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Full Length Article

# Susceptibility and fitness cost of *Aedes albopictus* on their survivability after the exposure to the insecticide

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ABSTRACT

The present study was undertaken to investigate the lethal and sub-lethal effects of insecticides on the life history of *Aedes albopictus* mosquitoes from urban and sub-urban areas in Penang, Malaysia. The female mosquitoes aged two to five days old were exposed to; (1) diagnostic dose (lethal dose) at 5% malathion and 0.75% permethrin; and (2) sub-lethal concentration of 1.5% malathion, and 0.2% permethrin, respectively and adult female survivors from sub-lethal concentrations were evaluated for fitness parameters. Fecundity, fertility, adult longevity, development time and survival of mosquitoes were the crucial point in their life history had studied. *Aedes albopictus* species from urban Sg. Dua strain has developed high resistance towards 0.75% permethrin and was confirmed on resistance after 24 h of reading. While sub-urban Batu Maung strain is still susceptible to both 5% malathion and 0.75% permethrin. At the sub-lethal dose, we discovered 0.2% permethrin insecticide have significantly more effects on the fitness cost of *Ae. albopictus* as compared to 1.5% malathion; with decreasing on fecundity, lesser time was needed to reach each development stages, and more male adult was emerged compared to female mosquitoes for both urban and sub-urban strains. Whereas, malathion insecticide only affected the number of eggs laid by the parent mosquitoes and the development time to reach adult stages. Even though, *Ae. albopictus* mosquitoes had developed resistance towards permethrin resulted in decreasing mortality, but subsequent effects on their fitness cost still continued on the first generation. Thus, it will benefit in reducing the transmission of mosquito-borne diseases.

## Introduction

The main dengue vector control method in Malaysia is targeting mostly on adult mosquitoes using permethrin, deltamethrin and malathion insecticides and also larviciding with temephos and *Bacillus thuringiensis israelensis* (*Bti*) (MOH, 2011). However, recent trends in dengue vector control in the Western Pacific Region (WPR) country, including Malaysia have shifted from relying entirely on insecticides to the source reduction, biological control, and environmental management through cooperation with community participation. This is due to the wide-scale outdoor spraying which of questionable effectiveness since many mosquitoes may be inaccessible and would be unaffected (Chang et al., 2011).

Vector-borne disease resurgent resulted in the insecticide resistance in mosquitoes as a part of the high usage of insecticides. Evidence of the resistance development towards permethrin and temephos has been recorded in both *Aedes albopictus* and *Aedes aegypti* in Kuala Lumpur and Pulau Pinang, Malaysia (Nazni et al., 2009; Chan et al., 2011). Comparing on species, *Ae. albopictus* showed a higher proportion of individual resistance towards malathion and dieldrin compared to *Ae. aegypti* mosquitoes in Malaysia (Ishak et al., 2015). It is possible at the present of some insecticides; survivors will correspondingly induce and rebuild population more rapidly than expected, and causing resurgences in disease incidence (Sutherst, 2004). Survived mosquitoes after exposure, even exposed to the low concentration of pesticide benefited itself in term of size of the adult, which has been associated with high fecundity and high longevity of adults (Briegel and Timmermann, 2001).

Insecticide resistance is generally believed to arise from selection acting on random variation such as pre-adaptive (Hamdan et al., 2005; Nazni et al., 1998) or by increasing the mutation rates (Wood et al., 1984; Hamdan et al., 2005). Besides, the massive usage of different

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insecticide classes for strategies of vector control in such a long time has contributed to the insecticide resistance in dengue vector of mosquitoes (Brown, 1986; Hemingway and Ranson, 2000). Lately, many researchers reported the presence of kdr-like resistance inferred from cross resistance between DDT and pyrethroids, which act on the same site within the sodium channel (Dong, 2007; Silva et al., 2014; Li et al., 2015). This kdr-like resistance took over the binding site on the sodium channel and inhibited the activation of DDT and pyrethroid insecticide (Hemingway and Ranson, 2000).

Survivor of the insecticide treatment may be managing to detoxify the pesticide more efficiently, but could give passive effects as the treatment may elevate fluctuating asymmetry (Mpho et al., 2001), interpreted as a symptom of increased in developmental instability or reduced of developmental homeostasis (Markow, 1995; Leamy and Klingenberg, 2005). Exposure to temephos insecticide increased fluctuating asymmetry in males *Culex quinquefasciatus* by reducing wing trait size and increased mortality rate without any effect on the development time (Mpho et al., 2001). The same pattern of effect was also observed in *Aedes albopictus* (Wiwatanaratanabutr and Kittayapong, 2006). Whereas, *Aedes aegypti* population showed a lower reproductive capacity when treated with organophosphate (Belinato and Martins, 2016), which the population was reduced due to the lower number of laid eggs in proportion to the amount of ingested blood. This condition can directly influence the pathogen loads ingested and the vector competence itself (Belinato et al., 2012; Otali et al., 2014).

Despite the resistance, problems occurred in *Ae. albopictus* Skuse mosquitoes, we aim to understand the fitness cost of *Ae. albopictus* after exposure to both malathion and permethrin insecticides. We investigate how they altered their development, longevity, and survivability of their progenies to become successful in nature as less is known about their biological strategies to survive after the exposure to the insecticides.

## Materials and methods

### *Mosquito colonies*

Three different strains of *Ae. albopictus* were used throughout this study; (1) the laboratory strain of Vector Control Research Unit (VCRU) served as susceptible strain, (2) wild strain of sub-urban Batu Maung, and (3) wild strain of urban Sungai Dua. Eggs of *Ae. albopictus* of wild strains were collected by placing ovitraps at Batu Maung sub-urban area (5°17′03.4″N, 100°16′46.5″E) and Sungai Dua urban area (5°21′02.8″N, 100°18′03.7″E). Both urban and sub-urban sites were known to be the hot-spot dengue areas and received frequent fogging using chemical insecticides; malathion and permethrin. The VCRU strain is considered as susceptible strain, in which this strain has been cultured for > 500 generations since the 1970s at Vector Control Research Unit, Universiti Sains Malaysia (5°21′21.7″N, 100°18′05.8″E).

