The Development of Resistance and Susceptibility of *Aedes Aegypti* Larvae and Adult Mosquitoes against Selection Pressure to Malathion, Permethrin and Temephos Insecticides and its Cross-Resistance Relationship against Propoxur

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ABSTRACT In a response to mosquito-borne disease outbreak from time to time, the susceptibility of a medically important mosquito, *Aedes aegypti* was assessed to determine resistance development against chemical insecticides malathion, permethrin and temephos. World Health Organization (WHO) standard procedures were used namely, larval bioassay to determine the susceptibility of lethal concentration (LC) and adult bioassay to determine the lethal time (LT) in malathion, permethrin and temephos of selected resistant strains. These mosquito strains were bred in the Insectarium, Division of Medical Entomology, Institute for Medical Research (IMR), Malaysia. Colonies of these mosquito strains were established from the larvae by subjecting to selection pressure which yield 50% - 70% mortality level to the subsequent 10 generations. The rate of resistance development and resistance ratio (RR) was calculated by LC50 and LT50 values for larval bioassay and adult bioassay respectively. The lab bred *Aedes aegypti* 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 exhibited the following insecticides shown degree of potency or effectiveness to larvae of *Aedes aegypti* when comparison made on its resistance ratio (RR) in ascending order permethrin > malathion > temephos. It was suggested that temephos is a promising chemical larvicidal agent for the control of *Aedes aegypti* larvae. In contrast, malathion and permethrin were the effective adulticide agent for the control of adult *Aedes aegypti*. There was some degree of a cross-resistance relationship against propoxur in these three strains

ABSTRAK Berikutan kejadian penyakit bawaan nyamuk dari masa ke masa, kerentanan nyamuk yang penting dari segi perubatan, *Aedes aegypti* telah diselidiki untuk penentuan perkembangan kerintangan terhadap insektisid kimia seperti malathion, permethrin dan temephos. Prosedur piawai Badan Kesihatan Sedunia (WHO) seperti bioasei larva untuk penentuan kerentanan kepekatan (LC) dan bioasei dewasa untuk penentuan waktu maut (LT) dengan malathion, permethrin dan temephos bagi beberapa strain rintang terpilih. Strain nyamuk ini telah dibela di Insektari, Bahagian Entomologi Prubatan, Institut Penyelidikan Perubatan, (IMR), Malaysia. Koloni nyamuk ini telah dibela dari larva dengan mengenakan tekanan pemilihan yang menghasilkan 50% - 70% tahap kematian hingga kepada 10 generasi seterusnya. Kadar perkembangan kerintangan dan nisbah kerintangan dikira mengikut nilai LC50 dan LT50 bagi bioasei larva dan bioasei dewasa. *Aedes aegypti* belaan makmal telah digunakan sebagai strain rentan untuk perbandingan. Ujian bioasei dewasa telah dijalankan menggunakan dos diagnostik 5.0% malathion, 0.75% permethrin dan dengan 0.1% temephos. Kesemua keputusan bioasei telah dikenakan analisa probit. Keputusan darjah potensi insektid atau kecekapannya terhadap larva *Aedes aegypti* berbanding dengan nisbah kerintangan mengikut turutan menaik adalah permethrin > malathion > temephos. Dicadangkan temephos sebagai agen larvisid kimia bagi kawalan larva *Aedes aegypti*. Sebaliknya, malathion dan permethrin adalah agen insektisid bagi kawalan *Aedes aegypti* dewasa. Terdapat beberapa darjah kerintangan silang terhadap propoxur bagi ketiga-tiga strain tersebut.

(Resistance development, resistance ratio, lethal concentration, lethal time, bioassay larvae & adults)

INTRODUCTION

The development of mosquito resistance to chemical insecticides is making the control of mosquitoes and the diseases they transmit more difficult [1]. Mosquito resistance to chemical insecticides which are widely used to control them is a major global problem today. Insecticide resistance is especially serious in disease vector and nuisance mosquitoes, occurring at least 83 anopheline and culicine species [2]. Such resistance when widespread may hamper vector control programmes, rendering them highly ineffective as a tool for control.

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 of exposure to insecticides. Also involved are ecological influences such as the isolation, inbreeding and reproductive potential of the insect population.

