

INSECTICIDE SUSCEPTIBILITY TESTS OF *ANOPHELES MINIMUS S.L.*, *Aedes aegypti*, *Aedes albopictus*, AND *Culex quinquefasciatus* IN NORTHERN THAILAND

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Abstract. The susceptibility of *Anopheles minimus s.l.*, *Aedes aegypti*, *Ae. albopictus*, and *Culex quinquefasciatus* to insecticide in northern Thailand was monitored by using the WHO standard susceptibility test. One- to two-day old female mosquitos, which were reared from wild caught females or immature stages, were exposed to discriminating dosages of insecticides for recommended exposure periods, and the 24-hour mortality recorded. The results revealed that, in general, *An. minimus s.l.* was still susceptible to DDT and permethrin, except in some areas where a slight increase in tolerance to DDT was observed. *Ae. aegypti* and *Ae. albopictus* were both highly resistant to DDT, but in some areas the former was also resistant to permethrin and deltamethrin. *Cx. quinquefasciatus* was resistant to DDT and etofenprox, with a slight increase in tolerance to permethrin, deltamethrin, malathion and fenitrothion. No resistance to lambda-cyhalothrin was detected in any of the species studied.

INTRODUCTION

Since the first trial of DDT residual spray to control malaria in Chiang Mai Province, northern Thailand, in 1949, it was used primarily country-wide, with great success in interrupting or reducing malaria transmission throughout the country (Malikul, 1988). Several organophosphate and carbamate compounds, which are mainly used for agricultural purposes, have also been tried, but their application for public health is limited to a small scale and short period. However, temephos has been used for controlling dengue vectors for over 50 years. Like elsewhere, one of the adverse effects of insecticide use in Thailand is that many mosquito species have been reported resistant to DDT as well as to other insecticides (WHO, 1992). In addition, due to public concern about its adverse effects on the environment and health, DDT has not been imported since 1995 and its use had decreased over time until recently, when it ceased to be used in malaria control (Chareon-

viriyahpap *et al.*, 1999). In the early 1990s, pyrethroids, mainly permethrin and deltamethrin, and to a lesser extent lambda-cyhalothrin and etofenprox, were introduced for malaria and dengue control, particularly in the impregnation of bednets (Somboon *et al.*, 1995) and indoor/outdoor sprays. At present, pyrethroids are the main insecticides used in controlling vector-borne diseases throughout the country. Therefore, some concern has been expressed as to the possibility that mosquitos may develop resistance to pyrethroids due to selection pressure. Moreover, those already resistant to DDT may rapidly become resistant to pyrethroid because of the possibility of cross-resistance (Miller, 1988).

The review by Chareonviriyaahpap *et al.* (1999) provides useful information on the use of insecticides and the insecticide resistance of mosquito vectors in Thailand, although not all of the species of mosquitos that have been reported resistant to insecticides were cited. It should be noted that most of the data collected were originally from the reports of susceptibility tests performed routinely by the Offices of Vector-Borne Diseases Control (formerly Ma-

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alaria Centers) located throughout the country, and based on the standard WHO contact test using discriminating concentrations. However, it is apparent that some results, especially those of the primary malaria vector, *An. minimus s.l.*, which was reported resistant to DDT and permethrin, are difficult to conclude for several reasons. For example, (1) in tests carried out on wild-caught females and in the field with varying temperatures, pyrethroids, as well as DDT, had negative temperature coefficients of toxicity (lower toxicity at higher temperature). When using a discriminating dose to detect pyrethroid resistance, tests in the range of 22-28°C would give reliable results (Hodjati and Curtis, 1999). In the daytime of the rainy and hot seasons in Thailand, temperatures over 30°C are common and might lead to the decreased toxicity of pyrethroid; (2) there is no strong evidence to confirm the mosquitos' physiological resistance. The operational criterion of resistance has usually been taken as the survival of 20% or more of the individuals tested at the currently known diagnostic concentrations, using WHO test kits in the field (WHO, 1992). With regard to *An. minimus s.l.*, however, a mortality of 96-100% after 1 hour exposure to 4% DDT or 0.25% permethrin papers was commonly recorded throughout the country, with 80-95% mortality being found on a few occasions in some locations. This reduced susceptibility was rarely observed over time in the same areas (Suwonkerd and Prajakwong, 1995). Unlike *An. annularis* observed in several areas, a mortality of 1-66% was commonly encountered after exposure to 4% DDT for 1 hour, providing there was more clear evidence of the resistance status of this species (Suwonkerd and Prajakwong, 1995); (3) recent investigations revealed that no DDT-resistant *An. minimus* had been detected, although DDT had been widely applied since its first use in Thailand in 1949 (Ismail and Pinichpongse, 1980; Patipongse, 1986); (4) no insecticide-resistant *An. minimus* has been reported in other countries where DDT has been applied.