Ovitraps were placed in the selected sites and were collected after three days introduced in the field. This is to allow the adult mosquitoes to lay eggs in the ovitraps and long enough to avoid the emergence of adults. Normally, *Aedes* mosquitoes may reach adulthood within 7–8 days based on temperature and humidity. The water and the paddle (for attachment of the eggs) from the ovitrap cans were brought to the laboratory for culturing purpose. The selection of locations for the ovitraps is following the guideline by Yap and Thiruvengadam (1979), which ovitraps were located near other potential breeding sites such as containers, except tires. This is because tires are black in colour and often contains water. Thus, the mosquitoes will be less attracted to the ovitraps. Ovitraps also located under partially or totally shaded areas, behind or beside premises which suitable for resting or breeding areas, and avoid being located under the direct sunlight.

### *Mosquitoes rearing*

The egg and larvae collected from both field areas were reared separately for each urban and sub-urban strains under laboratory condition with a temperature of 28 ± 2 °C and 70–80% relative humidity (RH). The larvae and eggs found in each ovitrap were reared in a plastic container sized 120 × 175 × 20 mm with 1000 ml of seasoned water. Seasoned water is the water that left standing for > 24 h to reduce the chlorine content. The culture was maintained by feeding with larval food at 1 mg/ daily. Larval food was made of dog biscuit, bovine liver, yeast, and milk powder in the ratio of 2:1:1:1. The larvae were reared until they emerged into an adult and confined in an adult cage. The non-blood feeding female adult mosquitoes aged 2–5 days was prepared for the adult bioassay test. The adult mosquitoes only provided with 10% sucrose solution soaked on a cotton ball until the test was performed.

### *Insecticides adult bioassay test for the WHO and sub-lethal doses*

In this adult bioassay test, the parent generation ($F_0$) of non-bloodfed *Ae. albopictus* female mosquitoes aged 2–5 days old collected from both field and VCRU strains were used in this study. Two insecticide impregnated papers consisted of malathion and permethrin were used for this study at two different concentrations; (1) WHO test dose of 5% malathion and 0.75% permethrin, and (2) sub-lethal dose of 1.5% malathion and 0.2% permethrin. The WHO test dose is a standard dose approved by the WHO to determine the resistance level of the mosquitoes and the impregnated paper was supplied by the Vector Control Research Unit, Universiti Sains Malaysia. While, in understanding the fitness cost of *Ae. albopictus'* life history after the exposure to insecticides, we exposed a different set of female adults to the sub-lethal concentrations of 1.5% malathion and 0.25% permethrin. Our pilot test found the 50% mortality dose for VCRU susceptible mosquito strains was at 1.5% for malathion and 0.2% for permethrin. Therefore, this dose was chosen, and this sublethal dose will allow at least 50% of the mosquitoes to survive for the purpose to observe the next progeny developments ($F_1$). For the control group, the mosquitoes were exposed to the negative control (plain paper without any insecticide), OP- carbamate control (consisted of 648 mg olive oil), and OC-Control (consisted of 648 mg risella oil).

An adult bioassay test was conducted following the standard method by the WHO (2013). A total of 150 female adult mosquitoes was used for a total of six replicates (25 mosquitoes per replicate) for each insecticide. Only two replicates were tested for control groups. A total of 24 tubes were prepared to test the insecticide susceptibility and eight tubes for control set. A set of an adult bioassay test consisted of one slide, four clips, and two tubes marked as holding and exposure tubes. A sheet of clean white paper sized 12 cm × 15 cm was rolled into a cylinder shape, inserted into the holding tubes and fastened into position using a silver clip at both ends. The tube was attached to the slides. A total of 25 *Ae. albopictus* female adult mosquitoes were then introduced into the holding tube. Once the female mosquitoes were transferred, the slide unit was closed and positioned in an upright for an hour. Mosquitoes' viability was observed during that an hour acclimation time, and any of dead or abnormal mosquitoes were replaced with a new individual.

An exposure tube was prepared in the same way as the holding tube with the selected treated impregnated paper, either malathion or permethrin. The treated impregnated paper consisted of insecticides were secured in placed using copper clips at both ends. The exposure tubes were then attached to the vacant position on the slides and slide unit opened. The mosquitoes are blown gently from holding tube into the exposure tube. Once all the mosquitoes were in the exposure tubes, the slide unit was closed, and the holding tube was detached from the slide. Mosquitoes were kept in the exposure tube and set in a vertical position for an hour. During that time, the knockdown of mosquitoes was observed and recorded for every five minutes to an hour.

After an hour, the exposed mosquitoes were transferred into a paper cup sized 59 mm × 89 mm × 120 mm and provided with 10% sucrose solution on a cotton ball. After 24 h, the mortality and the number of alive mosquitoes were counted and recorded. An adult mosquito is considered alive if it is able to fly, regardless of the number of the remaining legs. While, any knocked-down mosquitoes, which is not responsive are considered moribund and counted as dead. Each insecticide of malathion and permethrin was tested separately for both WHO and sublethal doses.

*Life history traits of F1 generation after the exposure to sublethal doses*

Mosquito batch from the sub-lethal dose of 1.5% malathion and 0.2% permethrin were proceeded to be cultured for the next study. The replicates used in this study were varied based on the surviving number of adults in the adult bioassay test using the sub-lethal concentrations. After completion of the adult bioassay test, each alive individual mosquito was transferred separately into a paper cup to prepare for the next step of fecundity and fertility tests. The adult female mosquitoes were provided with a bloodmeal and a cone shape filter paper as an oviposition substrate. A moist cotton ball soaked with 10% sucrose solution was provided throughout the study to keep the mosquitoes' alive. Filter papers containing eggs were air dried at least 24 h under the room temperature and counted under a compound microscope (Hornby et al., 1994). Filter paper containing the eggs were removed daily and changed with the new one. The number of eggs was recorded daily for seven days or until the female died.