There has been a large increase in the number of insecticide-resistant culicine species and also an increase in the geographical areas involved. According to the 22nd Report of the WHO Expert Committee on Insecticides, [3] of 41 species exhibiting resistance, 35 are resistant to DDT, 26 to dieldrin and 49 to organophosphorus and carbamate compounds. About 17 culicine species are resistant to all three groups of insecticides. The increase in the number of species with multiple resistances to organophosphorus compounds is of particular importance, since these constitute the main group of insecticides used for larviciding, which is the principal attack measure against most of these species.

Resistance of *Aedes aegypti* to chlorinated hydrocarbons is general in tropical America and South-East Asia. Resistance is rapidly increasing in Africa and in the Pacific Islands, mainly to dieldrin. Organophosphorus resistance has been recorded in the field in a number of places in tropical America and in South Vietnam, but multiple resistance was not detected and the resistant strains could not be colonized. The reports of organophosphorus resistance in New Caledonia, Malaysia, Congo and Thailand should be confirmed, as the tests are not yet conclusive [4].

Since insecticides, particularly organophosphate (OP), carbamate and pyrethroid are still an integral part of vector management strategies, evaluation of vector management programs must be done regularly to determine the rate at which they are contributing or enhancing resistance development [5]. 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 [6].

The objective of this study was to evaluate the characterization of the resistance development to malathion, permethrin and temephos due to selection pressure in *Aedes aegypti* larval population and to determine whether selection induces cross-resistance in adult population to propoxur. Such knowledge is essential in defining future control strategies against this medically important mosquito.

Mosquito

Adult mosquitoes and larvae i.e. *Aedes aegypti* were bred in the Insectarium of Division of Medical Entomology, IMR, Malaysia. Adult females were supplied with wire caged white mouse for blood feeding. Three days after feeding, a piece of moist filter paper in a porcelain bowl half-filled with water was introduced for oviposition of *Aedes aegypti*. Eggs laid were allowed to hatch in a tray of tap water. The 1st and 2nd instar larvae of *Aedes aegypti* were fed on liver powder while the the 3rd and 4th instar larvae were fed on small piece of half-cooked liver. The larvae that emerged were used for the test undertaken. The subsequent 10 generations of larval stage were subjected to selection pressure. To compare the resistance level of the resistant strains of *Aedes aegypti* strain which was reared for about 30 years were used as standard susceptible strain. This strain has not been exposed to any insecticide or biological control agent.

Insecticides

Malathion and temephos (Organophosphates i.e. OP) 93.3% a.i. and sand granule formulation (1% weight/ weight, Abate ®) both were patented by Cynamide, Co. U.S. Permethrin (pyrethroid) 10.9% a.i. (patented by Shell, Malaysia) were used in this study.

Selection pressure

The larval stages were subjected to selection pressure against malathion, permethrin and temephos at every generations (thousand of late 4^{th} instar larvae were treated in 1 litre capacity beaker). The concentration chosen for the selection was one that gave 50% mortality and the surviving larvae were reared and the selections were continued for 10 successive generations.

Bioassay test for mosquito larvae

This test was conducted according to WHO standard larval susceptibility bioassay procedure [6]. Twenty-five early fourth instars larvae were selected and the bioassay was conducted in disposable paper cups of 300ml capacity. Stock solution of the insecticide was prepared for *Aedes aegypti* malathion at 500 mg/L , permethrin 1000 mg/L and temepos 50 mg/L Each insecticide consisted of five different concentrations in three replicates with different ascending volume and three controls without insecticide. The prepared stock solution of insecticide was added into 150 ml dechlorinized tap water which is kept overnight. After introducing the larvae into paper cup, 100 ml tap water was added to make the final volume as 250 ml. Larval mortality was recorded after 24 hours of exposure. Moribund larvae, if any, counted as dead. Larvae that survived were collected and subjected to selection pressure with the concentration of the respective insecticides which indicated 50% of larval mortality was reared and colonies were established from adults that emerged.

Bioassay test for adult mosquitoes

Non-blood fed females aged 2-5 days old from each malathion, permethrin and temephos selected *Aedes aegypti* 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 period which was 1 hour for malathion, 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 (LC50) for larvae and lethal time (LT50) values for each strain and insecticide was calculated using the Probit Analysis Program [7]. Based on the LC50 and LT50 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 [8].

