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Adult and larval insecticide susceptibility status of *Culex quinquefasciatus* (Say) mosquitoes in Kuala Lumpur Malaysia

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Abstract. The susceptibility of *Culex quinquefasciatus* to chemical insecticides in two field sites in Kuala Lumpur was evaluated using the WHO standard susceptibility test. Less then 7days old female mosquitos, reared from wild caught females were exposed to discriminating dosages of insecticides at recommended exposure periods. The larval bioassay were conducted using the multiple concentrations and the LC50 value was determined. The results indicated that cyfluthrin is the most effective among all the insecticides tested with LT50 value of 29.95 min and 28.59 min, for the strain from Ampang Hill and Pantai Dalam, respectively. It was surprisingly to note that both these field strains showed 0% mortality when tested against malathion and DDT. The LC50 value indicated that both strains were highly resistant to malathion with resistance ratio of 17,988 folds and 14,053 folds, respectively. This concludes that resistance at larval stages is extremely high compared to adult stages.

INTRODUCTION

The process of rapid urbanization and unplanned growth of cities has resulted into man made mosquito proliferation habitats promoting the breeding of a variety of disease vectors, and consequently enhanced disease transmission. Lack of adequate drainage and in many areas even the provision of drainage, and water stagnation is promoting the breeding of *Culex quinquefasciatus* and the spread of filariasis due to *Wuchereria bancrofti* (WHO, 1992).

Lymphatic filariasis, known as elephantiasis, puts at risk more than a billion people in more than 80 countries. Over 120 million have already been affected by it, over 40 million of them are seriously incapacitated and disfigured by the disease. One-third of the people infected with the disease live in India, one third are in Africa and most of the remainder are in South Asia, the Pacific and the Americas (WHO, 2005). In South –East Asian countries, it is one of most major public health problems and eight out of the ten countries in the Region are known to be endemic for filariasis (WHO, 2004). In Malaya filariasis was only first observed by Daniels (1908) who examined blood films from 100 patients and found *W. bancrofti* microfilaria (mf) in 3 of them, two Indians and one Chinese, all were immigrants. Study conducted by Leicester in 1908 stated that *Cx. quinquefasciatus* could be the vector of urban filariasis in Malaysia (Lee, 2005).

In Malaysia, there is no specific control programme for filariasis vectors. The disease is managed through mass application of chemotherapy. The mosquito *Cx. quinquefasciatus* is regarded as a nuisance mosquito. The extensive use of malathion and permethrin for dengue control and also in agricultural pest control would have indirectly contributed to pressure for selection of resistance to organo-phosphorous and

pyrethroid compounds in *Cx. quinquefasciatus* (Lee, per.com). The objective of the present study is to determine the susceptibility of *Cx. quinquefasciatus* to several commonly used larvicides and adulticides in the Federal Territory of Kuala Lumpur.

MATERIALS AND METHODS

Mosquitoes

Adult female *Cx. quinquefasciatus* were collected from two different localities namely, Pantai Dalam and Ampang Hill in Kuala Lumpur using the bare leg catch technique. The field mosquitoes were brought to the insectary and were blood - fed using white mice. Three days after bloodmeal the mosquitoes were set for egg laying. The larval stages were fed with ground mouse pellet. There were large number of larvae produced. A portion was used for larval bioassay and another portion of these were further reared to adult stages for adult bioassay .The adults were provided with 10% sugar solution with vitamin B complex. All susceptibility test for adult as well as larvae were conducted on filial generation 1 (F1).

Insecticides

The insecticides used for larval bioassay were two organophosphates (technical grade 93.3% a.i malathion, and 95.6% a.i temephos) and one pyrethroid (a commercial grade 10.9% a.i permethrin). The rational of using these selected insecticides for larval bioassay was that malathion and permethrin has been frequently used for dengue outbreaks control and temephos is always used as larvicide for container - breeding *Aedes* control.

The insecticides used in the adult susceptibility test were diagnostic dosages of WHO impregnated papers obtained from the Vector Control Research Unit in University of Science, Penang. The adults were tested against two organophosphates (5% malathion, 1% fenitrothion), three pyrethroids (0.75% permethrin, 0.05% lambdacyhalothrin (lambda), 0.15% cyfluthrin), one organochlorine (4% DDT) and one carbamate (0.1% propoxur).