The fertility by *Ae. albopictus* were observed after the exposure to the insecticides. The eggs from a different individual were soaked in 700 ml of seasoned water separately and the first instar larvae hatched was recorded daily. As some of the group may delay in hatching mode, the observation was kept up to 30 days.

To observe the development and survival of the larvae, the same batch of eggs was allowed to develop until becoming an adult, and the data were recorded daily. The hatched larvae were placed in plastic container sized 120 × 175 × 20 mm with 700 ml volume of water and fed with larval food at 1 mg/daily. Once it reached the pupal stage, they were pipetted out and then placed in a cage.

When each of the tested adults emerged, the adult mosquitoes' longevity of $F_1$ generation was observed and recorded daily until dead. The sex of mosquitoes was identified either female or male. The experiment was done separately for all insecticides tested.

*Statistical analysis*

All of the data were analyzed using analysis in SPSS version 20.0 (IBM, 2011), and data were log-transformed prior to the statistical analysis to fulfill the assumption of required tests. To obtain knockdown time 50 ($KdT_{50}$) and knockdown time 95 ($KdT_{95}$), data from the adult bioassay test were analyzed using probit analysis. $KdT_{50}$ was defined as the time required to knock down 50% of the mosquitoes (Ocampo et al., 2011), whereas $KdT_{95}$ was the time required for 95% knockdown of mosquitoes. Then, the values of the resistance ratio (RR) were determined using the $KdT_{50}$ and $KdT_{95}$ values. RR values were then evaluated by dividing the knockdown time of the field strain to the susceptible strain. The mosquito population was considered susceptible ($RR < 3$), low resistance ($3 < RR < 5$), moderate resistance ($5 < RR < 10$) and high resistance ($RR > 10$) (Mazzarri and Georghiou, 1995). The VCRU susceptible strain was used as the reference, with 100% mortality for all insecticides tested.

To confirm the susceptibility status of mosquitoes, the percentage of mortality after the 24 h exposure to insecticides in the adult bioassay test was characterized using WHO criteria where mosquitoes are considered as (1) susceptible, if the percentage mortality was between 98 and 100%, (2) incipient resistant if mortality was 80–98% and (3) resistant if mortality was < 80% (WHO, 1998). The difference between strains was analyzed using one-way ANOVA for each insecticide by using a number of mortality. The susceptibility status was only determined using mosquitoes tested on WHO test dose.

The fecundity, fertility, and longevity data from the sub-lethal test dose were summarized in descriptive statistics (mean value) and analyzed by using one-way ANOVA to check their significant differences of treatment between strains and insecticides. The log-transformed values of fecundity, fertility, and longevity were served as dependent variables, whereas strains and treatments as the independent variable. To analyze the differences for each developmental stage of mosquitoes starting from egg to adult were used MANOVA by life stage (four larval stages, pupa, and adults) as the dependent variable, while strains and insecticide treatments become dependant variables. Tukey's Post-hoc was used to determine the significant differences between each life stage. The growth changes of larval development depend on the form and size; which the first instar is about 1 mm length and fourth instar approximately 8 mm (Schaper and Hernandez-Chavarria, 2006). Kaplan – Meier survival analysis with 95% CIs was performed to estimate the surviving population of *Ae. albopictus* at each life stages. The status of mosquitoes was defined as 0 for alive and 1 as dead. The sex ratio between male and female was analyzed using Chi-square analysis with an assumption of normal approximates at ratio 1:1 male to female.

## Results

*Susceptibility status using WHO test dose on Aedes albopictus of the urban and sub-urban area strains to the insecticides*

The results of the adult insecticide bioassays conducted on the WHO lethal dose of 5% malathion for *Aedes albopictus* after an hour test indicated the urban Sungai Dua strain exhibited $RR_{95}$ at 1.32 folds ($KdT_{50}$ = 52.21 min) and Batu Maung with $RR_{95}$ at 2.12 folds ($KdT_{50}$ = 65.34 min). In which, suggested both strains are still susceptible to malathion in comparison to VCRU susceptible strain ($KdT_{95}$ = 67.80 min; non-overlapping 95% CIs; Table 1).

Surprisingly, the urban Sungai Dua strain has developed significantly high resistance towards permethrin at a dose of 0.75% with 27.41 folds of $RR_{95}$ compared to VCRU susceptible strain (non-overlapping 95% CIs). However, the sub-urban Batu Maung still susceptible towards 0.75% permethrin ($RR_{95}$ = 1.19 fold).

As shown in Table 1, the 24 h mortality results had confirmed that the VCRU susceptible strain is still susceptible to malathion, but showed a slight incipient resistant status towards permethrin (97.33% mortality). After 24 h, the susceptibility status of Batu Maung strain changed from susceptible (at one hour adult bioassay test) to incipient resistance with mortality > 80% for both 5% malathion and 0.75% permethrin. Whereas, Sungai Dua strain was confirmed to develop resistance towards 0.75% permethrin insecticide after 24 h with mortality below than 11%, and incipient resistance was detected for 5% malathion (Table 1).

*The mortality rate of Aedes albopictus after exposure to sub-lethal doses*

In order to proceed to the next objective, all strains were exposed to sub-lethal dose at 0.2% permethrin and 1.5% malathion. The 24 h mortality results confirmed the VCRU strain was the most susceptible straian which we expected to have about 50% mortality after the exposure to sub-lethal dose. The total of individual replicates (survived individual) for this study was 25 for negative control, 25 for OP control, and 25 for OC control for all strains (derived from 2 bioassay tests). For permethrin insecticides, a total replicate was 17 for VCRU strain (5 tests), 50 for Batu Maung strain (6 tests) and 118 for Sungai Dua strain (6 tests). While, 12 for VCRU strain (5 tests), 91 for Batu Maung strain (6 tests) and 93 for Sungai Dua strain (6 tests) were used for malathion insecticide. Hereafter, the individual replicates were known as replicate

**Table 1**
Knockdown time (min) $KdT_{50}$, $KdT_{95}$ and mortality after 24 h exposure to WHO diagnostic dose of 5% malathion and 0.75% permethrin against female adult *Ae. albopictus* for 1 h exposure for susceptible VCRU, Sungai Dua urban, and Batu Maung sub-urban strains.