Table 1. LC50 (mg/L) values in insecticide test of early fourth instar larvae of resistant strains of *Aedes aegypti* of 10 subsequent generation exposed continuously for 24 hours selection pressure to malathion, permethrin and temephos

GENERATION	MALATHION LC 50 (MG/L) (95% C.L.)	RESISTANCE RATIO (RR- S*)	GENERATION	PERMETHRIN LC 50 (MG/L) (95% C.L.)	RESISTANCE RATIO (RR- S*)	GENERATION	TEMEPHOS LC 50 (MG/L) (95% C.L.)	RESISTANCE RATIO (RR- S*)
Susceptible	0.1035 (0.0944 - 0.1128)	-	Susceptible	0.0415 (0.0305 – 0.0566)	-	Susceptible	0.0104 (0.0095 – 0.0114)	-
F43	0.3355 (0.3355 – 0.3544)	3.2	F39	0.0525 (0.0079 – 0.0702)	1.3	F42	0.0280 (0.0249 – 0.0352)	2.7
F44	0.3898 (0.3552 – 0.4733)	3.8	F40	0.0686 (0.0526 – 0.0897)	1.7	F43	0.0105 (0.0014 – 0.0399)	1.0
F45	0.2725 (0.2543 – 0.2835)	2.6	F41	0.1506 (0.1222 – 0.2461)	3.6	F44	0.0305 (0.0267 – 0.0414)	2.9
F46	0.2241 (0.1666 – 0.2551)	2.2	F42	0.1770 (0.1641 – 0.2037)	4.3	F45	0.0154 (0.0069 – 0.0311)	1.5
F47	0.1507 (0.0946 – 0.1816)	1.5	F43	0.1652 (0.1543 – 0.1770)	4.0	F46	0.0136 (0.0111 – 0.0150)	1.3
F48	0.2295 (0.2162 – 0.2431)	2.2	F44	0.2490 (0.2242 – 0.3260)	6.0	F47	0.0184 (0.0164 – 0.0250)	1.8
F49	0.1548 (0.1158 – 0.1773)	1.5	F45	0.2560 (0.2220 – 0.2961)	6.2	F48	0.0056 (0.0000 – 0.0097)	0.5
F50	0.2181 (0.2040 – 0.2357)	2.1	F46	0.1314 (0.1170 – 0.1476)	3.2	F49	0.0138 (0.0132 – 0.0143)	1.3
F51	0.1571 (0.1414 – 0.1686)	1.5	F47	0.1193 (0.1031 – 0.1360)	2.9	F50	0.0101 (0.0053 – 0.0122)	1.0
F52	0.1707 (0.1598 – 0.1809)	1.6	F48	0.0922 (0.0762 – 0.10150)	2.2	F51	0.0108 (0.0099 – 0.0115)	1.0

* Resistance ratio to susceptible strain

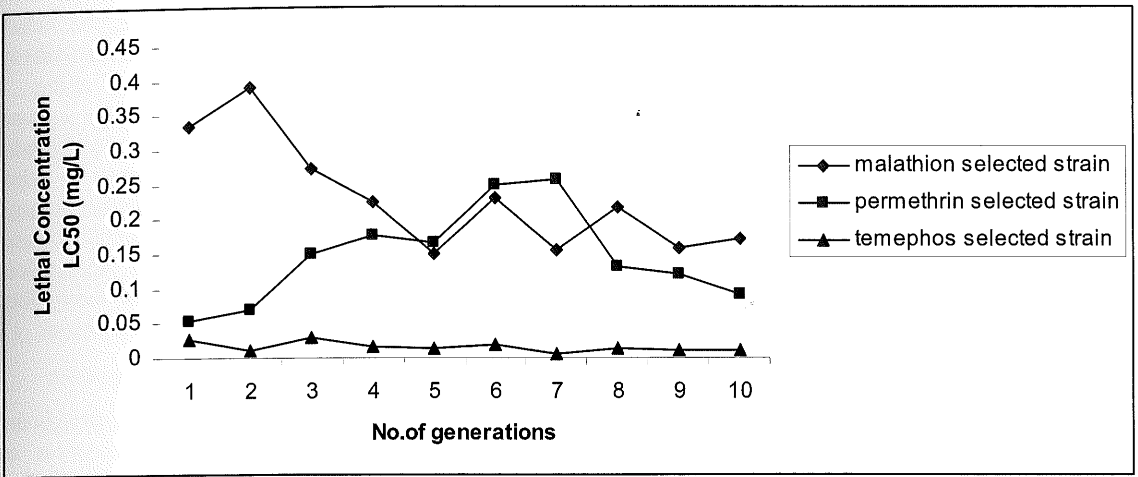

Figure 1. LC50 values of malathion, permethrin and temephos selected *Aedes aegypti* for larval bioassay at the 10 subsequent generations.

