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Resistance development and insecticide susceptibility in *Culex quinquefasciatus* against selection pressure of malathion and permethrin and its relationship to cross-resistance towards propoxur

Selvi, S.[1], Edah, M.A.[1], Nazni, W.A.[2], Lee, H.L.[2] and Azahari, A.H.[2]
[1]Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur
[2]Entomology Division, Infectious Diseases Research Centre (IDRC), Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur

Abstract. To determine resistance level and characterize malathion and permethrin resistance in *Culex quinquefasciatus*, two methods were used namely: WHO procedures of larval bioassay to determine the susceptibility of lethal concentration (LC) and adult bioassay to determine the lethal time (LT) which are resistant to malathion and permethrin. These mosquito strains were bred in the Insectarium, Division of Medical Entomology, IMR. Thousands of late fourth instar larvae which survived the selection pressure to yield 50% mortality of malathion and permethrin were reared and colonies were established from adults that emerged. Larvae from these colonies were then subjected to the subsequent 10 generations in the test undertaken for malathion resistant strain (F61 – F70) and permethrin resistant strain (F54 – F63). Selection pressure at 50% - 70% mortality level was applied to the larvae of each successive generation. The rate of resistance development and resistance ratio (RR) were calculated by LC_{50} for larval bioassay and LT_{50} value for adult bioassay. The lab bred *Cx. quinquefasciatus* was used as a susceptible strain for comparison purpose. The adult bioassay test was carried out by using diagnostic dosages of malathion 5.0%, permethrin 0.75% and with propoxur 0.1%. All bioassay results were subjected to probit analysis. The results showed that LC_{50} for both malathion (F61 – F70) and permethrin (F54 – F63) resistant *Cx. quinquefasciatus* increased steadily to the subsequent 10 generations indicating a marked development of resistance. The adult female malathion resistant strain have developed high resistance level to malathion diagnostic dosage with resistance ratio 9.3 to 9.6 folds of resistance. Permethrin resistance ratio remained as 1.0 folds of resistance at every generation. It was obvious that malathion resistance developing at a higher rate in adult females compared to permethrin. Female adults exposed to 2 hours of exposure period for propoxur 0.1% showed presence of cross-resistance among the both strains of mosquitoes towards propoxur and it was indicated by 70%–100% mortality at 24 hours post-recovery period.

INTRODUCTION

Culex quinquefasciatus is one of the most common mosquitoes found in human habitations in the Tropics and Subtropics of the world. In most of its range the females intensely anthrophophilic, fed actively only at night and it causes nuisance (Richard & David, 1959) and are vectors of urban filariasis and Japanese encephalitis. Insecticide resistance is an increasing problem in vector control programmes. Insecticide resistance has continually challenged entomologists who seek means to retard its development or soften its impact on financially limited control programmes (Brattsten *et al.*,1986). Insecticide resistance is especially serious in disease vector and nuisance mosquitoes occurring at least in 83 anopheline and culicine species (Georghiou & Pasteur, 1978). Recently, resistance has been documented in 428 species of arthropods. Such resistance when widespread may

adversely hamper vector control programmes, rendering them highly ineffective as a tool for control. These documentations were mostly done via the conventional detection methods using World Health Organization (WHO) standard testing procedure mainly based on susceptibility tests which are dosage-mortality (bioassay) experiments done in the laboratory (Lee *et al.*,1996).

The principal factors on which the development of insecticide resistance in insect populations depends on various aspects. If the genetic potentiality for development of resistance to a given insecticide is present, the rate at which development proceeds will depend on certain obviously important factors such as the frequency of resistance genes and their dominance, the selection pressure and the previous history for exposure to insecticide. Also involved are ecological influences such as the isolation, inbreeding and reproductive potential of the insect population.

Since insecticides, particularly organophosphate (OP) and carbamate are still an integral part of vector management strategies, evaluation of vector management programmes must regularly be done to determine the rate at which they are contributing or enhancing resistance development (Brown & Brogdon, 1987). In this case, continuous monitoring of resistant mosquito populations may play an important role in trying to come up with management strategies that will prevent or minimize the development of resistance to effective insecticides (Lee & Tadano, 1994).