| Strain | $KdT_{50}$(min*) | RR | Status** | $KdT_{95}$(min*) | RR | Status** | Status** | Status*** |
|---|---|---|---|---|---|---|---|---|
| | 5% Malathion (WHO test dose) | | | | | | | |
| VCRU(susceptible strain) | 41.62 (39.54–43.78) | 1.00 | S | 67.80 (61.94–77.01) | 1.00 | S | 99.33 ± 0.62 | S |
| Urban Sungai Dua | 52.21 (50.07–54.74) | 1.25 | S | 89.80 (81.60–102.07) | 1.32 | S | 97.33 ± 1.83 | IR |
| Sub-urban Batu Maung | 65.34 (62.40–58.85) | 1.57 | S | 144.00 (129.32–164.13) | 2.12 | S | 88.67 ± 3.09 | IR |
| 0.75% Permethrin (WHO test dose) | | | | | | | | |
| VCRU(susceptible strain) | 23.08 (11.07–32.44) | 1.00 | S | 48.98 (34.34–215.97) | 1.00 | S | 97.33 ± 1.83 | IR |
| Urban Sungai Dua | 320.38 (223.05–599.97) | 13.88 | HR | 1342.76 (690.17–4344.06) | 27.41 | HR | 11.33 ± 3.63 | R |
| Sub-urban Batu Maung | 24.79 (20.32–28.90) | 1.07 | S | 58.53 (47.40–84.27) | 1.19 | S | 96.67 ± 3.09 | IR |

* $KdT_{50}$ = times required to knock down 50% of the population exposed.
** Resistance ratio (RR) = the knock down time of the field strain/the susceptible strain; Susceptible (RR < 3), low resistance (3 < RR < 5), moderate resistance (5 < RR < 10) and high resistance (RR > 10).
*** using WHO criteria: Susceptible (mortality between 98 and 100), incipient resistant (mortality between 80 and 98%), and resistant (mortality < 80%).

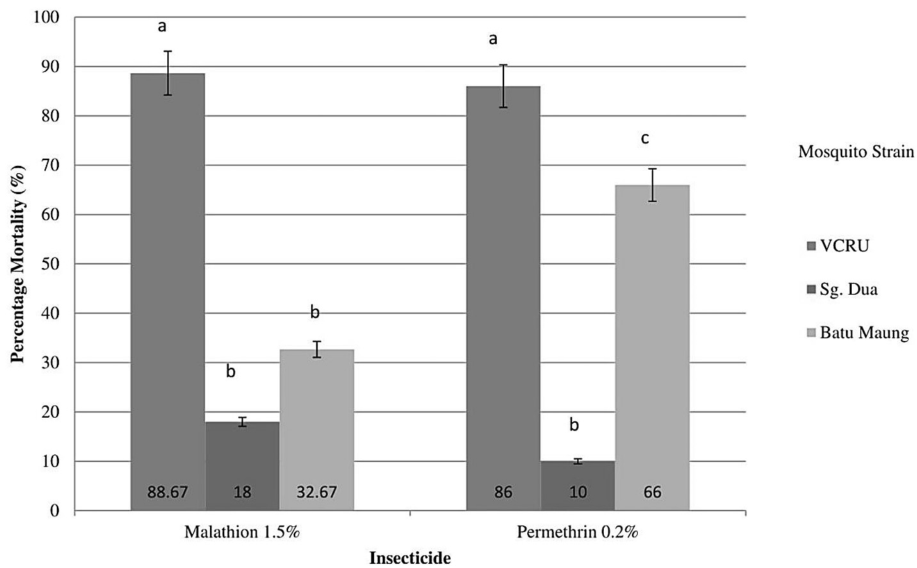


**Fig. 1.** Mortality of *Ae. albopictus* after 24 h exposure to sub-lethal dose of 1.5% malathion and 0.2% permethrin for susceptible VCRU, urban Sungai Dua and sub-urban Batu Maung strains.

for the next study. Exposure to sub-lethal doses of 1.5% malathion and 0.2% permethrin had resulted in 88.67% and 86.0% mortality, respectively. Whereas, the Sungai Dua strain showed more survival for both insecticides as compared to Batu Maung strain. The percentage of mortality among mosquito strains significantly differed after exposure to permethrin between all strains ($P < .05$), but both of the field strains showed no significant difference towards malathion ($P = .172$; Fig. 1).

*Effects of sub-lethal insecticide dose on Aedes albopictus' life history*

*Effects of insecticide on the longevity of Aedes albopictus*

Based on the results shown in Table 2, mosquito from urban Sg. Dua areas showed longer longevity with 15.51 ± 0.94 days after treated with 0.2% permethrin, but showed no significant differences with other strains ($P > .05$). The same results were given after treating with malathion with 15.26 ± 2.08 days for Sg. Dua strains which significantly longer than VCRU strain (7.88 ± 0.51 days; F = 9.494, df = 2, $P = .00$; Table 2). The Batu Maung strain had significantly double the times in longevity (15.29 ± 1.48 days) as compared to negative control of Sg. Dua strain (F = 4.492, df = 2, $P = .014$), but no significant differences detected when treated with permethrin and malathion. Thus, it suggested that the longevity of Batu Maung strains was not significantly influenced by the insecticides. Whereas, Sg. Dua strain showed no significant difference longevity in all treatment (F = 1.526, df = 4, $P = .197$), even though the day of longevity is lower when treated with negative control (7.83 ± 1.80 days) compared to other treatments.