RESULTS

Larval bioassay

The results of larval bioassay against three different insecticide selected *Aedes aegypti* was shown in Table 1. The differences in the susceptibility of three different insecticide selected *Aedes aegypti* were clearly shown as indicated by their lethal concentration, LC50 values. Based on the LC50 values, malathion selected strain had the highest LC50 value at second generation (F44) with LC50 0.3898 mg/L, temephos selected strain at third generation (F44) 0.0305 mg/L and permethrin selected strain at seventh generation (F45) 0.2560 mg/L.

The resistance ratios in malathion selected strain varied from 1.5 folds to 3.8 folds, in temephos 0.5 folds to 2.9 folds and in permethrin 1.3 folds to 6.2 folds. From the results obtained it was found that permethrin resistance is developing in a higher degree compare to malathion and temephos. In the organophosphate insecticides group, temephos had a higher insecticidal activity than malathion when compared to its resistance ratio. Meanwhile permethrin was the least toxic among these two insecticides tested on *Aedes aegypti* larvae.

From Figure 1 it was noted that the development of resistance in the 3 different insecticide selected strains were inconsistent and gradual slopes of LC50 values indicating that these strains were comprised of heterozygous population in response to these insecticides. According to the resistance ratio (RR) obtained at LC50 it was evident that permethrin selected strain was highly resistant towards permethrin with 6.2 folds of resistance followed by malathion and temephos selected strain with the RR values 3.8 folds and 2.9 folds.

Adult bioassay for malathion

The susceptibility test of adult mosquitoes to diagnostic dosage for malathion 5.0% impregnated paper showed a variety of susceptibility to malathion when compared with the susceptible strain. It showed increase in LT50 values in the minutes range of 16.9 to 45.4 minutes (Table 2). At the first generation (F43) it was observed that this strain has the highest level of malathion resistance. The resistance ratio after ten generations of selection pressure increased from 0.8 to 2.1 folds of resistance compared with the susceptible strain. As shown in Table 2 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 1.7 fold from generation F43 to F52.

Adult bioassay for permethrin

The resistance ratios at 50% lethal time, LT50 for permethrin showed various susceptibility to permethrin impregnated paper compared with susceptible strain with a range of 10.8 to 16.7 minutes as shown in Table 3.This strain had a highest resistance ratio at fourth generation (F42) with the resistance ratio 1.7 folds.

Adult bioassay for cross-resistance

Cross-resistance in malathion resistant strain treated against propoxur with diagnostic dosage 0.1% showed an increase of resistance from 0.8 to 1.9 folds of resistance (Table 4) after selection pressure to 10 generations. The LT50 values varied from 31.2 to 80.2 minutes. The permethrin selected strains LT50 values with range of 23.2 to 52.5 minutes and the resistance ratio has not indicated drastic increase (Table 5) and it was similar to malathion LT50 values. Meanwhile in temephos selected strain the LT50 values ranged from 27.7 to 40.9 minutes and the cross-resistance ratio was two times compared with the other two insecticides from 0.6 to 2.1 folds of resistance (Table 6).

24 hours post-exposure treatment

At 24 hours recovery period malathion impregnated paper at 5.0% and permethrin at 0.75% diagnostic concentration caused higher mortality rate in range of 98.3% to 100% of mortality in both malathion and permethrin selected strain. Based on the evaluation criteria, it was considered that 100% mortality as susceptible, while at a mortality range of 90%-99% indicate that the status of resistance needs to be verified and mortality below 90% is considered resistant [9, 10]. Meanwhile in cross-resistance to propoxur it was observed that malathion selected strain at the generation F43, F46, F48, F49 and F50 caused mortality rate lesser than 90%. These five generations indicated that there is a presence of a resistant gene in these populations which induced cross-resistance to propoxur. Permethrin and temephos selected strains showed 80% to 98.3% and 75% to 98.3% of mortality respectively. These suggested that both the strains have slowly developed a certain degree of cross-resistance to propoxur.

