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Current insecticide susceptibility status of Malaysian *Anopheles maculatus* Theobald to malathion, permethrin, DDT and deltamethrin

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Abstract. Chemical insecticides are still considered as important control agents for malaria vector control. However, prolonged use of these chemicals may select mosquito vectors for resistance. In this study, susceptibility status of adult *Anopheles maculatus* collected from 9 localities in peninsular Malaysia, viz., Jeli, Temerloh, Pos Banun, Senderut, Jeram Kedah, Segamat, Kota Tinggi, Kluang and Pos Lenjang were determined using the standard WHO bioassay method in which the adult mosquitoes were exposed to standard insecticide impregnated papers malathion, permethrin, DDT and deltamethrin – at pre-determined diagnostic dosage. Deltamethrin was most effective insecticide among the four insecticides tested, with the LT50 of 29.53 min, compared to malathion (31.67 min), DDT (47.76 min) and permethrin (48.01 min). The effect of all insecticides on the laboratory strain was greater (with all insecticides demonstrated LT50 < 1 hour) than the field strains (deltamethrin 32.7, malathion 53.0, permethrin 62.0, DDT 67.4 min). *An. maculatus* exhibited low degree of resistance to all test insecticides, indicating that these chemical insecticides are still effective in the control of malaria vector.

INTRODUCTION

Human malaria is truly a disease of global proportions and is one of the most broadly distributed vector-borne infections. Although malaria in Malaysia is already under control, malaria persists in a number of problematic foci, such as aboriginal areas, tribal villages found in cleared hilly jungles, and in communities working in agricultural and land development (Rohani *et al*., 1994). Ministry of Health Malaysia reported that in 2012, 4726 malaria cases occurred in Malaysia with 16 deaths (VBDCP, 2012). *Anopheles maculatus* Theobald is the principal vector of human malaria in peninsular Malaysia (Reid, 1968; Loong *et al*., 1988; Vythilingam *et al*., 1995). The other malaria vectors are *Anopheles sundaicus*, *Anopheles campestris*, *Anopheles donaldi* and *Anopheles cracens* especially along the Malaysia-Thailand border (VBDCP, 1988).

At present, chemical control of malaria in Malaysia is carried out using deltamethrin for indoor residual spraying, temephos as a larvicide and permethrin to impregnate bed nets (VBDCP, 2012). During the Malaria Eradication Programme, indoor residual spraying of DDT emulsifiable concentrate (EC) at $2g/m^2$ has been extensively used since 1967. Extensive use of residual insecticide spraying for malaria vector control has selected anopheline mosquitoes resistant to insecticides. Due to the removal of DDT from public health usage worldwide, except in designated countries, the Vector Borne Disease Control Programme has switched to the usage of pyrethroids in 1998 till today.

Pyrethroids are particularly suitable for the public health purposes because of their quick knockdown effects, high insecticidal potency, relatively low mammalian hazard at operational doses, non-bioaccumulation due to rapid degradation in the environment (Elliot *et al.*, 1978). Extensive use of pyrethroids in turn, led to development of pyrethroid resistance in many arthropods, including anopheline mosquitoes (Enayati *et al.*, 2003). Despite the extensive and wide usage of insecticides for many years in this country, very few reports on the resistance status of the malaria vector species are available (Loong *et al.*, 1989; Rohani *et al.*, 1994, 1995). Therefore, there is an urgent need to update insecticides resistance data in malaria vector populations.

The objective of this study was to determine the susceptibility status of *Anopheles maculatus* Theobald to the insecticides that are currently being used in the malaria control programmes, namely deltamethrin, permethrin, and malathion. Tests on DDT susceptibility were also conducted due to historical use of DDT in residual spraying.

MATERIALS AND METHODS

Nine study localities were selected in peninsular Malaysia where incidence of malaria is prevalent, namely States of Pahang, Kelantan, Negeri Sembilan, Johor and Perak (Figure 1). Probe surveys were carried out in areas endemic for malaria based on the reports submitted by District Health Department to the Vector-borne Diseases Control Programme, MOH. All these localities were either Malay or arboriginal villages where blood surveys had revealed high malaria rates in the human populations.