Aedes aegypti, the primary vector of dengue viruses in urban areas of Thailand, has long been known to be resistant to DDT (Neely,

1966). Resistance to malathion, temephos and fenitrothion has also been reported (Chareonviriyahpap *et al*, 1999). At present, pyrethroids are widely used for controlling adult mosquitos at household (aerosol canisters) and community level (fogging and ULV). However, little is known about whether this vector species in Thailand develops resistance to commercially available pyrethroids, because the resistance of mosquitos to DDT may confer cross-resistance to pyrethroids, as mentioned above. Moreover, the insecticide susceptibility status of *Ae. albopictus*, which is considered to be the vector of dengue viruses in sub-urban and rural areas of Thailand, is poorly documented.

Culex quinquefasciatus, although not regarded as a vector of any diseases in Thailand, is a potential vector of the filarial worm, *Wuchereria bancrofti*, in the laboratory (Jitpakdi *et al*, 1998). Increased immigration of laborers from Myanmar and people from endemic areas along the Thai-Myanmar border to cities in Thailand is very worrying because of filariasis being introduced by some immigrants carrying *W. bancrofti* microfilariae (Swaddiwudhipong *et al*, 1996). This mosquito species has been reported resistant to DDT, dieldrin, malathion and temephos (Yasuno *et al*, 1967; Chareonviriyahpap *et al*, 1999), but up-to-date information concerning its susceptibility to insecticides is limited.

This report presents the results of insecticide susceptibility tests of *An. minimus s.l.*, *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus* in northern Thailand, based on the standard WHO contact test using discriminating concentrations under controlled temperatures.

MATERIALS AND METHODS

Mosquito collection

Adult female *Anopheles minimus s.l.* were collected (over 50 individuals/site) from human and animal bait collections at 7 villages in northern Thailand. All collecting villages had a long history of DDT residual spraying. After confirmation of their morphological characteristics according to Harrison (1980), they were

pooled and allowed to lay eggs, and reared to adults in an insectary maintained at 25-27°C and 70-80% relative humidity with a photoperiod of 14:10 h L:D. One to two day old F1 progeny females were randomly selected and tested. *Aedes aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus* were collected at larval and pupal stages from their breeding habitats at several locations in northern Thailand. They were transferred to the insectary and reared to adults. One- to two-day old emerging females were randomly selected and tested.

Insecticide susceptibility test

The insecticide susceptibility test followed the standard WHO contact test (1975). Not more than 20 females (per exposure tube) were exposed to the diagnostic dosages of standard WHO insecticide papers, with exposure times according to the insecticide used, as recommended by WHO (1992) and this study's modifications (Table 1). When tested with pyrethroid papers, the exposure tubes were held horizontally. In each test, a number of mosquitos, as controls, were exposed to paper without insecticide. After exposure, they were provided with 2% sugar solution and the 24-hour mortality rate was recorded. If the mortality in the control groups was over 5%, but less than 20%, correction of the mortality was made by applying the Abbot formula. When mortality in the controls was over 20%, the tests were discarded. Data of the tested samples were pooled if no mortality was observed in the controls.

RESULTS

Tests of F1 progeny *Anopheles minimus s.l.* females that originated from the villages of Phrae, Mae Hong Son, Chiang Mai and Lampang Provinces (Table 2) mostly showed 100% mortality after exposure to 4% DDT papers, except those from Lampang, where the mortality was 96.8%. No survival after exposure to 0.25% permethrin papers was observed in any test.

Table 3 presents the mortality of the emerging *Ae. aegypti* females after exposure to the insecticide papers. It is clearly seen that this species was resistant to DDT and permethrin at all collecting sites, with mortality ranging from 0-20.6% and 8.3-96.0%, respectively. It should be noted that lower mortality after exposure to permethrin was observed in the Muang districts (8.3-40.8%), which are much more urbanized than the other districts (69.9-96.0%). Deltamethrin resistance was detected only in the towns of Chiang Mai and Nan Provinces with mortality rates of 61.2% and 73.9%, respectively. One test of etofenprox in Nan Province showed a mortality of 75.0%, suggesting that this vector may be resistant to this insecticide in other areas as well. No resistance to lambda-cyhalothrin or fenitrothion was detected in any of the sites studied.

Tests of two populations of *Ae. albopictus* in Chiang Mai Province showed that this species was resistant to DDT, with mortality ranging from 64.7-81.2% (Table 4), but it is still susceptible to permethrin and fenitrothion.