DISCUSSION

From the results obtained it was shown that permethrin resistance was developing at a higher rate compared to malathion and temephos resistant larvae. This shows that malathion and temephos selected *Aedes aegypti* larvae was less resistant compared to permethrin insecticide used in this study. This finding is parallel to an earlier study by Paeporn [11] showing low levels of resistance to temephos after 19 generations of selection with the resistance ratio of 4.82 and 4.07 folds. The results exhibited the following insecticides shown degree of potency or effectiveness to larvae of *Aedes aegypti* when comparison made on its resistance ratio (RR) in ascending order permethrin > malathion > temephos. Similar study [12] with *Aedes aegypti* collected from Tortola, the British Virgin Islands, which showed low levels of resistance to other organophosphate and carbamate insecticides and a high level of resistance to the permethrin and moderate level of resistance to malathion, fenithrothion and chlorpyrifos in the thirty-four strains of *Aedes aegypti* larvae from 17 Caribbean countries [13].

All the larvae strains exhibited a significant decline in the resistance after subjection of selection pressure towards LC50 values after few generations. It was not clear why such variation on the LC50 values was found and probably this could be contributed by heterozygous genes in the population which caused quick dilution of resistant genotypes resulting in the decline of resistance level. However it can be verified by conducting biochemical test and polyacrylamide gel electrophoresis.

Adult bioassay results exhibited permethrin and malathion as the most potent insecticide to produce high level of mortality rate in adults. In contrast, continuous selection pressure on permethrin and malathion can cause resistance development at higher rates in larvae. Analysis of the results of this study obviously indicating that gene expression was more active in larvae compared to adults in comparison with the resistance ratio results of malathion and permethrin in 24 hours post-treatment [14]. Therefore, it was evident that resistance does not depend upon one or the other stages of mosquitoes.

Results indicated that presence of cross-resistance among the three 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 share either a metabolic pathway or target site. From the results obtained it was found that malathion selected strain directly influenced cross-resistance to propoxur with the lowest mortality rate.

Table 2. LT50 (min) values and 24 hours post-exposure mortality of malathion selected *Aedes aegypti* adult female mosquitoes of 10 subsequent generation exposed against WHO diagnostic dosage of malathion 5.0%

MALATHION 5.0% (1 Hour of Exposure Time)					24 HOURS POST-EXPOSURE MORTALITY (%)
SPECIES/ STRAIN *Aedes aegypti*	**GENERATION**	**LT50 (MG/L) 95% (C.L)**	**REGRESSION LINE**	**RESISTANCE RATIO (RR- S*)**	
Susceptible	F948	23.4 (15.1 -36.2)	Y = 4.73x - 48.76	-	100
Resistant	F43	45.4 (43.5 – 47.5)	Y = 6.77x -73.93	1.9	98.3
Susceptible	F949	20.5 (19.4 -21.7)	Y = 4.67x – 47.82	-	100
Resistant	F44	27.2 (25.9 – 28.4)	Y = 7.67x – 82.67	1.3	100
Susceptible	F950	16.4 (15.2 – 17.8)	Y= 4.96x – 50.61	-	100
Resistant	F45	33.7 (29.9 – 37.0)	Y = 9.7x – 106.84	2.1	100
Susceptible	F951	19.0 (18.7 – 19.4)	Y = 12.71x – 151.05	-	100
Resistant	F46	37.5 (36.2 – 38.8)	Y= 9.40x – 103.77	2.0	100
Susceptible	F952	21.8 (21.3 – 22.4)	Y= 9.13x – 98.54	-	100
Resistant	F47	24.7 (23.4 – 25.9)	Y= 7.57x – 81.23	1.1	98.3
Susceptible	F953	21.8 (21.2 – 22.4)	Y= 8.61x – 92.59	-	100
Resistant	F48	23.2 (21.9 – 24.5)	Y= 6.49x – 68.70	1.1	98.3
Susceptible	F954	20.7 (20.1 – 21.3)	Y= 8.03x – 85.90	-	100
Resistant	F49	22.0 (20.9 – 23.1)	Y= 8.87 – 95.61	1.1	100
Susceptible	F955	15.2 (14.7 – 15.6)	Y= 7.82x – 82.44	-	100
Resistant	F50	27.1 (26.0 – 28.2)	Y= 9.81x – 107.18	1.8	100
Susceptible	F956	20.7 (20.2 – 21.1)	Y= 11.65x – 126.78	-	100
Resistant	F51	16.9 (15.7 – 18.1)	Y= 5.09x – 52.15	0.8	98.3
Susceptible	F957	23.9 (23.2 – 24.8)	Y= 7.40x – 79.21	-	100
Resistant	F52	27.3 (26.0 – 29.0)	Y= 7.68x -82.83	1.1	98.3

