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Baseline susceptibility to malathion and permethrin in field-collected *Culex quinquefasciatus* Say from Penang, Malaysia

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Abstract. Adult susceptibility of field-collected *Culex quinquefasciatus* to malathion and permethrin was determined using a standard World Health Organization (WHO) bioassay method with discriminating concentration generated locally. Knockdown times (KTs) of each strain in response to malathion and permethrin were generated and used as basis for insecticide susceptibility comparison. Eggs were collected from several locations in Penang, Malaysia (Universiti Sains Malaysia campus, Sg Dua, Gelugor, Bukit Mertajam, Bayan Baru and Bayan Lepas) and reared till adulthood before they were tested. Low malathion and permethrin resistance were found in adult *Cx. quinquefasciatus* from all locations except for strains from Bayan Baru and Bukit Mertajam, which were susceptible to malathion and permethrin, respectively. Bioassay results were also used to estimate resistant genotype frequencies of the field populations.

INTRODUCTION

The *Culex quinquefasciatus* (Say) mosquito is an important urban nuisance mosquito in many parts of the world. Current control efforts rely heavily on the use of public health and household insecticides. Extensive usage and heavy reliance on insecticides has led to the development of insecticide resistance in this species worldwide (Brown & Pal, 1973). In Malaysia, insecticide resistance in this species was first reported in the 1950s when organochlorine resistance (DDT, dieldrin and BHC) was detected (Reid, 1955; Wharton, 1958). Organophosphate resistance in *Cx.. quinquefasciatus* in Malaysia to fenthion had been reported by Thomas (1970). Recently, there have been many reports of resistance of field collected strains of this species from central part of Peninsular Malaysia to several organophosphate and carbamate insecticides, namely malathion and temephos (Lee, 1990; Lee & Tadano, 1994) and propoxur (Lee *et al.*, 1992; Lee & Hamimah, 1992).

In northern Peninsular Malaysia, only one report on insecticide resistance in *Cx. quinquefasciatus* had been documented, where two strains from Penang demonstrated organophosphate and pyrethroid resistance (Setakana, 1989). Here we report our findings on the resistance status of *Cx. quinquefasciatus*, collected from several locations in Penang, Malaysia, to permethrin and malathion using the recommended WHO contact-exposure method for adult mosquito. Gene frequencies of all strains were also estimated using Hardy-Weinburg equilibrium expression.

MATERIALS AND METHODS

Strains

Mosquito eggs were collected from six locations in Penang (Universiti Sains Malaysia campus [USM campus], Sg Dua, Gelugor, Bukit Mertajam, Bayan Lepas and Bayan Baru) in dark enamel trays containing polluted water (5000 ppm of guinea pig dung solution). They were then brought back to the laboratory and reared under conditions of 26 ± 2°C, 70 ± 5% and 12-hour photoperiod, till adulthood for insecticide bioassays. A susceptible strain (VCRU), which has been cultured under insecticide-free condition for more than 15 years, was used for comparison.

Preparation of impregnated papers

Technical grade insecticides of malathion

(purity: 94.6%) and permethrin (purity: 93.7%) were used. Based on specification laid down by World Health Organization, chromatography papers (Schleiher & Schill 2034) were used as the base for insecticide-impregnation. Impregnation solution was prepared in a mixture of oil/insecticide (silicone oil for permethrin; olive oil for malathion) and acetone (at ratio of 1:2). The mineral oils were needed to spread the insecticides evenly throughout the whole paper to prevent crystalization of insecticides on paper fibre. Cut chromatography papers (total area: 180 cm^2) were each impregnated under conditions of 26 ± 2°C and 65 ± 5% R.H. with 2 ml of impregnation solution; each containing a known concentration of insecticide. For control papers, they were impregnated with oil/acetone mixture. The impregnated papers were left overnight prior to bioassay.

Determination of discriminating concentration

Discriminating concentration (DC) is defined as the insecticidal concentration that killed all susceptible individuals. Insecticide bioassay using one DC is more efficient in detecting the presence of resistant individuals in the population than bioassays utilizing a series of concentrations (Roush & Miller, 1986). The method used to obtain in this study was the WHO standard contact method for adult mosquitoes (Busvine, 1971).