*Effects of insecticide on fecundity and fertility of Aedes albopictus*

Varies in the number of eggs derived from the treatment is due to the effects of insecticides on *Ae. albopictus* mosquitoes. The eggs ranged laid from the survived female mosquitoes varied between 7 and 122 eggs for negative control, 7–125 eggs for OC control and 8–132 eggs for OP control for all mosquito strains (VCRU, Batu Maung, Sungai Dua). For permethrin, a ranged of 8–122 eggs for VCRU strain, 3–86 eggs for Batu Maung strain and 5–115 eggs for Sungai Dua strain. While, for malathion, the ranged was between 29 and 90 for VCRU, 4–112 for

**Table 2**
Longevity, fecundity, fertility and sex ratio of mosquito progenies ($F_1$ generation) treated with sublethal dose insecticides in adult bioassay tests.

| Treatment | Strain | n | Longevity (mean day ± SE) | Fecundity (mean ± SE) | Fertility (mean ± SE) | n | Sex ratio |
|---|---|---|---|---|---|---|---|
| Negative Control | VCRU(susceptible strain) | 25 | $10.00 \pm 0.92^{a,A}$ | $56.54 \pm 3.68^{a,A}$ | $0.94 \pm 0.49^{a,A}$ | 37 | 1:2.36 |
| | Urban Sungai Dua | 25 | $7.83 \pm 1.80^{a,A}$ | $19.39 \pm 5.62^{b,A}$ | $0.14 \pm 0.10^{a,A}$ | 6 | 1:2 |
| | Sub-urban Batu Maung | 25 | $15.29 \pm 1.48^{b,A}$ | $21.86 \pm 3.91^{b,A}$ | $0.82 \pm 0.47^{a,A}$ | 34 | 1:1 |
| OC-Control | VCRU(susceptible strain) | 25 | $9.69 \pm 0.63^{a,A}$ | $42.45 \pm 5.95^{a,AB}$ | $3.91 \pm 1.4^{ab,AB}$ | 107 | 1:1.14 |
| | Urban Sungai Dua | 25 | $15.50 \pm 2.10^{a,A}$ | $29.27 \pm 6.92^{b,AB}$ | $0.12 \pm 0.08^{a,A}$ | 4 | 1:3 |
| | Sub-urban Batu Maung | 25 | $10.49 \pm 0.45^{a,B}$ | $23.36 \pm 5.43^{b,AB}$ | $6.80 \pm 2.80^{b,B}$ | 203 | 1:0.60 |
| OP-Control | VCRU(susceptible strain) | 25 | $6.31 \pm 0.30^{a,B}$ | $42.38 \pm 4.40^{a,AB}$ | $6.69 \pm 2.07^{a,B}$ | 141 | 1:0.88 |
| | Urban Sungai Dua | | $17.50 \pm 2.84^{b,A}$ | $21.61 \pm 7.04^{b,AB}$ | $0.15 \pm 0.08^{b,A}$ | 4 | 1:1 |
| | Sub-urban Batu Maung | | $12.17 \pm 2.78^{b,AB}$ | $14.43 \pm 3.59^{b,AB}$ | $0.38 \pm 0.16^{b,A}$ | 12 | 1:1 |
| 0.2% Permethrin | VCRU(susceptible strain) | 17 | $10.71 \pm 1.63^{a,A}$ | $36.29 \pm 9.66^{a,B}$ | $0.41 \pm 0.23^{a,AB}$ | 7 | 1:6 |
| | Urban Sungai Dua | 118 | $15.51 \pm 0.94^{a,A}$ | $24.10 \pm 2.69^{a,B}$ | $1.11 \pm 0.38^{a,A}$ | 117 | 1:0.63 |
| | Sub-urban Batu Maung | 50 | $13.36 \pm 1.87^{a,A}$ | $5.60 \pm 2.29^{b,B}$ | $0.86 \pm 0.76^{a,A}$ | 33 | 1:0.74 |
| 1.5% Malathion | VCRU(susceptible strain) | 12 | $7.88 \pm 0.51^{a,B}$ | $24.53 \pm 7.36^{ab,B}$ | $1.47 \pm 1.40^{a,AB}$ | 17 | 1:1.43 |
| | Urban Sungai Dua | 93 | $15.26 \pm 2.08^{b,A}$ | $20.26 \pm 2.46^{a,AB}$ | $0.25 \pm 0.11^{a,A}$ | 23 | 1:0.53 |
| | Sub-urban Batu Maung | 91 | $15.00 \pm 0.71^{b,A}$ | $15.02 \pm 2.95^{b,AB}$ | $0.70 \pm 0.44^{a,A}$ | 69 | 1:0.73 |

*Means within a column.
**Means followed by the same small letter indicated no significant difference between strain ($P > .05$)
*Means within a row followed by the same capital letter indicated no significant differences between treatments ($P > .05$).
*There was different ranges of fecundity and fertility after exposure to sub-lethal doses.

Batu Maung and 6–76 for Sungai Dua strains. The VCRU strain mosquitoes show less affected by 1.5% malathion with the highest fecundity of 24.53 ± 7.36' while the lowest is Batu Maung strain with 15.02 ± 2.95 eggs (Table 2). A significantly lower number of fecundity was found in Batu Maung strains (15.02 ± 2.95 eggs) as compared to Sungai Dua strain (20.26 ± 2.46 eggs) after treated with 1.5% malathion ($P = .013$; Table 2). The same results were detected when treated with 0.2% permethrin, with the lowest number of eggs' laying eggs was given by Batu Maung strain at 5.60 ± 2.29 and showed significant differences with other strains (F = 1 3. 69, df = 2, $P < .05$; Table 2). However, the treatment with 0.2% permethrin and 1.5% malathion did significantly influence the number of eggs laid as compared in all control treatments ($P < .05$).

The mean percentage of hatchability (fertility) in both urban Sungai Dua and sub-urban Batu Maung strains after treated with 0.2% permethrin and 1.5% malathion was found significantly lower than the VCRU strain with the highest fertility was about 15.37% hatchability ($P > .05$; Table 2). Significantly higher percentage of fertility was detected in OP-control of VCRU strain at 15.79% (6.69 ± 2.07) as compared to other strains (F = 11.852, df = 2, $P < .05$). Whereas, OC-control gave 29.10% fertility rate for Batu Maung strain with 6.80 ± 2.80 eggs hatched from 23.36 ± 5.43.