Figure 1. Map of peninsular Malaysia showing the nine localities where *Anopheles maculatus* adults were collected for the susceptibility study

Adult female mosquitoes were collected from these areas using human landing catches (HLC) or cattle bait trap (CBT). HLC was conducted after obtaining informed consent from the collectors. Captured mosquitoes were identified and those unfed were given a blood-meal from white mice. The fed mosquitoes were kept for egg-laying and eggs collected were allowed to hatch and larvae reared at 27 ± 2°C and relative humidity (RH) of 80 ± 10% at a photoperiod of 12:12. The resulting F1 or F2 female adults from each area were used for testing. The Jeram Kedah strain F58-F70 served as a laboratory standard susceptible strain against which the field strains were compared. This strain has been maintained in the insectary for more than 10 years without exposure to chemical or biological agents.

Sugar-fed female *An. maculatus* aged between 3-6 day old were tested for susceptibility to deltamethrin, permethrin, malathion and DDT using WHO adult testing kits with diagnostic dose of 0.05% deltamethrin, 0.75% permethrin, 5% malathion and 4.0% DDT (WHO, 1998). Into each kit, 15 females *An. maculatus* were released and exposure test kits were covered with black cloth to ensure that they rest on the impregnated papers. Cumulative mortality was recorded at interval of 15 minutes until a period of 2 hours or 90% mortality. Final mortality was further recorded after 24 hours of holding period. Each diagnostic dosage consisted of six replicates and three replicates of control. In each test, 90 mosquitoes were tested for each exposure period.

Appropriate controls were made along with the tests, and mortality was corrected by Abbott's formula. All tests were conducted under laboratory conditions of 27 ± 2°C and relative humidity (RH) of 80 ± 10%. The criteria for interpretation of insecticides susceptibility test results were based on those recommended by WHO (1998). The data were analyzed by probit analysis using version 5 of SAS/STAT.

The data were analysed on two aspects. Firstly, LT50 value, which states the time required to kill 50% of the population was calculated and secondly the resistance ratio (RR) were then obtained by dividing the LT50 of the tested field strain with LT50 value of the standard susceptible laboratory strain.

RESULTS AND DISCUSSION

The susceptibility test was undertaken between May 2009 and November 2011. Table 1 shows the lethal times for 50% knockdown (LT50) of laboratory strain (F58 – F70) and field collected population against four insecticides: 0.05% deltamethrin, 0.75% permethrin, 4.0% DDT and 5% malathion. The LT50 value for laboratory strain against deltamethrin, malathion, DDT and permethrin was 29.53, 31.67, 47.76 and 48.01 minutes, respectively. These results clearly showed that deltamethrin was the most effective insecticide among the four tested. The LT50 values against DDT and permethrin were shown to be almost twice that shown by deltamethrin.

The LT50 values of field collected *Anopheles maculatus* against deltamethrin, malathion, DDT and permethrin were slightly different from the laboratory strain in the order of insecticide effectiveness: deltamethrin was still the most effective (32.7 min), followed by malathion (53.0 min), permethrin (62.0 min) and DDT (67.4 min). The LT50 of the laboratory strain was achieved sooner (< 1 hour) than the field strain for all insecticides tested.

With LT50 value of 29.53 min for laboratory strain against deltamethrin, LT50 value of strain from Temerloh (31.51min.) and Senderut (49.56 min.) could suggest low resistance to deltamethrin, while other localities; with almost similar LT50 values as the laboratory strain (26.76–28.35 min.) when exposed to deltamethrin; could suggest susceptibility to deltamethrin.

LT50 values for laboratory strain against malathion, DDT and permethrin was 31.67, 47.76 and 48.01 minutes respectively (Table 1). Compared with field strains from all localities, the LT50 was higher than the laboratory strain when exposed to malathion, DDT and permethrin, which indicated the possibility of resistance to malathion (40.18–86.20 min.), DDT (52.67–96.64 min.)