WHO Larval Bioassay

The larval susceptibility test was conducted according to WHO(1981a). Twenty – five early 4th instar larvae of the mosquito strains were introduced into 250 ml of water containing various concentrations of insecticides. The insecticides used were malathion, temephos and permethrin. These insecticides were tested at 5 different concentrations with 3 replicates per concentration. In the control experiments, less than 1% ethanol was used in 250 ml of water since ethanol was the solvent used in the experiment. Mortality of the test was recorded at the end of 24 h and subjected to probit analysis to obtain the LC50 value.

WHO Adult Bioassay

The bioassay procedure of WHO (WHO, 1981b) was employed against all the mosquitoes species. Sugar - fed less than 7 days old adult female mosquitoe were used. Batches of 15 adult mosquitoes were exposed to insecticide-impregnated papers in standard WHO test tubes lined with the papers. All test were undertaken at 25°C ± 2°C. The mosquitoes were exposed to the diagnostic dosages at the respective exposure period. Cumulative mortality counts were recorded at every 5 minutes for the respective exposure periods. Results were recorded every five minutes until the respective exposure periods of the different insecticides. After the exposures periods, the mosquitoes were transferred into a clean paper cup and provided with sugar solution. The test mosquitoes and the controls were held for a 24-h recovery period and the mortality was recorded. If the control mortality was between 5% and 20%, the percentage mortalities should be corrected by Abbott's (1925) formula. All data were subjected to a probit analysis computer programme and LT50 for each insecticide from the different location was obtained (Raymond,

1985). Resistance ratio of all species was calculated.

RESULTS AND DISCUSSION

The LT50 values of *Cx. quinquefasciatus* adult mosquitoes laboratory and field strains exposed to various group of insecticides is shown in Table 1. A quick perusal of the data indicates that the most toxic diagnostic dosage against susceptible adult *Cx. quinquefasciatus* mosquitoes is permethrin with LT 50 value of 10.78 min. In the field strains, Ampang Hill and Pantai Dalam, cyfluthrin is the most effective among all the insecticides with LT50 value of 29.95 min and 28.59 min, respectively. Field strain of *Cx. quinquefasciatus* showed 0% mortality against WHO 4% malathion and 5% discriminating dosage. This indicates that this species is highly resistant to malathion and DDT. Both the field strains in Kuala Lumpur are under heavy pressures from organophosphorous compounds on adults through indoor and outdoor house spraying of malathion in dengue prone areas. In the bioassays, no mortalities occurred for any mosquitoes after exposure to control papers.

The resistance ratio of the insecticides in decending order for both the Ampang Hill and Pantai Dalam strains is DDT = malathion > fenitrothion > propoxur > permethrin > lambdacyhalothrin > cyfluthrin. Both strains has similar trend in toxicity. From the LT50 value though the pyrethroids takes the shortest time for mortality the resistant ratio (RR) value showed that *Cx. quinquefasciatus* has developed high level of resistance to permethrin in the Ampang Hill and Pantai Dalam with RR value of 12.20 and 10.95 respectively.

Table 1. Resistance status of field strain *Culex quinquefasciatus* adults to various groups of insecticides

Strains Insecticides	LT_{50} (min)	95% (Confidence Limit of LT_{50})	LT_{90} (min)	RF_{50}	RF_{90}
Susceptible strain					
fenitrothion	63.4	68.0 – 106.66	180.12	–	–
malathion	37.65	39.65 – 44.0	55.44	–	–
cyfluthrin	16.48	18.41 – 82.0	31.20	–	–
lambdacyhalothrin	24.75	2.0 – 27.06	46.84	–	–
permethrin	10.78	13.61 – 60.0	22.15	–	–
DDT	454.48	97.0 – 928.81	4735.83	–	–
propoxur	84.17	91.25 – 96.0	161.53	–	–
Ampang Hill					
fenitrothion	202.57	149.78 – 698.81	508.53	3.19	2.82
malathion	100% R*	–	–	100	–
cyfluthrin	29.95	26.69 – 33.41	91.54	1.82	2.90
lambdacyhalothrin	34.38	32.03 – 36.91	65.99	1.39	1.41
permethrin	78.15	69.46 – 87.72	270.29	7.20	12.20
DDT	100% R*	–	–	100	
propoxur	144.41	113.32 – 224.34	676.45	1.72	4.19
Pantai Dalam					
fenitrothion	612.08	251.24 – 9128.44	5170.62	9.65	28.70
malathion	100% R*	–	–	100R*	
cyfluthrin	28.59	25.86 – 31.23	66.91	1.73	2.14
lambdacyhalothrin	36.43	34.0 – 39.0	66.78	1.47	1.43
permethrin	79.82	73.07 – 87.49	242.46	7.40	10.95
DDT	100% R*	–	–	100R*	
propoxur	172.37	129.22 – 278.15	710.97	2.05	4.40