*Effects of insecticide on the development of Aedes albopictus*

The developmental period of mosquitoes ($F_1$) after treated with insecticides were monitored started from eggs until they emerged into the adult stage, including the duration of the four larval instars, pupae and adult. The MANOVA test by using Wilks'- Lambda indicated the development period of all four stages of larvae, pupae and adults have significant interaction effect between insecticide treatments and mosquito strains on the combined variables with F (40, 3459.401), $P = .00$, Wilks' Lambda = 0.415.

Derived from the table of test between-subject effects of MANOVA, the result showed that the development period of *Ae. albopictus* mosquito did not significantly differ among all strains in the adult stage (F = 0.634, df = 2, 797, $P = .531$). Significant effect on the development of mosquitoes showed only at larval stages of the second instar, third instar and fourth instar larvae and pupae stage after treated with 0.2% permethrin and 1.5% malathion, which showed lesser time to reach from one instar to another instar as compared to VCRU strains ($P < .05$; Fig. 2). However, to reach the adult stage after treated with 1.5% malathion, mosquitoes from Batu Maung strain significantly had longer development time at 10.87 ± 1.75 days as compared to VCRU strain (8.82 ± 0.12 days; F = 15.181; df = 2; $P < .05$; Fig. 2(e)).

*Effects of insecticide on the survivability of Aedes albopictus*

The survival analysis indicated the survival of *Ae. albopictus* after treated with three types of control and two insecticides. When treated with permethrin insecticide (Fig. 3d) and malathion (Fig. 3e), Sungai Dua strain mosquitoes survived longer and prolonged their lifespan with > 53 days as compared to other strains, and log-rank test showed significant differences between strains ($X^2 = 12.622$, df = 2, $P < .05$).

While, between all three controls (negative, OP-Control and OC-control), the Batu Maung strain showed the longest survival time as compared to other strains. There were significant differences when treated with OP- control ($P < .5$; Fig. 3b), but had no significant effect on survival of adult *Ae. albopictus* when treated with negative and OC-control (F = 0.067, $P = .552$; Fig. 3a, Fig. 3c). Whereas, Sungai Dua strains exhibited the lowest survival time in < 25 days for all control treatment.

*Effects of insecticide on the sex ratio of Aedes albopictus*

There was no significant difference in the sex ratio of three strain mosquitoes ($P > .05$) when treated with malathion, but significant difference was found when exposed to permethrin ($X^2 = 6.097$, df = 2, $P = .047$; Table 2) which less female was developed as compared to male mosquitoes with only 65 (41.0%) emerged as female mosquito from 157 total mosquitoes.

## Discussion

Investigations regarding the fitness cost of mosquitoes towards insecticide generally performed through comparison of biological parameters such as developmental and survival rate, fecundity, fertility, and sex ratio under controlled the laboratory condition. The sub-urban Batu Maung strain was found more susceptible due to the fact that this strain was collected from the rural habitat which is under less selection pressure of insecticides. While, urban Sungai Dua strain is more confined to urban settings with higher exposure to insecticide due to the high number of dengue cases, either during to fogging operation by Minister of Health (Rohani et al., 2011) or may due to more household insecticide exposure used by the residence (Nkya et al., 2013). The evidence of incipient resistance status towards permethrin and malathion by *Ae. albopictus* mosquitoes has been previously recorded in Penang (Chan et al., 2011; Ishak et al., 2015). However, our study recorded higher resistance status for permethrin on *Ae. albopictus* collected from the urban Sungai Dua areas. This could be due to the extensive use of the higher frequency of insecticides used during fogging operations and household insecticides by residence, and in this study