Susceptible – laboratory strain (S)
Resistant – selection pressure strain (R)

* Resistance ratio to susceptible strain

Table 3. LT50 (min) values and 24 hours post-exposure mortality of permethrin selected *Aedes aegypti* adult female mosquitoes of 10 subsequent generation exposed against WHO diagnostic dosage of permethrin 0.75%

	PERMETHRIN 0.75% (1 HOUR OF EXPOSURE TIME)				**24 HOURS POST-EXPOSURE MORTALITY (%)**
SPECIES/ STRAIN *Aedes aegypti*	**GENERATION**	**LT50 (MG/L) 95% (C.L)**	**REGRESSION LINE**	**RESISTANCE RATIO (RR- S*)**	
Susceptible	F948	13.5 (13.1 – 13.8)	Y= 9.37x – 99.28	-	100
Resistant	F39	12.7 (11.7 – 13.8)	Y= 5.40x – 54.99	0.9	100
Susceptible	F949	12.8 (12.1 – 13.4)	Y= 5.83x – 59.74	-	100
Resistant	F40	14.6 (14.0 – 15.1)	Y= 6.45x – 67.03	1.1	100
Susceptible	F950	13.7 (13.2 – 14.2)	Y= 7.03x – 73.32	-	100
Resistant	F41	10.8 (9.9 – 11.4)	Y= 4.3x – 42.42	0.8	100
Susceptible	F951	6.5 (5.4 – 7.3)	Y= 3.34x – 31.10	-	100
Resistant	F42	10.9 (10.2 -11.5)	Y= 5.32x – 53.74	1.7	100
Susceptible	F952	19.1 (18.7 – 19.60)	Y= 8.53x – 91.25	-	100
Resistant	F43	16.7 (12.1 – 22.9)	Y= 5.37x – 55.28	0.9	100
Susceptible	F953	14.1 (13.6 – 14.6)	Y= 6.75x – 70.22	-	100
Resistant	F44	7.7 (6.2 – 9.2)	Y= 2.92x – 26.78	0.5	100
Susceptible	F954	9.3 (8.1 – 10.5)	Y= 3.26x – 30.76	-	100
Resistant	F45	14.9 (14.0 – 16.0)	Y= 5.39x – 55.24	1.6	100
Susceptible	F955	8.1 (6.7 – 9.6)	Y= 3.85x – 37.0	-	100
Resistant	F46	12.5 (11.6 – 13.3)	Y= 3.25x – 31.05	1.5	100
Susceptible	F956	9.9 (8.6 – 11.3)	Y= 4.08x – 39.84	-	100
Resistant	F47	13.6 (12.6 – 14.8)	Y= 5.19x – 52.19	1.4	98.3
Susceptible	F957	13.9 (13.5 – 14.4)	Y= 8.09x – 85.18	-	100
Resistant	F48	12.7 (11.7 – 13.8)	Y= 5.14x – 55.10	0.9	100

Susceptible – laboratory strain (S)
Resistant – selection pressure strain (R)

* Resistance ratio to susceptible strain

Table 4. Cross-resistance susceptibility LT50 (min) values and 24 hours post-exposure mortality of malathion selected *Aedes aegypti* adult female mosquitoes of 10 subsequent generation exposed against WHO diagnostic dosage of propoxur 0.1%