R* - Highly resistance i.e. zero mortality 24 h after exposure

The organochlorine insecticide, DDT is the least effective insecticide because in the susceptible strain the LT 50 value is 454.48 min which is considered to be high. Though, *Culex* is highly resistant to DDT and malathion in both the field strains however, in the susceptible strain the LT 50 value for malathion is 37.65 min. This indicates that if the mosquitoes are kept insecticide – free for a long period the resistance can be reversed. Fenitrothion which belongs to organophosphate group also exhibits very high resistance in Pantai Dalam strain with a resistance ratio of 28.70 folds. The emergence of resistance in this strain could be due to the usage of this chemical in agricultural sector since fenitrothion is not being used in the Malaysian Vector Control Programme.

The LC50 values of *Cx. quinquefasciatus* larvae against the insecticide malathion and temephos is shown in Table 2. From this table it shows clearly that both the Ampang Hill strain and Pantai Dalam strain are highly resistant to malathion with resistant ratio of 17,988 folds and 14,053 folds respectively. It also indicates that though the larvae are highly resistant to malathion its resistance ratio to temephos was 3.18 folds. This study indicates that there is no cross resistance of malathion against temephos in the larval stage. The existence of malathion resistance in Malaysian adult and larval *Cx. quinquefasciatus* has been confirmed by biochemical test (Lee, 1990; Lee *et al.*, 1992).

Although larviciding induces more larval resistance than adult resistance and adulticiding may produce more adult resistance than larvae, resistance is not restricted to one or the other stage. The larval test by its very nature is more sensitive than the adult test in detecting change in susceptibility level: roughly, a 2 fold increase in adult LC50 is accompanied by a 10 fold increase in larval LC50 and a 4 fold adult by a 100 fold increase in larval LC50. (Brown, 1986). Again roughly, a population may be termed resistant when its larval LC50 has increased by 10 times (Knipling, 1950).

Resistance to organophosphates and carbamate insecticides has been reported in many parts of the world. Bisset *et al.* (1994) reported that *Cx. quinquefasciatus* has shown resistance in the Eastern, Central and Western part of Cuba against malathion and carbamate. Similarly, in Brazil, Bracco *et al.* (1997) has shown that the mosquito is resistant to malathion fenitrothion and carbamate. They suggested insecticide management program should be developed. In the African *Cx. quinquefasciatus*, the mosquitoe larvae were resistant to chlorpyrifos and temephos with resistance ratio of 3 to 6 folds and 3 to 18 folds, respectively (Chandre *et al.*, 1998). In Florida, Liu *et al.* (2004) indicated that the *Cx. quinquefasciatus* larvae were 4-70 folds resistant to malathion, 13 – 940 folds resistant to permethrin and 200-830 folds resistant to resmethrin. Their study

Table 2. Resistance status of field strain larvae of *Culex quinquefasciatus* to various groups of insecticides

Strains Insecticides	LC_{50} (mg/L)	95% (Confidence Limit of LC_{50})	LC_{90} (mg/L)	RF_{50}	RF_{90}
Susceptible strain					
malathion	0.0078	0.0062 – 0.0097	0.035	–	–
temephos	8.70	5.21 – 14.52	12.69	–	–
Ampang Hill					
malathion	140.31	122.32 – 160.93	493.67	17988.46	14104.86
temephos	26.3	24.00 – 30.28	40.43	3.02	3.18
Pantai Dalam					
malathion	109.62	94.32 – 124.91	367.42	14053.85	10497.71

indicated that resistance to pyrethroids are extremely high and suggested that the mosquitoes should be managed by using microbial control agents or spinosad.

It is suggested that insecticide – resistant *Cx. quinquefasciatus* mosquitoes are less likely to transmit filariasis than their insecticide – susceptible counterparts (McCarroll *et al*., 2000). This phenomenon will probably eliminate filariasis globally since the *Culex* mosquitoes are becoming resistant very rapidly today.

Since it has been shown that the *Culex* mosquitoes are able to develop high level of resistance to all major group of insecticides, it would be valuable if the insecticides are used on rotational basis to slow down the selection pressure of insecticides against the mosquito species. The data obtained from this study can be used in making timely management decisions about the judicious choice of pesticides in a vector control program.

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