Knowing the proportion of resistant phenotypes will provide proper timing of insecticide application while confirmation and characterization of the resistance mechanisms will give possible choice of the insecticide to be used to retard the rapid evolution of resistance.

The objectives of this study were to determine the rate of resistance development to the insecticide malathion (OP) and permethrin (pyrethroid) in the presence of selection pressure and to verify whether it will result in cross-resistance to propoxur in those strains. Such knowledge is essential in defining future control strategies against this medically important mosquito.

MATERIALS AND METHODS

Mosquitoes

Adults of *Cx. quinquefasciatus* were bred in the Insectarium of Division of Medical Entomology, IMR. Adults females were supplied with caged mouse for blood feeding. Three days after feeding, a porcelain bowl half-filled with tap water was introduced for oviposition. Eggs laid were allowed to hatch in a tray of tap water containing ground mice pelletes. The larvae that emerged were used for the tests. The subsequent 10 generations of larval stage were subjected to selection pressure. To compare the resistance level of the resistant strains of *Cx. quinquefasciatus*, laboratory bred Penang strain reared for about 30 years were used as a standard susceptible strain. This strain has not been exposed to any insecticide or biological control agent.

Insecticides

Malathion 93.3% a.i. (Cynamide) and permethrin 10.9% a.i. (Shell) were used in this study.

Bioassay test for mosquito larvae

This test was conducted according to WHO (1981) larval susceptibility bioassay procedure. Twenty-five early fourth instar larvae were selected and the bioassay was conducted in disposable paper cups of 300ml capacity. Stock solution of the insecticide was prepared as for malathion 2,500 mg/L and permethrin was 1000 mg/L. Each insecticide consisted of five different concentrations in three replicates with serial dilution and three controls without insecticide. The prepared stock solution of insecticide was added into 150ml deionized tap water which was kept overnight. After introducing the larvae into paper cup, 100 ml water was added to

make the final volume as 250ml. Larval mortality was recorded after 24 hours of exposure. Moribund larvae if any were counted as dead.

Selection pressure test for mosquito larvae

The larval stages were subjected to selection pressure against malathion and permethrin at every 10 generations (thousands of late fourth instar larvae were treated in 1 litre capacity beaker together with the larvae that survived from larval bioassay test) to the concentration which yielded 50% mortality (LC_{50} in 24 hours) and the surviving larvae were reared to the next generation from the adults that emerged.

Bioassay test for adult mosquitoes

The female adults from each malathion and permethrin resistant *Cx. quinquefasciatus* mosquitoes were used in the test. Fifteen 10% sucrose fed females less than seven days old from each of the strains in four replicates and two controls were used. A diagnostic test using standard WHO Test Kits was conducted by exposing to papers impregnated with malathion 5.0%, permethrin 0.75% and propoxur 0.1%. Exposed mosquitoes were covered with black cloth to make sure they would be resting on the impregnated paper. Exposure tubes with permethrin impregnated papers were laid horizontally throughout the test. Cumulative mortality was recorded after every 5 minutes for all the test insecticides with their respective exposure periods which were 3 hours for malathion and 2 hours for permethrin and propoxur. Mosquitoes that survived the exposure period were then kept in holding tubes to observe the effect of post-treatment and mortality was recorded after 24 hours. Cotton pads soaked in 10% sugar solution were provided during the 24 hours holding period.

Insecticide impregnated papers

Malathion 5.0%, permethrin 0.75% and propoxur 0.1% impregnated papers were purchased from Vector Control Research Unit, Penang, Malaysia.

Data analysis for susceptibility test

Lethal concentration (LC_{50}) for larvae and lethal time (LT_{50}) values for each strain and insecticide was calculated using the Probit Analysis Program (Raymond, 1985). Based on the LC_{50} and LT_{50} values resistance ratio (RR) was determined by the ratio of resistant strain to the ratio of susceptible strain by adopting the method of Brown & Pal (1971).