Table 1. LT50 (minutes) values of deltamethrin, permethrin, malathion and DDT against adult *Anopheles maculatus*

Insecticide/Strain	LT50 (min)	Range*	Slope
deltamethrin 0.05%			
Laboratory strain	29.53	25.64<LT50<33.67	5.78±1.02
Temerloh, Pahang	31.51	25.75<LT50<39.91	3.19±0.74
Pos Senderut, Pahang	49.56	43.87<LT50<58.40	4.71±0.89
Pos Banun, Perak	27.58	23.54<LT50<30.79	3.79±0.54
Kota Tinggi, Johor	26.76	23.19<LT50<30.50	5.00±0.80
Pos Lenjang, Pahang	28.35	24.46<LT50<31.71	5.26±0.84
permethrin 0.75%			
Laboratory strain	48.01	37.99<LT50<87.62	3.24±0.93
Jeli, Kelantan	60.29	56.33<LT50<64.05	9.61±1.32
Temerloh, Pahang	55.50	51.27<LT50<59.35	9.81±1.73
Senderut, Pahang	76.81	69.72<LT50<85.47	4.89±0.10
Pos Banun, Perak	58.01	50.12<LT50<64.73	5.191±1.38
Segamat, Johor	58.21	45.20<LT50<73.92	7.50±1.62
Kota Tinggi, Johor	75.13	66.82<LT50<85.04	3.34±0.48
Kluang, Johor	60.10	55.41<LT50<65.34	8.80±1.75
Pos Lenjang, Pahang	52.11	35.77<LT50<91.21	3.47±1.12
DDT 4.0%			
Laboratory strain	47.76	30.84<LT50<74.53	3.65±1.09
Jeli, Kelantan	54.16	46.46<LT50<63.76	3.08±0.48
Temerloh, Pahang	96.64	82.91<LT50<144.12	4.30±1.15
Pos Banun, Perak	57.02	40.07<LT50<79.89	7.19±2.18
Segamat, Johor	53.63	49.50<LT50<58.21	6.14±0.84
Kota Tinggi, Johor	65.57	59.18<LT50<72.52	5.35±0.11
Kluang, Johor	92.77	83.85<LT50<105.38	4.42±0.66
Pos Lenjang, Pahang	52.67	38.51<LT50<84.35	3.78±1.19
malathion 5%			
Laboratory strain	31.67	29.02<LT50<34.32	6.21±1.16
Jeli, Kelantan	89.20	70.90<LT50<149.14	2.94±0.68
Temerloh, Pahang	63.12	54.86<LT50<87.59	5.20±1.40
Pos Banun, Perak	41.01	22.68<LT50<70.89	5.68±2.25
Segamat, Johor	46.90	43.41< LT50<50.33	8.19±1.10
Kota Tinggi, Johor	45.32	41.25<LT50<49.25	6.84±0.96
Kluang, Johor	46.74	27.49<LT50<81.63	5.36±1.95
Pos Lenjang, Pahang	42.71	31.07<LT50<48.17	5.77±1.14

*Confidence interval is 95%

and permethrin (52.11–76.81 min.). Josiane *et al.* (2006) also found that adults *Anopheles gambiae* in Cameroon exhibited high level of resistance against DDT and permethrin which was suggested then to be due mainly to knockdown resistance (kdr) mutations. It was also reported that *An. gambiae* was highly susceptible to deltamethrin. In our case, few field strains (Pos Banun, Kota Tinggi and Jeram Kedah) presented similar finding.

Table 2 shows resistance ratio (RR) of the test population. If the RR is more than 1, some degree of resistance is suspected (Rohani *et al.*, 1995). Based on this evaluation criterion, adults *An. maculatus* collected from all study localities suggested low degree of resistance to permethrin with

Table 2. Comparative 50% knockdown times (LT50) and 24 hours post-exposure mortality of F1/F2 *Anopheles maculatus* exposed to 0.05% deltamethrin, 0.75% permethrin, 5% malathion and 4.0% DDT