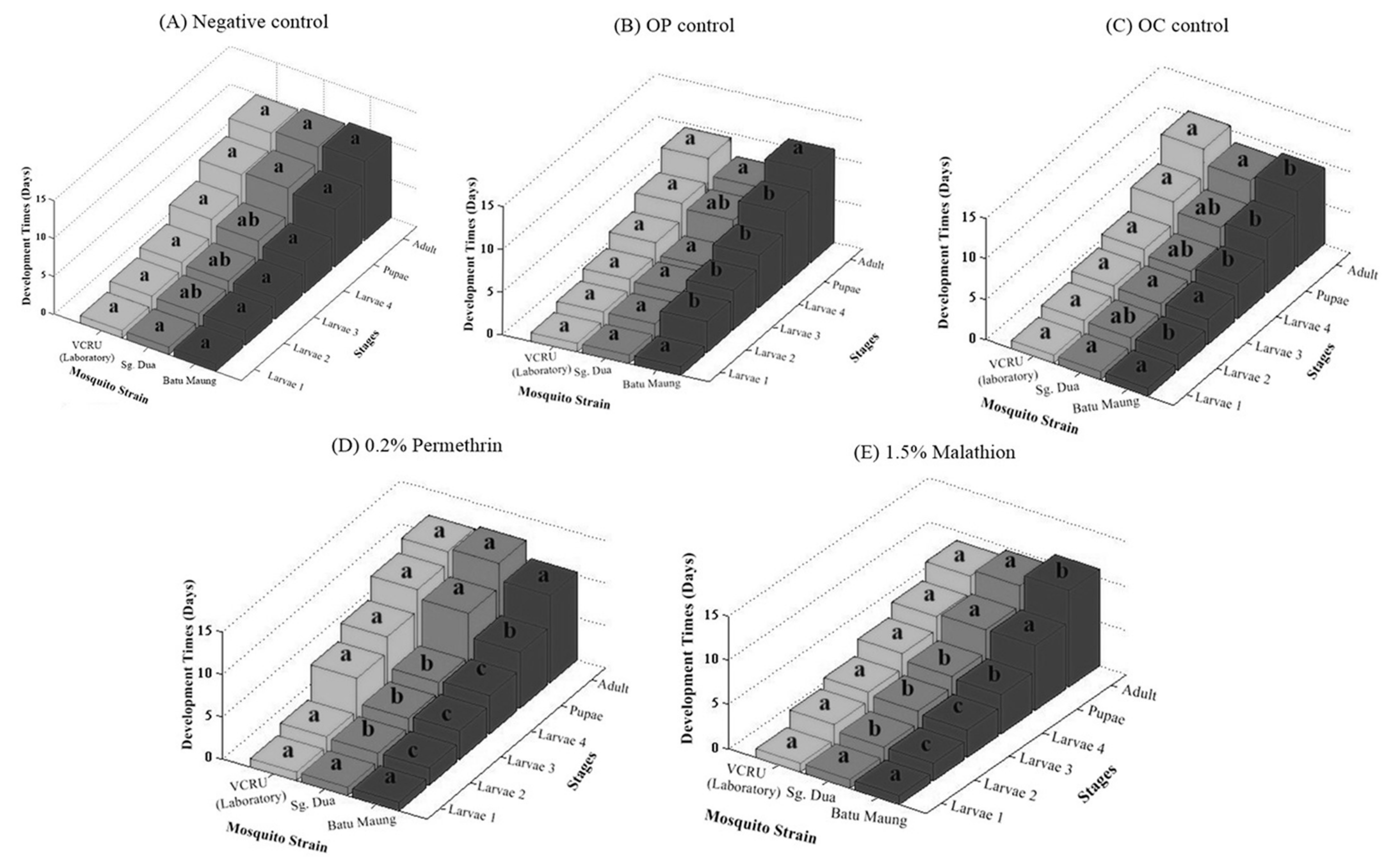


**Fig. 2.** The development days needed to reach stage-specific for different strains of mosquitoes after treated with (A) negative control, (B) OP control, (C) OC control, (D) 0.2% Permethrin, and (E) 1.5% Malathion. Same letter represents no significant difference between mosquito strain at $P < .05$ using MANOVA test.

site. Chemical insecticide spraying is designed to reduce disease transmission by decreasing the target infectious adult mosquitoes and its populations by reducing their longevity (Chang et al., 2011). Therefore, the extensive use of spray and unsystematic fogging operations may eventually contribute to the resistance towards permethrin.

The average longevity duration of *Ae. albopictus* started from oviposition to adult emergence until deaths were 19.3 days with a range of 10 to 46 days as studied by Chan (1971) and Medlock et al. (2006). Interestingly, our study found the longevity of *Ae. albopictus* mosquitoes became shorter when treated with both permethrin and malathion insecticides with a range between 7.88 days ± 0.51 to 15.26 days ± 2.08 as one of the effects on their fitness cost. In this situation, it is probably due to the delayed effects of insecticides that disrupt the *Ae. albopictus'* body system. However, Martins et al. (2012) suggested the shortening in the longevity seemed not to be generally associated with insecticide resistance in field populations. In *Ae.aegypti* the fitness handicaps towards temephos are proportional to the resistance level. (Belinato et al., 2012).

Our study found that *Ae. albopictus* mosquitoes from each strain either have a lower fecundity rate or alternatively decreased egg laying ability when treated with malathion. This might occur due to the lower rate of insemination after the exposure to insecticides, same to those that happened in a resistant field strain of *Ae. aegypti* females studied by Mebrahtu et al. (1997) and Belinato et al. (2012). Mebrahtu et al. (1997) have detected *Ae. aegypti* generation derived from permethrin resistant parent showed a reduction in larval hatching rates. There are several possible reasons on why this phenomenon may occur; insecticides may have a direct toxic effect which has been left unmetabolized in the body of insecticide-treated parent and this effect have been transmitted to eggs (Sanil and Shetty, 2012), or insecticide acting on the central nervous system of mosquitoes that cause a disturbance in the neurosecretory system which associated with the reproductive physiology in the parent generation (Lee, 2000). Besides, in this study, we found only *Ae. albopictus* that exposed to malathion insecticides gave a significant deleterious effect to the progeny and caused fitness handicaps. In which, this organophosphate insecticide (malathion) affect the neurotic system in insects which directly disturbed their peripheral intoxication, hormonal balance and metabolism process in insects that can affect behavior and physiology of insects such as courtship and oviposition. Later on, effects on mosquito eggs fertilization and ovulation (Haynes, 1988; Becker et al., 2003; Ali et al., 2006) and interferes with neuromuscular of insect (Becker et al., 2003).

Time of development is a primary aspect of fitness in disseminating mosquito populations (Martins et al., 2012) and important for epidemiological implications. In which, insecticides may have modified some of the morphological and developmental traits in resistant individuals. In our study, exposure to permethrin and malathion has fastened the development time at larval stages. Rapid development will favor a high pupal population size, and larvae tend to less subjected to parasitic infections and desiccation. In contrast, the presence of resistance alleles of organophosphate (OP) in *Culex pipiens* altered longer developmental time of larval stage and induced a shorter wing length about up to 6% of the normal length (Bourguet et al., 2004). Mosquito larvae tend to pupate faster as their environment increased in toxicity as a self- preservation mechanism (Shaalan et al., 2005). However, we found out after treated with malathion, the pupae needed more time to reach the adult stage with longer development time had occurred at the adult stage. The presence of malathion resulted in lengthened development time of mosquitoes and significantly reduced survivorship (Agnew and Koella, 1999; Muturi et al., 2011) same as happened to our $F_1$ mosquito generation of Batu Maung strain. Reducing energy use and nutrition intake will generate energy trade-offs between insecticide resistance