RESULTS

Larval bioassay

The results showed that LC_{50} for both malathion (F61 – F70) ten generations and permethrin (F54–F63) ten generations of resistant strains of *Cx. quinquefasciatus* increased steadily indicating a marked development of resistance rate (Figure 1) though it showed various susceptibility with higher and lower values of LC50 (mg/L) throughout the subsequent 10 generations. After subjection to selection pressure with malathion and permethrin, it was found that permethrin resistance was developing at a higher rate compared to malathion (Figure 1). Malathion and permethrin resistant strains have the highest level of resistance values, with LC50: 1.762 mg/L at 9^{th} generation and 3.979 mg/L at 6^{th} generation respectively (Table 1 & Table 2). The LC50 after 10 generations of malathion selection was 1.132 mg/L and permethrin selection was 2.136 mg/L. It was found that larvae resistance ratio of malathion selected strain ranged from 79.9 to 91.3 folds of resistance at LC50. Meanwhile, the resistance ratio at LC50 between permethrin selected strain and susceptible strain was 32.8 to 41.3 folds of resistance.

Adult bioassay for malathion

The susceptibility test of adult mosquitoes to diagnostic concentration for malathion 5.0% impregnated paper showed a variety of susceptibility to malathion when

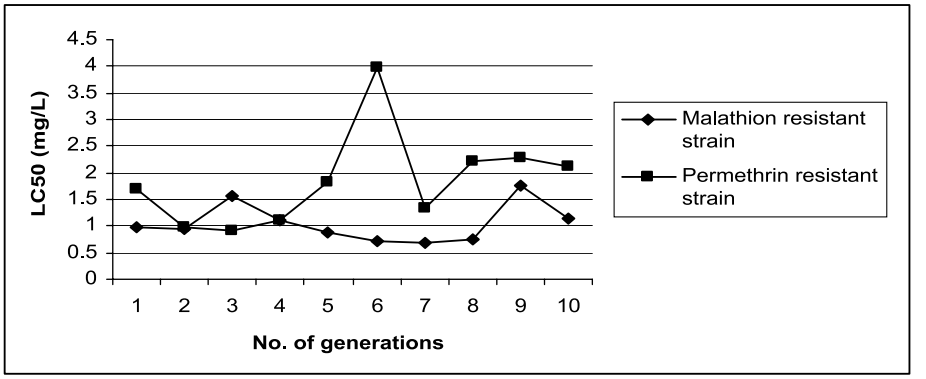

Figure 1. LC_{50} values of malathion and permethrin resistant *Culex quinquefasciatus* for larval bioassay at the 10 subsequent generations.

Table 1. LC_{50} (mg/L) values in insecticide test of early fourth instar larvae of laboratory and resistant strains of *Culex quinquefasciatus* of 10 subsequent generation exposed continuously for 24 hours to malathion

MALATHION				
Species/ Strain *Culex quinquefasciatus*	Generation	LC_{50} (mg/L) 95% (C.L)	Regression line	Resistance Ratio (RR)
Susceptible	F 685	0.0124 (0.0014 – 0.00259)	Y= 1.61x – 6.42	–
Resistant	F 61	0.9902 (0.9057 – 1.1835)	Y= 4.18x – 36.76	79.9
Resistant	F 62	0.9348 (0.9017 – 0.9731)	Y= 8.71x – 81.88	75.4
Resistant	F 63	1.5788 (1.2962 – 2.9828)	Y= 4.68x – 42.74	127.3
Resistant	F 64	1.1003 (0.9422 – 13.968)	Y= 2.01x – 15.18	88.7
Resistant	F 65	0.8763 (0.8094 – 0.9225)	Y= 6.46x – 59.23	70.7
Resistant	F 66	0.7196 (0.5508 – 0.8021)	Y= 4.95x – 43.81	58.0
Resistant	F 67	0.6707 (0.0051 – 0.8268)	Y= 2.27x – 17.30	54.1
Resistant	F 68	0.7632 (0.3425 – 1.3645)	Y= 4.83x – 42.73	61.5
Resistant	F 69	1.7621 (1.4440 – 4.7648)	Y= 3.82x – 34.12	142.1
Resistant	F 70	1.1319 (1.0426 – 1.1959)	Y= 5.42x – 49.52	91.3

Table 2. LC_{50} (mg/L) values in insecticide test of early fourth instar larvae of laboratory and resistant strains of *Culex quinquefasciatus* of 10 subsequent generations exposed continuously for 24 hours to permethrin