Strain	Insecticide	LT50	*RR	*Mortality (%)
Laboratory strain	0.05% deltamethrin	29.53	–	100
	0.75% permethrin	48.01	–	100
	5% malathion	31.67	–	100
	4.0% DDT	47.76	–	100
Jeli, Kelantan	0.75% permethrin	60.29	1.26	100
	5% malathion	89.20	2.82	100
	4.0% DDT	54.16	1.13	96.7
Temerloh, Pahang	0.05% deltamethrin	31.51	1.06	100
	0.75% permethrin	55.50	1.16	100
	5% malathion	63.12	1.99	100
	4.0% DDT	96.64	2.02	100
Senderut, Pahang	0.05% deltamethrin	49.56	1.68	100
	0.75% permethrin	76.81	1.20	100
Pos Banun, Perak	0.05% deltamethrin	27.58	0.94	100
	0.75% permethrin	58.01	1.20	100
	5% malathion	41.01	1.27	100
	4.0% DDT	57.02	1.08	100
Segamat, Johor	0.75% permethrin	58.21	1.21	100
	5% malathion	46.90	1.48	100
	4.0% DDT	53.63	1.99	98.0
Kota Tinggi, Johor	0.05% deltamethrin	26.76	0.91	100
	0.75% permethrin	75.13	1.56	100
	5% malathion	45.32	1.43	100
	4.0% DDT	65.57	1.37	100
Kluang, Johor	0.75% permethrin	60.10	1.25	100
	5% malathion	46.74	1.48	100
	4.0% DDT	92.77	1.94	100
Pos Lenjang, Pahang	0.05% deltamethrin	28.35	0.97	100
	0.75% permethrin	52.11	1.08	100
	5% malathion	42.71	1.35	100
	4.0% DDT	52.67	1.11	100

*RR = resistance ratio, *mortality (%) = 24 hours post-exposure.

RR value between 1.16–1.56. Similar result was observed when malathion (RR value: 1.27–2.82) and DDT (RR value: 1.08–2.02) were used. The low degree of resistance in *An. maculatus* against DDT might be due to DDT being previously used in residual spraying, or/and because of the occurrence of cross-resistance of pyrethroids usage. Cross-resistance may have occurred as a result of similar actions of DDT and pyrethroids on the voltage-dependent sodium channel of nerve axons (Bloomquist, 1996).

Exposure to deltamethrin also indicated similar result but only seen in two localities, Temerloh (RR value: 1.06) and Pos Senderut (RR value: 1.68) while strains from the rest of the localities (Pos Banun, Kota Tinggi and Pos Lenjang) suggested that they were

susceptible to deltamethrin with RR value < 1.0.

Table 2 also showed 24 hour post-exposure adult mortality in all study localities concerned. Our findings showed 100% mortality for all strains when tested against deltamethrin, permethrin and malathion. Test against DDT however, showed that the mortality of strain from Jeli and Segamat was < 100% which was 96.7% and 98.0% respectively. Other localities (Temerloh, Pos Banun, Kota Tinggi, Kluang and Pos Lenjang) presented 100% mortality rates. *Anopheles maculatus* from Jeli and Segamat were collected from Malay villages which were near to the rubber and oil palm plantations where agriculture pesticides are frequently used. Hence, some degree of resistance to DDT could be due to cross resistance with agricultural pesticides. Reports from various parts of the world have indicated that mosquito control has become more difficult in areas of intensive agriculture (Diabate *et al*., 2002; Overgaard, 2006).

Based on the WHO (1998) evaluation criteria (100% mortality as susceptible, 90–99% mortality indicate status of resistance need to be verified and mortality <90% as resistant), it could be suggested that adults of *An. maculatus* collected from all study localities were susceptible to permethrin, malathion, deltamethrin and DDT, although two localities (Jeli and Segamat) when exposed to DDT showed mortality which require resistance verification (< 100%).

This study therefore provided clear evidence that the use of chemical insecticides in the control of malaria vector is still effective in Malaysia. On the other hand, the data also suggested the presence of low degree of resistance towards all four insecticides as shown by the LT50 and RR obtained. Data obtained from other similar studies presented similar trend of outcomes (Rohani *et al.*, 1994, 1995). Biochemical test is necessary to confirm the susceptibility/ resistance status of malaria vectors.

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