diptera: Culicidae) in Guangzhou, China. PLoS ONE, 15(3), e0229829.