PERMETHRIN				
Species/ Strain *Culex quinquefasciatus*	Generation	LC_{50} (mg/L) 95% (C.L)	Regression line	Resistance Ratio (RR)
Susceptible	F 685	0.05167 (0.04648 – 0.06094)	Y= 4.19x – 31.52	–
Resistant	F 54	1.6973 (1.5478 – 1.8761)	Y= 3.62x – 32.02	32.8
Resistant	F 55	0.9845 (0.5344 – 1.7913)	Y= 2.38x – 18.78	19.1
Resistant	F 56	0.9062 (0.7764 – 1.0269)	Y= 2.75x – 22.39	17.5
Resistant	F 57	1.0929 (0.9159 – 1.2687)	Y= 2.07x – 15.77	21.2
Resistant	F 58	1.8394 (1.6458 – 2.0473)	Y= 2.92x – 24.99	35.6
Resistant	F 59	3.9794 (3.3522 – 5.5503)	Y= 3.80x – 35.30	77.0
Resistant	F 60	1.3358 (–)	Y= 1.73x – 12.52	25.9
Resistant	F 61	2.2160 (2.0536 – 2.3714)	Y= 4.62x – 42.82	42.9
Resistant	F 62	2.2747 (2.1270 – 2.4203)	Y= 5.11x – 47.93	44.0
Resistant	F 63	2.1355 (1.9499 – 2.3024)	Y= 4.15x – 37.89	41.3

compared with the susceptible strain. However it showed increase in LT50 values in the range 255.5 to 26010.6 minutes and the decrease of LC50 values in each successive generations was not consistent for malathion (Table 3). At the fourth generation (F64) it was observed that this strain has the highest level of malathion resistance. The resistance ratio after 10 generations of selection pressure increased from 9.3 to 9.6 folds of resistance compared with susceptible strain. As shown in Table 3 after 10 generations of selection, malathion resistance level had induced decrease in the percentage of 24 hours adult post-exposure mortality at the rate of 2.9 fold from generations F61 to F70.

Adult bioassay for permethrin

The resistance ratios at 50% lethal time (LT50) for permethrin showed various susceptibilities to permethrin impregnated paper compared with susceptible strain with a range of 17.0 to 30.8 minutes as shown in Table 4. After the subjection to selection pressure it was found that this strain had the highest value of permethrin resistance ratios in each successive generation and remained unexpectedly

Table 3. LT_{50} (min) values and 24 hours post-exposure mortality of malathion resistant *Culex quinquefasciatus* adult female mosquitoes of 10 subsequent generations exposed against WHO diagnostic dosage of malathion 5.0%

MALATHION 5.0% (3 Hours of Exposure Time)					24 hours post-exposure mortality (%)
Species/ Strain *Culex quinquefasciatus*	Generation	LT_{50} (mg/L) 95% (C.L)	Regression line	Resistance Ratio (RR)	
Susceptible	F 657	21.0 (20.2 – 21.7)	Y= 5.55x – 57.80	–	100
Resistant	F 61	7557.4 (–)	Y= 1.03x – 9.29	–	68.3
Susceptible	F 658	27.4 (25.9 – 28.9)	Y= 6.54x – 69.80	–	100
Resistant	F 62	255.5 (229.5 – 446.4)	Y= 12.14x – 145.67	9.3	45
Susceptible	F 659	31.4 (30.3 – 32.4)	Y= 11.34x – 125.32	–	100
Resistant	F 63	1648.5 (711.6 – 17041.6)	Y= 0.96x – 7.69	52.5	48.3
Susceptible	F 660	38.3 (37.3 – 39.4)	Y= 12.5x – 139.73	–	98.3
Resistant	F 64	26010.6 (–)	Y= 0.73x – 5.53	–	40
Susceptible	F 661	32.0 (30.5 – 33.2)	Y=13.11x – 145.81	–	100
Resistant	F 65	571.8 (283.4 – 22548960000)	Y= 2.34x – 24.86	17.9	38.3
Susceptible	F 662	31.5 (28.0 – 35.3)	Y= 8.15x – 88.67	–	100
Resistant	F 66	274.2 (233.1 – 374.7)	Y= 4.11x – 46.12	8.7	36.7
Susceptible	F 663	43.4 (41.8 – 44.9)	Y= 9.39x – 104.31	–	100
Resistant	F 67	326.9 (234.2 – 36349.2)	Y= 5.50x – 63.85	7.5	26.7
Susceptible	F 664	31.2 (30.2 – 32.2)	Y= 13.45x – 149.63	–	100
Resistant	F 68	403.4 (288.9 – 1024.5)	Y= 3.21x – 35.48	12.9	21.7
Susceptible	F 665	43.0 (41.7 – 44.3)	Y= 11.31x – 126.54	–	100
Resistant	F 69	280.0 (218.1 – 11563.3)	Y= 8.28x – 98.07	6.5	11.7
Susceptible	F 666	34.1 (33.0 – 35.2)	Y= 12.76x – 142.17	–	100
Resistant	F 70	326.1 (267.1 – 457.4)	Y= 3.09x – 33.66	9.6	23.3