PROPOXUR 0.1% (1 HOUR OF EXPOSURE TIME)					24 HOURS POST-EXPOSURE MORTALITY (%)
SPECIES/ STRAIN *Aedes aegypti*	GENERATION	LT50 (MG/L) 95% (C.L)	REGRESSION LINE	RESISTANCE RATIO (RR- S*)	
Susceptible	F948	22.7 (5.4 – 94.8)	Y= 4.36x – 44.51	-	96.7
Resistant	F43	80.2 (68.5 – 138.4)	Y= 7.66x – 86.20	3.5	26.7
Susceptible	F949	19.8 (19.0 – 20.6)	Y= 4.64x – 47.42	-	100
Resistant	F44	37.0 (35.5 – 38.6)	Y= 7.00x – 76.61	1.9	96.7
Susceptible	F950	21.9 (21.2 – 22.6)	Y = 6.67x – 70.61	-	100
Resistant	F45	37.8 (36.0 – 39.8)	Y= 5.39x – 57.40	1.7	100
Susceptible	F951	38.3 (36.8 – 39.8)	Y= 6.98x – 75.86	-	90
Resistant	F46	65.2 (60.1 – 75.7)	Y= 6.72x – 74.40	1.7	31.7
Susceptible	F952	29.8 (27.8 – 31.9)	Y= 7.72x – 85.53	-	98.3
Resistant	F47	41.2 (39.6 – 42.9)	Y= 7.22x – 78.90	1.4	91.7
Susceptible	F953	49.5 (47.6 – 51.6)	Y= 8.28x – 91.79	-	81.7
Resistant	F48	43.4 (41.4 – 45.7)	Y = 5.82x – 62.70	0.9	88.3
Susceptible	F954	34.2 (32.8 – 35.7)	Y= 6.39x – 63.70	-	100
Resistant	F49	43.5 (41.0 – 46.5)	Y = 4.38 – 45.98	1.3	81.7
Susceptible	F955	30.4 (29.0 – 31.9)	Y= 5.32x – 56.07	-	100
Resistant	F50	52.8 (50.8 – 55.5)	Y= 8.43x – 93.84	1.7	81.7
Susceptible	F956	41.5 (39.4 – 43.9)	Y= 4.98x – 52.84	-	80
Resistant	F51	31.2 (29.6 – 32.8)	Y= 5.68x – 60.31	0.8	93.3
Susceptible	F957	35.9 (33.9 -37.7)	Y= 5.95x – 63.76	-	98.3
Resistant	F52	46.1 (44.3 – 48.1)	Y= 7.39x – 81.18	1.3	93.3

Susceptible – laboratory strain (S)
Resistant – selection pressure strain (R)

* Resistance ratio to susceptible strain

Table 5. Cross-resistance susceptibility LT50 (min) values and 24 hours post-exposure mortality of permethrin selected *Aedes aegypti* adult female mosquitoes of 10 subsequent generation exposed against WHO diagnostic dosage of propoxur 0.1%

PROPOXUR 0.1% (1 HOUR OF EXPOSURE TIME)					24 HOURS POST-EXPOSURE MORTALITY (%)
SPECIES/ STRAIN *Aedes aegypti*	**GENERATION**	**LT50 (MG/L) 95% (C.L)**	**REGRESSION LINE**	**RESISTANCE RATIO (RR- S*)**	
Susceptible	F948	22.7 (5.4 – 94.8)	Y= 4.36x – 44.51	-	96.7
Resistant	F39	39.0 (22.8 – 66.6)	Y= 6.70x – 72.66	1.7	91.7
Susceptible	F949	19.8 (19.0 – 20.6)	Y= 4.64x – 47.42	-	100
Resistant	F40	28.7 (27.0 – 30.6)	Y= 6.62x – 70.86	1.4	91.7
Susceptible	F950	21.9 (21.2 – 22.6)	Y = 6.67x – 70.61	-	100
Resistant	F41	42.2 (40.1 – 44.5)	Y= 5.38x – 57.52	1.9	90
Susceptible	F951	38.3 (36.8 – 39.8)	Y= 6.98x – 75.86	-	90
Resistant	F42	29.6 (27.7 – 31.5)	Y= 41.0x – 42.01	0.8	95
Susceptible	F952	29.8 (27.8 – 31.9)	Y= 7.72x – 85.53	-	98.3
Resistant	F43	52.5 (49.7 – 56.1)	Y= 6.0x – 65.33	1.8	83.3
Susceptible	F953	49.5 (47.6 – 51.6)	Y= 8.28x – 91.79	-	81.7
Resistant	F44	46.3 (39.3 – 54.6)	Y= 51.5x – 55.08	0.9	80
Susceptible	F954	34.2 (32.8 – 35.7)	Y= 6.39x – 63.70	-	100
Resistant	F45	23.2 (22.0 – 24.3)	Y= 7.6x – 81.37	0.7	100
Susceptible	F955	30.4 (29.0 – 31.9)	Y= 5.32x – 56.07	-	100
Resistant	F46	24.3 (23.1 – 25.4)	Y= 7.98x – 85.82	0.8	100
Susceptible	F956	41.5 (39.4 – 43.9)	Y= 4.98x – 52.84	-	80
Resistant	F47	28.1 (26.7 – 29.5)	Y= 6.34x – 67.59	0.7	98.3
Susceptible	F957	35.9 (33.9 -37.7)	Y= 5.95x – 63.76	-	98.3
Resistant	F48	39.0 (22.8 – 66.6)	Y= 6.71x – 72.78	1.1	93.3