Table 1
Diagnostic dosages (%) and exposure times (hour).

Species	Insecticide						
	DDT	Fenitrothion	Malathion	Permethrin	Delta-methrin	Lambda-cyhalothrin	Etofenprox
<i>Ae. aegypti</i>	4/0.5	1/1		0.25/1	0.025/1	0.1/1	0.25/1
<i>Ae. albopictus</i>	4/0.5			0.25/1			
<i>An. minimus s.l.</i>	4/1			0.25/1			
<i>Cx. quinquefasciatus</i>	4/4	1/2	5/1	0.25/1	0.025/1	0.1/1	0.25/1

Table 2
Mortality of F1 progeny of *Anopheles minimus s.l.* females recorded at 24 hours after exposure to 4% DDT and 0.25% permethrin (PER).

Site of collection	Month/Year of test	Insecticide	No. tested	Mortality (%)	No. control tested	Control mortality (%)
Phrae Province						
Ban Huai Rong,	Sep 1997	DDT	40	100	40	0
Rong Kwang district	Aug 1998	DDT	60	100	60	0
		PER	60	100	60	0
Mae Hong Son Province						
Ban Huai Pong Kan Nai,	Nov 1997	DDT	164	100	100	0
Muang district		PER	48	100	40	0
Ban Phra Ko Lo,	Nov 1997	DDT	223	99.6	100	0
Muang district		PER	60	100	60	0
Ban Huai Sai Kao,	Nov 1997	DDT	44	100	40	0
Muang district		PER	60	100	60	0
Chiang Mai Province						
Ban Muang Na,	Nov 1997	DDT	106	100	100	0
Chiang Dao district		PER	82	100	80	0
Lampang Province						
Ban Den U-dom,	Dec 1997	DDT	62	96.8	60	0
Thoen district						
Chiang Mai Province						
Ban Pang Mai Daeng,	Sep 2000	DDT	80	100	80	0
Mae Taeng district		PER	75	100	60	0

Table 5 shows the mortality of the emerging *Cx. quinquefasciatus* females after exposure to the insecticide papers. This species was resistant to DDT at all collecting sites, with mortality ranging from 2.0-68.8%. Two populations tested with etofenprox, one in Chiang Mai Province and the other in Nan Province, were both resistant, with 70.4% and 66.7% mortality, respectively. Mortality ranging from 93.5-100% after exposure to permethrin, deltamethrin, fenitrothion and malathion papers was observed, except in Nan Province where a mortality of 47.1% after exposure to malathion was found. Lambda-cyhalothrin appeared to be the only one among the insecticides tested that gave 100% mortality.

DISCUSSION

This study provides evidence that, in general, *An. minimus s.l.* in northern Thailand

is still susceptible to DDT, except in some areas where this species may have already developed resistance to it. However, resistant populations are probably low in frequency. Prapanthadara *et al* (2000) detected a low frequency of DDTase based DDT resistance in an *An. minimus* population in the area adjacent to one of this study's collecting sites, *ie* Ban Huai Rong, Rong Kwang district, Phrae Province, but no DDT resistance was detected in this study. A collecting site in this study was also adjacent to the area where the permethrin-resistant *An. minimus* was reported, as reviewed by Chareonviriyahpap *et al* (1999), but no permethrin-resistant populations were detected in any sites of this study. Resistance to permethrin, if any, might occur infrequently and in limited areas, which would have little effect, at least up to now, on the efficacy of pyrethroids that are currently used mainly in the malaria control program in Thailand. However, regular monitoring of pyrethroid resistance is needed.

Table 3

Mortality of *Aedes aegypti* females reared from wild larvae and pupae collected from several provinces of northern Thailand, 24 hours after exposure to 4% DDT, 0.25% permethrin (PER), 0.025% deltamethrin (DEL), 0.1% lambda-cyhalothrin (LAM), 0.25% etofenprox (ETO), and 1% fenitrothion (FEN).

Site of collection	Month/Year of test	Insecticide	No. tested	Mortality (%)	No. control tested	Control mortality (%)
Chiang Mai Province						
Chiang Mai city, Muang district	Aug 1997	DDT	62	1.6	40	0
		PER	132	8.3	40	0
		DEL	333	61.2	120	0
		LAM	50	100	50	0
		FEN	32	100	30	0
Ban Pang Mai Daeng, Mae Taeng district	Aug 1997	DDT	25	0	10	0
		PER	93	69.9	40	0
		DEL	74	100	40	0
		FEN	17	100	10	0
Lampang Province						
Lampang city, Muang district	Aug 1997	DDT	34	20.6	20	0
		PER	98	40.8	40	0
		DEL	40	100	20	0
		FEN	30	100	20	0
Nan Province						
Nan city, Muang district	Aug 1997	DDT	11	0	10	0
		PER	43	30.2	20	0
		DEL	23	73.9	10	0
Na Noi district	Sep 1997	PER	101	96.0	20	0
Pua district	Sep 1997	PER	81	93.8	30	0
Tha Wang Pha district	Jun 2000	ETO	20	75.0	10	0

Table 4

Mortality of *Aedes albopictus* females reared from wild larvae and pupae collected from several provinces of northern Thailand, 24 hours after exposure to 4% DDT, 0.25% permethrin (PER), and 1% fenitrothion (FEN).