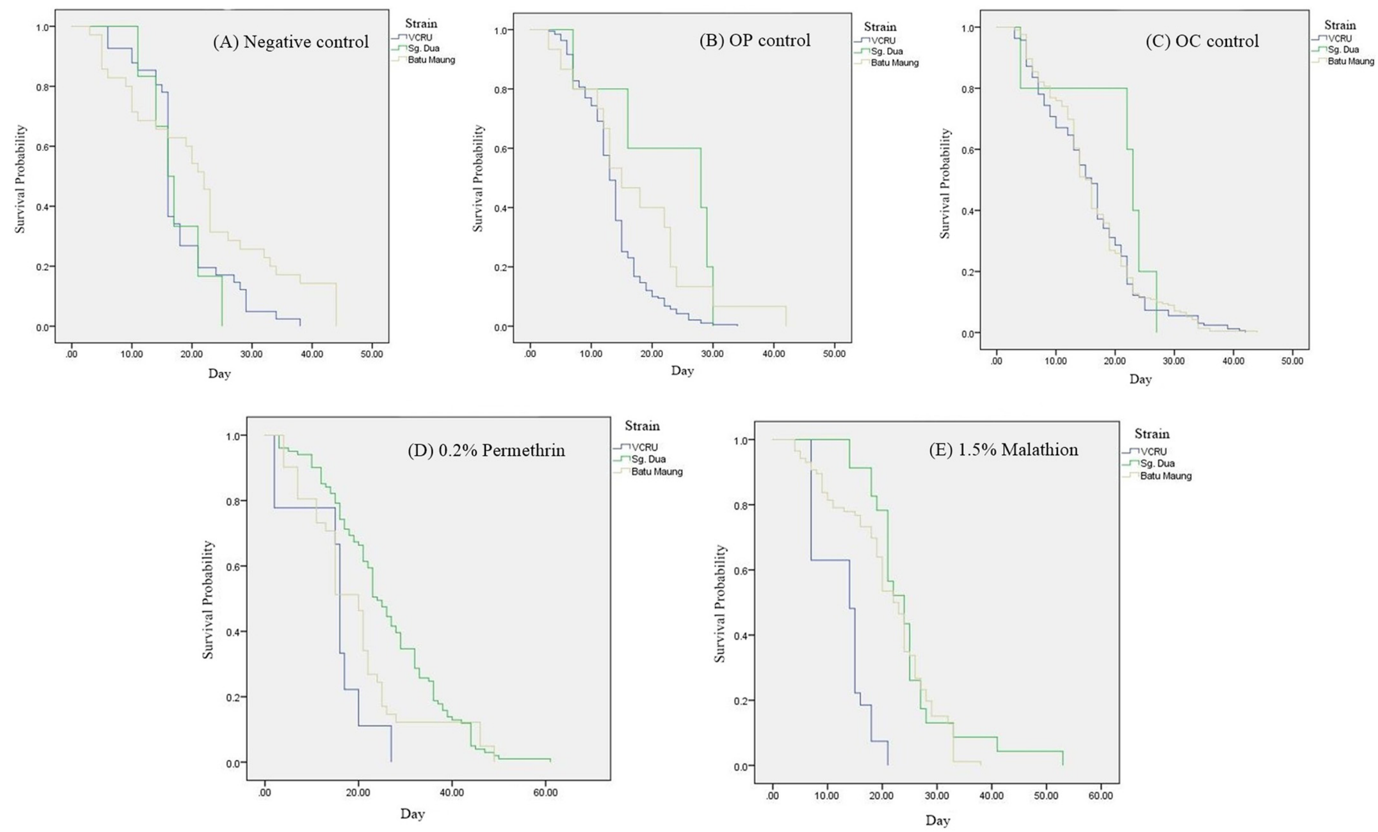


**Fig. 3.** The survival rate of *Aedes albopictus* after treated with (A) negative control, (B) OP control, (C) OC control, and insecticides of (D) 0.2% Permethrin, and (E) 1.5% Malathion.

and key life history traits. The large variations in fitness cost have been reported depending on species of insect, their genetic background and resistance mechanism (Plernsub et al., 2013). For examples, studied by Belinato et al. (2012) found out *Ae. aegypti* in Brazil which highly resistant to temephos exhibited decreases in bloodmeal acceptance, amount of ingested blood, egg production and frequency of inseminated females.

The individual mosquitoes that survived from the sub-lethal effect of insecticides, in turn, benefited with higher fecundity (Blackmore and Lord, 2000), and may enhance their survival (Boone and Semlitsch, 2001) which can influence pathogen transmission (Muturi et al., 2011). This condition seems to apply to the Sungai Dua mosquitoes strain when treated with permethrin. First ($F_1$) generations of Sungai Dua strain appears to inherit a resistance gene from their parents as mentioned in Lee (2000) study, the mosquito that developed resistance towards insecticide resulted in higher survival. Resistant genes are usually autosomal, as a result, resistance is inherited equally to both genders of mosquitoes (Sutherland et al., 1967).

The mode of sex determination in *Aedes* mosquitoes normally approximates 1:1 male to female. Females mosquito carried homogametic sex, *mm* and male carried heterogametic sex, *Mm*. The male parent has a crucial role in determining the sex ratio in progeny and given the normal segregation, which an equal number of males and females should occur (McClelland, 1962; Meiklejohn and Tao, 2010). However, *Ae. albopictus* in our study exposed to permethrin caused more male to emerge, but no effects on the sex ratio when treated with malathion which the cause is unknown. We postulate that the male sex genes might be less prone and affected by the insecticides compared to female gen in our mosquito strains. A study by Shaalan et al. (2005) showed that the sex ratio of sub-lethal emergent adult's mosquitoes tended to favor female. However, few studies suggested that insecticides may not significantly affect the sex ratio of mosquito populations (Aguilera et al., 1995; Shaalan et al., 2005; Sanil and Shetty, 2012).

## Conclusion

Sub-lethal treatments of either malathion or permethrin profoundly affected a variety of reproductive parameters in *Ae. albopictus*. The field strain of sub-urban Batu Maung mosquitoes was found more susceptible compared to urban Sungai Dua strain due to selection pressure of insecticides in their habitat, which sub-urban strain received less number of frequency of fogging activities. In this study, we discovered permethrin insecticide affecting more on the fitness cost of *Ae. albopictus* compared to malathion; such as developed a significant resistance towards WHO dose and sub-lethal dose, especially to Sungai Dua wild strain, the decrease of number laid of eggs, a lesser development time to reach each stage, also male adult emerged more than female mosquitoes. While, malathion insecticide still susceptible to all strains, and only effects towards fecundity by female mosquitoes and development time to reach of each stage. Even though mosquitoes developed resistance towards permethrin, which had caused less mortality, but their fitness cost still affected and thus helping to reduce the transmission of vector-borne diseases.

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