Susceptible – laboratory strain (S)
Resistant – selection pressure strain (R)

* Resistance ratio to susceptible strain

Table 6. Cross-resistance susceptibility LT50(min) values and 24 hours post-exposure mortality of temephos selected *Aedes aegypti* adult female mosquitoes of 10 subsequent generation exposed against WHO diagnostic dosage of propoxur 0.1%

PROPOXUR 0.1% (1 HOUR OF EXPOSURE TIME)					24 HOURS POST-EXPOSURE MORTALITY (%)
SPECIES/ STRAIN *Aedes aegypti*	GENERATION	LT50 (MG/L) 95% (C.L)	REGRESSION LINE	RESISTANCE RATIO (RR- S*)	
Susceptible	F948	22.7 (5.4 – 94.8)	Y= 4.36x – 44.51	-	96.7
Resistant	F42	33.2 (31.7 – 35.0)	Y= 4.27x – 44.18	1.5	96.7
Susceptible	F949	19.8 (19.0 – 20.6)	Y= 4.64x – 47.42	-	100
Resistant	F43	42.4 (40.3 – 44.9)	Y= 5.0x – 53.12	2.1	90
Susceptible	F950	21.9 (21.2 – 22.6)	Y = 6.67x – 70.61	-	100
Resistant	F44	42.1 (39.8 – 44.7)	Y= 4.73x – 49.97	1.9	80
Susceptible	F951	38.3 (36.8 – 39.8)	Y= 6.98x – 75.86	-	90
Resistant	F45	29.5 (27.7 – 31.4)	Y= 4.35x – 44.88	0.8	85
Susceptible	F952	29.8 (27.8 – 31.9)	Y= 7.72x – 85.53	-	98.3
Resistant	F46	34.9 (33.4 – 36.4)	Y= 6.78x – 73.27	1.2	90
Susceptible	F953	49.5 (47.6 – 51.6)	Y= 8.28x – 91.79	-	81.7
Resistant	F47	28.0 (26.5 – 29.5)	Y= 5.75x – 60.8	0.6	98.3
Susceptible	F954	34.2 (32.8 – 35.7)	Y= 6.39x – 63.70	-	100
Resistant	F48	39.7 (37.9 – 41.6)	Y= 5.88x – 63.17	1.2	75
Susceptible	F955	30.4 (29.0 – 31.9)	Y= 5.32x – 56.07	-	100
Resistant	F49	27.7 (25.7 – 29.5)	Y= 4.62x – 47.88	0.9	100
Susceptible	F956	41.5 (39.4 – 43.9)	Y= 4.98x – 52.84	-	80
Resistant	F50	31.8 (30.3 – 33.2)	Y = 6.97x – 75.18	0.8	100
Susceptible	F957	35.9 (33.9 -37.7)	Y= 5.95x – 63.76	-	98.3
Resistant	F51	40.9 (39.2 – 42.4)	Y= 8.93x – 98.69	1.1	91.7

Susceptible – laboratory strain (S)
Resistant – selection pressure strain (R)

* Resistance ratio to susceptible strain

CONCLUSION

In conclusion the findings of this present study indicated that permethrin (pyrethroid) selection of resistance was developing at a higher rate compared to malathion and temephos (organophosphates) based on the LC50 values and resistance ratios. According to the degree of potency that has been shown earlier in this study, it was suggested that temephos is a promising chemical larvicidal agent for the control of *Aedes aegypti* larvae. In contrast, malathion and permethrin were the effective adulticide agent for the control of adult *Aedes aegypti.* There was some degree of cross-resistance relationship against propoxur in these three strains. However biochemical and gel electrophoresis (SDS PAGE) studies needed to verify the spectrum of the cross-resistance involved.

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