Twenty susceptible sucrose-fed adult female *Cx. quinquefasciatus* (VCRU strain), aged 3 – 5 days were aspirated into the holding tube before being blown into the exposing tube with its inner surface lined with insecticide-impregnated paper. Mosquitoes were exposed for 4 hours for malathion and 1 hour for permethrin, respectively. For permethrin, the exposing tube was placed horizontally so that all knocked down mosquitoes were still in contact with the insecticide during the exposure time. After the exposure time, the mosquitoes were kept in a holding tube and provided with a wet cotton bung containing 10% sucrose solution. Mortality was scored at 24 hours post-exposure. Experiments were done for a series of insecticidal concentration (malathion: 0.1 – 1.6%; permethrin: 0.2 – 1.6%) and each concentration was replicated 5X. Mortality data within 10 – 90% range were subjected to analysis using a software of probit analysis developed by Finney (1971). DC was generated by multiplying LC_{95} by 2.

Susceptibility test against field strains

All field strains of mosquitoes were exposed to the insecticide-impregnated paper (containing DC) according to the method described earlier. They were assessed for knockdown at selected time intervals up to the earlier stated exposure period. Knockdown data were subjected to probit analysis to determine KT_{50} and KT_{95} values for both malathion and permethrin. From KT values obtained, resistance ratios (RR) were calculated by dividing the KT_{50} or KT_{95} for each strain with the corresponding knockdown time of the laboratory susceptible strain. Mortality data at 24 hours were used for estimation of resistant gene frequency. The number of replicates done depended on the number of adult mosquitoes that emerged from the collected eggs.

Estimation of resistant gene frequency

Malathion and permethrin-resistant gene frequencies were estimated with Hardy-Weinburg expression (Falconer, 1981) by assuming that the resistant gene was inherited monofactorially and that the laboratory susceptible strain consisted of only susceptible homozygotes. The frequency of S allele in all field strains was then estimated by calculating the proportion of dead individuals (SS). The frequency of resistant gene (R) can then be determined, since $R = 1 - S$.

RESULTS AND DISCUSSION

Two criteria influenced the efficiency of bioassay in the determination of DC, i.e., the insecticidal exposure time set, and the amount of insecticide contacted by the insects (Tabashnik *et al.*, 1993). Both criteria will affect the cost-effectiveness of the test. For example, if the malathion exposure time was set at 1 hour, a great amount of insecticide will be needed, and

thus will not be cost-effective. Insecticidal exposure time may also affect the reliability of bioassay results. Having a shorter insecticidal exposure time will reduce the influence of factors other than insecticidal action on the insects, in addition to time-saving. However, if the exposure time is too short, it may be difficult to differentiate the susceptible from the resistant individuals. This problem usually arises when conducting assays with slow acting insecticides (Tabashnik *et al.*, 1993).

Discriminating concentrations generated for both malathion and permethrin in this study were 3.8% (equivalent to 0.042 mg/cm^2) (for 4 hours exposure) and 4.2% (equivalent to 0.047 mg/cm^2) (for 1 hour exposure), respectively. Different exposure times were used for the two insecticides after taking into account the above mentioned factors. The DC generated from this study was higher than those proposed by World Health Organization (i.e., 5% for malathion and 0.25% for permethrin, for 1-hour exposure time). Genetical conditions may have contributed to differences in DCs. We strongly feel that it is important to generate local DC, instead of using those proposed by World Health Organization, unless the local investigators have similar WHO susceptible strain and testing conditions.

Low malathion resistance were detected in the five field population collected, with Sg Dua strain demonstrating the highest resistance level (RR_{50} = 4.4x and RR_{95} = 24.1x) (Table 1). Bayan Baru strain was susceptible to malathion. Setakana (1989) reported moderate malathion resistance (8 – 11x) in *Cx. quinquefasciatus* collected from Prai and Penang Island using dose-response (LC) method on larval stages. In Penang, Malaysia, various organophosphate insecticides such as malathion, fenthion, fenitrothion and temephos has been used for public health vector control and agricultural practices. Thermal and cold fogging of malathion as adulticide for the control of *Aedes* in the dengue control programme in Penang has been practised since the 1960s. Brown (1960) had earlier reported a 5x increase in LC_{50} in a Penang strain of *Cx. quinquefasciatus* upon malathion selection for 5 generations. It is possible that malathion used for *Aedes* control may select *Culex* resistance to the insecticide after long-term usage.