Table 4. LT_{50} (min) values and 24 hours post-exposure mortality of permethrin resistant *Culex quinquefasciatus* adult female mosquitoes of 10 subsequent generations exposed against WHO diagnostic dosage of permethrin 0.75%

PERMETHRIN 0.75% (2 Hours of Exposure Time)					24 hours post-exposure mortality (%)
Species/ Strain *Culex quinquefasciatus*	Generation	LT_{50} (mg/L) 95% (C.L)	Regression line	Resistance Ratio (RR)	
Susceptible	F 657	19.5 (18.9-20.3)	Y = 8.19x – 87.47	–	100
Resistant	F 54	20.0 (18.3-21.9)	Y = 5.12x – 52.88	1.0	100
Susceptible	F 658	22.6 (22.0-23.3)	Y = 7.79x – 83.47	–	100
Resistant	F 55	27.3 (26.0-28.7)	Y = 5.95x – 63.02	1.2	100
Susceptible	F 659	22.6 (21.9-23.3)	Y = 7.64x – 81.47	–	100
Resistant	F 56	22.9 (21.9-24.0)	Y = 7.05x – 75.04	1.0	100
Susceptible	F 660	23.3 (21.8-24.9)	Y = 8.10x – 87.07	–	98.3
Resistant	F 57	23.7 (22.8-24.5)	Y = 7.22x – 77.13	1.0	100
Susceptible	F 661	21.2 (20.5-21.8)	Y = 6.68x – 70.69	–	100
Resistant	F 58	20.2 (17.2-23.7)	Y = 6.48x – 68.26	1.0	100
Susceptible	F 662	20.1 (19.5-20.8)	Y = 5.86x – 61.24	–	100
Resistant	F 59	22.0 (21.2-22.7)	Y = 8.32x – 89.35	1.1	100
Susceptible	F 663	22.9 (22.0-23.9)	Y = 7.55x – 80.76	–	100
Resistant	F 60	20.3 (18.6-22.0)	Y = 4.80x – 49.29	0.9	100
Susceptible	F 664	17.6 (17.1-18.2)	Y = 6.80x – 71.51	–	100
Resistant	F 61	30.8 (29.8-31.8)	Y = 8.58x – 93.55	1.8	98.3
Susceptible	F 665	24.4 (23.3-25.7)	Y = 10.20x – 111.21	–	100
Resistant	F 62	19.4 (18.5-20.2)	Y = 7.46x – 79.20	0.8	100
Susceptible	F 666	20.1 (19.1-21.2)	Y = 6.18x – 64.84	–	100
Resistant	F 63	17.0 (15.9-18.0)	Y = 4.98x – 50.93	0.8	100

unchanged with 1.0 folds of resistance compared with susceptible strain.

Adult bioassay for cross-resistance

Cross-resistance in malathion resistant strain treated against propoxur showed an increase of resistance ratio from 0.6 to 1.3 folds of resistance (Table 5) after seletion pressure to 10 generations. The LT50 values varied from 17.6 to 91.8 minutes. Whereas for permethrin resistant strains LT50 values with range of 27.2 to 97.1 minutes and the resistance ratio did not indicate drastic increase. These suggested that the cross-resistance to propoxur was present in these strains.