Site of collection	Month/Year of test	Insecticide	No. tested	Mortality (%)	No. control tested	Control mortality (%)	
Chiang Mai Province							
Chiang Mai city, Muang district	Sep 1997	DDT	16	81.2	10	0	
		Mae Taeng district	PER	20	100	10	0
			FEN	21	100	10	0
	Sep 1997	DDT	34	64.7	10	0	
Nan Province							
Na Noi district	Oct 1997	PER	38	100	20	0	

Table 5

Mortality of *Culex quinquefasciatus* females reared from wild larvae and pupae, 24 hours after exposure to 4% DDT, 0.25% permethrin (PER), 0.025% deltamethrin (DEL), 0.1% lambda-cyhalothrin (LAM), 0.25% etofenprox (ETO), 1% fenitrothion (FEN) and 5% malathion (MAL).

Site of collection	Month/Year of test	Insecticide	No. tested	Mortality (%)	No. control tested	Control mortality (%)
Chiang Mai Province						
Chiang Mai city, Muang district	Jan 1997	PER	58	96.5	40	0
		FEN	62	93.5	20	0
San Kampaeng district	Mar 1998	MAL	132	94.7	40	0
		DDT	31	3.2	20	0
		PER	54	98.1	20	0
		DEL	83	97.6	40	0
		LAM	28	100	20	0
		ETO	115	70.4	40	0
		FEN	25	96.0	20	0
	Apr 1998	MAL	76	96.1	40	0
		DDT	108	21.3	40	0
		PER	140	98.6	40	0
Feb 2000	FEN	107	95.3	40	0	
	MAL	140	100	40	0	
May 1998	LAM	100	100	40	0	
Lampang Province	Nov 1999	DDT	100	2.0	40	0
		PER	107	98.1	40	0
		LAM	80	100	40	0
		FEN	96	95.8	40	0
		MAL	98	98.9	40	0
Nan Province						
Tha Wang Pha district	May 2000	DDT	17	11.8	10	0
	Jun 2000	ETO	12	66.7	10	0
	MAL	17	47.1	10	0	

This study appears to be the first published report of pyrethroid-resistant *Ae. aegypti* in Thailand, suggesting some degree of ineffectiveness in permethrin and deltamethrin, which are currently used for controlling mosquitos in households or in the community. Although etofenprox, a pyrethroid insecticide, has not yet been widely applied for mosquito control in Thailand, *Ae. aegypti* has already been resistant to it. This may be explained by cross-resistance

among pyrethroids. Therefore, the use of pyrethroids to control this vector species should be carefully managed. In addition, regular monitoring of pyrethroid resistance is greatly needed.

Although the collecting sites of *Ae. albopictus* in this study were small and more investigations are needed, the result suggests that DDT-resistant *Ae. albopictus* is probably widely distributed. However, this species may be still susceptible to pyrethroid and organo-

phosphate compound.

Cx. quinquefasciatus was resistant to DDT and etofenprox. Increased DDTase activity and glutathione S-transferase activity have been reported in this species (Prapanthadara *et al*, 2000). The present study also suggests that in some areas, a low frequency of *Cx. quinquefasciatus* may have developed resistance to pyrethroids (permethrin and deltamethrin) and organophosphate compound (fenitrothion and malathion). Resistance to lambda-cyhalothrin has been detected in neither *Cx. quinquefasciatus* nor *Ae. aegypti*. This insecticide should be considered as the insecticide of choice in areas where the other insecticides are not effective.

In conclusion, this study has provided the present insecticide resistance status of important mosquito vectors in northern Thailand. The malaria vector, *An. minimus s.l.*, was in general susceptible to DDT and permethrin, except in some areas where a slight increase in tolerance to DDT was observed. The dengue vectors, *Ae. aegypti* and *Ae. albopictus*, were both highly resistant to DDT, but in some areas the former was resistant to some pyrethroids. The potential vector of Bancroftian filariasis, *Cx. quinquefasciatus*, was highly resistant to DDT, with a slight increase in tolerance to pyrethroids, malathion and fenitrothion.

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