On permethrin resistance, resistance levels were very low in all strains (<2x for both RR_{50} and RR_{95}) (Table 2). Bukit Mertajam strain was susceptible to permethrin, but the result should be treated with caution due to the low sample size. Low permethrin resistance in the other five strains may be due to non-target exposure. Amin & Hemingway (1989) found 1000x permethrin resistance in a *Cx. quinquefasciatus* strain from Saudi Arabia due to the extensive usage of DDT and pyrethroid usage in agricultural practice. They demonstrated cross-resistance between DDT and pyrethroids

Table 1: Baseline susceptibility to malathion and permethrin, and resistant gene frequencies (GF) of several Penang field strains of *Culex quinquefasciatus* mosquitoes

Strain	n	KT_{50} (min) (95% fiducial limit)	KT_{95} (min)	Regression slope	χ^2 (df)	RR_{50}	RR_{95}	GF
VCRU (lab)	200	60.2 (58.7 – 61.6)	125.2	5.17	1.98 (10)	-	-	0
USM campus	120	134.1 (127.9 – 141.2)	421.8	3.31	4.22 (10)	2.2	3.4	0.05
Bukit Mertajam	20	96.4 (89.1 – 104.1)	174.0	6.42	3.64 (7)	1.6	1.4	0
Sg Dua	60	264.7 (217.5 – 364.3)	3020.1	1.56	3.62 (10)	4.4	24.1	0.15
Gelugor	100	104.2 (99.2 – 109.5)	298.6	3.60	5.76 (10)	1.7	2.4	0.03
Bayan Baru	100	46.4 (43.1 – 49.6)	176.8	2.83	6.01 (9)	0.8	1.4	0.01
Bayan Lepas	60	87.5 (84.4 – 90.8)	155.5	6.59	3.37 (9)	1.4	1.2	0.03

Discriminating concentration = 0.042 mg/cm^2 (2 x LC_{95})

Table 2: Baseline susceptibility to permethrin and resistant gene frequencies (GF) of several Penang field strains of *Culex quinquefasciatus* mosquitoes

Strain	n	KT_{50} (min) (95% fiducial limit)	KT_{95} (min)	Regression slope	χ^2 (df)	RR_{50}	RR_{95}	GF
VCRU (lab)	300	9.6 (9.5 – 9.7)	13.2	12.00	9.00 (8)	-	-	0
USM campus	55	12.2 (11.9 – 12.6)	19.0	8.64	2.77 (10)	1.3	1.4	0.05
Bukit Mertajam	17	9.3 (8.6 – 9.8)	14.3	8.78	3.96 (6)	1.0	1.1	0
Sg Dua	80	14.0 (13.5 – 14.4)	23.2	7.43	7.98 (8)	1.5	1.8	0.05
Gelugor	100	12.1 (11.7 – 12.4)	20.1	7.38	11.08 (8)	1.3	1.5	0.02
Bayan Baru	100	13.1 (12.8 – 13.5)	23.0	6.78	13.13 (10)	1.4	1.7	0.01
Bayan Lepas	40	15.0 (14.4 – 15.6)	23.5	8.40	12.00 (8)	1.6)	1.8	0.06

Discriminating concentration = 0.047 mg/cm^2 (2 x LC_{95})

in their studies.

Gene frequency estimates for malathion- and permethrin-resistance ranged from 0 – 0.15 and 0 – 0.06, respectively in all field strains (Table 1 and 2). Sg Dua strain demonstrated the highest malathion-resistant gene frequency (0.15), while gene frequency for Bayan Lepas strain was the highest (0.06) for permethrin resistance. Weekly monitoring of malathion and permethrin resistance of the strain from USM campus indicated that gene frequencies varied and appeared to be time-dependent (data not shown) where the frequencies were seen to decrease with time over an 8-week period. Lower biotic potential in resistant individuals as compared to the susceptible individuals (Ferrari & Georghiou, 1981; Amin & White, 1984; El-Khatib & Georghiou, 1985) may be the explanation to this observation. Under non-insecticidal selection environment, susceptible individuals will produce more individuals than the resistant ones, and thus increase the proportion of susceptible individuals in the population with time. In addition, other factor such as migration may also influence the proportion of susceptible individuals in the field population.

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