24 hours post-exposure treatment

At 24 hours recovery period malathion impregnated paper at 5.0% diagnostic concentration caused the least mortality rate in malathion resistant adults in range 11.7% to 48.3% of mortality compared to permethrin at 0.75% diagnostic concentration which induced higher percentage of mortality ranged 98.3% to 100% (Table 3 & Table 4). However in cross-resistance to propoxur it was observed that malathion resistant strain at the generations F66 and F69 indicated 73.3% and 75% of mortality respectively. These 2 generations indicated that there is resistant gene in these

Table 5. Cross-resistance susceptibility LT_{50} (min) values and 24 hours post-exposure mortality of malathion resistant *Culex quinquefasciatus* adult female mosquitoes of 10 subsequent generations exposed against WHO diagnostic dosage of propoxur 0.1%

PROPOXUR 0.1% (2 Hours of Exposure Time)					24 hours post-exposure mortality (%)
Species/ Strain *Culex quinquefasciatus*	Generation	LT_{50} (mg/L) 95% (C.L)	Regression line	Resistance Ratio (RR)	
Susceptible	F 657	80.7 (78.9–82.6)	Y = 10.79x – 123.51	–	100
Resistant	F 61	46.9 (44.3–49.68)	Y = 5.57x – 60.01	0.6	98.3
Susceptible	F 658	66.6 (53.0–83.9)	Y = 3.26x – 33.55	–	91.7
Resistant	F 62	29.2 (24.7–34.5)	Y = 6.24x – 66.53	0.4	88.3
Susceptible	F 659	80.2 (77.4–83.4)	Y = 5.45x – 59.86	–	83.3
Resistant	F 63	27.2 (25.8–28.5)	Y = 6.77x – 72.41	0.3	100
Susceptible	F 660	37.6 (35.8–39.4)	Y = 5.41x – 57.62	–	93.3
Resistant	F 64	21.1 (20.4–21.7)	Y = 12.4x – 135.36	0.6	100
Susceptible	F 661	53.1 (49.8–56.6)	Y = 4.30x – 45.42	–	85
Resistant	F 65	28.2 (27.4–29.1)	Y = 9.56x – 104.46	0.5	100
Susceptible	F 662	29.7 (15.0–58.8)	Y = 2.67x – 25.63	–	93.3
Resistant	F 66	53.9 (52.0–55.9)	Y = 6.44x – 70.54	1.8	73.3
Susceptible	F 663	49.8 (46.6–53.2)	Y = 3.88x – 40.40	–	93.3
Resistant	F 67	17.6 (16.5–18.7)	Y = 6.54x – 68.54	0.4	100
Susceptible	F 664	31.0 (29.9–32.1)	Y = 6.90x – 74.29	–	100
Resistant	F 68	24.7 (22.7–26.8)	Y = 8.75x – 94.65	0.8	100
Susceptible	F 665	76.1 (73.8–78.6)	Y = 6.43x – 71.40	–	81.7
Resistant	F 69	91.8 (88.8–95.2)	Y = 6.28x – 70.14	1.2	75
Susceptible	F 666	30.7 (29.8–31.7)	Y = 8.81x – 96.23	–	98.3
Resistant	F 70	38.5 (32.0–46.2)	Y = 3.76x – 38.56	1.3	100

population which relatively induced cross-resistance to propoxur. Meanwhile, permethrin resistant strain showed 93.3% to 100% percentage of mortality. These suggested there was presence of cross-resistance in these strains to propoxur with malathion selected mosquitoes but no cross-resistance was observed against permethrin.

DISCUSSION

From the results obtained it was shown that permethrin resistance was developing at a higher rate compared to malathion in permethrin resistant larvae (Figure 1) This trend supports a similar study conducted by Nazni *et al.* (1998) and also in Cuba, where *Cx. quinquefasciatus* developed resistance to Cypermethrin when this pyrethroid was used in alternate cycles with malathion (Rodriquez *et al.*, 1993). There was no marked difference in the resistance pattern to malathion resistant strain in LC50 values where this strain only produced resistance at a lower rate. Both strains of larvae exhibited a significant reduction in resistance towards LC50 values after few generations. It was not clear why such variations on the LC50 value was found, however it can be

Table 6. Cross-resistance susceptibility LT_{50}(min) values and 24 hours post-exposure mortality of permethrin resistant *Culex quinquefasciatus* adult female mosquitoes of 10 subsequent generations exposed against WHO diagnostic dosage of propoxur 0.1%

PROPOXUR 0.1% (2 Hours of Exposure Time)					24 hours post-exposure mortality (%)
Species/ Strain *Culex quinquefasciatus*	Generation	LT_{50} (mg/L) 95% (C.L)	Regression line	Resistance Ratio (RR)	
Susceptible	F 657	80.7 (78.9–82.6)	Y = 10.79x – 123.51	–	100
Resistant	F 54	75.3 (72.5–78.1)	Y = 5.13x – 55.90	0.9	100
Susceptible	F 658	66.6 (53.0–83.9)	Y = 3.26x – 33.55	–	91.7
Resistant	F 55	80.2 (77.4–83.2)	Y = 5.45x – 59.86	0.6	100
Susceptible	F 659	80.2 (77.4–83.4)	Y = 5.45x – 59.86	–	83.3
Resistant	F 56	34.4 (30.5–38.9)	Y = 8.51x – 93.15	0.4	96.7
Susceptible	F 660	37.6 (35.8–39.4)	Y = 5.41x – 57.62	–	93.3
Resistant	F 57	39.8 (38.0–41.7)	Y = 5.94x – 63.92	1.1	100
Susceptible	F 661	53.1 (49.8–56.6)	Y = 4.30x – 45.42	–	85
Resistant	F 58	38.7 (36.1–41.6)	Y = 9.27x – 102.40	0.7	100
Susceptible	F 662	29.7 (15.0–58.8)	Y = 2.67x – 25.63	–	93.3
Resistant	F 59	32.2 (30.7–33.6)	Y = 7.11x – 76.80	1.1	100
Susceptible	F 663	49.8 (46.6–53.2)	Y = 3.88x – 40.40	–	93.3
Resistant	F 60	34.6 (33.2–36.0)	Y = 8.11x – 88.61	0.7	100
Susceptible	F 664	31.0 (29.9–32.1)	Y = 6.90x – 74.29	–	100
Resistant	F 61	43.2 (41.5–44.9)	Y = 7.78x – 85.50	1.4	93.3
Susceptible	F 665	76.1 (73.8–78.6)	Y = 6.43x – 71.40	–	81.7
Resistant	F 62	97.1 (75.3–257.6)	Y = 5.01x – 55.08	1.3	96.7
Susceptible	F 666	30.7 (29.8–31.7)	Y = 8.81x – 96.23	–	98.3
Resistant	F 63	27.2 (25.6–28.8)	Y = 5.07x – 53.00	0.9	100

verified by conducting biochemical test . It was obvious that selection pressure is important for maintaining resistance in the larval population. It is likely that the instability of resistance may be contributed by heterozygous population.

Adult bioassay results showed permethrin is the most potent insecticide to produce high level of mortality rate in adults. Continuous selection pressure on malathion can cause resistance development at a higher rate in adults compared to permethrin. Analysis of the results of this study obviously indicating that resistance gene expression was more active in larvae compared to adults in comparison with the resistance ratio results of malathion and permethrin in the 24 hours post-treatment. Similarly, Tadano & Brown (1996) reported a lower resistance ratio in adults compared to larvae after subjection to several insecticide.

Results indicated that presence of cross-resistance among both the strains in 24 hours post-recovery period. This is due to the selection by a certain insecticide of one or more genes will generally extend to other compounds that shares either a metabolic pathway or a target site. With the development of more cases of resistance this simple picture has now

become much more complex and consequently, predictions about cross-resistance spectra are much more difficult. One obvious reason for this is that different groups of genes can be selected with one insecticide. In the overall trend malathion resistant strain did not directly influence cross-resistance to propoxur and also there was no cross-resistance observed in permethrin resistant strain against propoxur.

In conclusion, the findings of this present study indicated that permethrin selection of resistance was developing at a faster rate compared to malathion based on the LC50 values and malathion was a promising chemical larvicidal agent for the control of *Cx. quinquefasciatus* larvae. In contrast, permethrin is the most potent adulticide to produce high level of mortality in adult *Cx. quinquefasciatus*. Meanwhile there was some low levels of cross-resistance relationship against propoxur in both the strains and biochemical study is needed to verify the spectrum of cross-resistance involved.

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