EFFICACY OF THREE INSECTICIDES AGAINST *ANOPHELES DIRUS* AND *ANOPHELES MINIMUS*, THE MAJOR MALARIA VECTORS, IN KANCHANABURI PROVINCE, THAILAND

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Abtract. We conducted this study to determine the insecticide susceptibility of two malaria vectors, *Anopheles dirus* and *Anopheles minimus* from Kanchanaburi Province, Thailand. The mosquitoes were collected and reared under laboratory conditions. The test was carried out on unfed F-1 female mosquitoes using a standard WHO testing protocol. The LD_{50} and LD_{90} of deltamethrin in both species were tested for by exposing the mosquitoes to various doses of deltamethrin for 1 hour. The lethal time was also tested among mosquitoes by exposing them to deltamethrin (0.05%), permethrin (0.75%) and malathion (5%), for different exposure times, ranging from 0.5 to 15 minutes. Percent knockdown at 60 minutes and mortality at 24 hours were calculated. The resistance ratio (RR) was determined based on the LD_{50} and LT_{50} values. LD_{50} of deltamethrin against *An.dirus* and *An.minimus* were 0.00077% and 0.00066%, respectively. LT_{50} values for deltamethrin (0.05%), permethrin (0.75%) and malathion (5%) against *An.dirus* and *An.minimus* were 1.20, 3.16 and 10.07 minutes and 0.48, 1.92 and 5.94 minutes, respectively. The study revealed slightly increased tolerance by both mosquito species, compared with laboratory susceptible strains, based on LD_{50} values. The two anopheline species had the same patterns of response to the three insecticides, based on LT_{50} values, although the LT_{50} values were slightly higher in the *An. dirus* population. Both *An. dirus* and *An. minimus* were fully susceptible to all the insecticides tested, with 100% mortality at 24 hours post-exposure. Deltamethrin was the most effective insecticide, followed by permethrin and malathion.

Keywords: *Anopheles dirus*, *Anopheles minimus*, malaria vector, insecticide susceptibility, Thailand

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INTRODUCTION

Despite decades of successful control programs and reductions in morbidity and mortality, malaria is still an important infectious disease in Thailand. According to the 2011 World Malaria

Report, 32,502 probable and confirmed malaria cases with 80 deaths were reported in 2010 in Thailand (WHO, 2011). *Anopheles dirus* and *An. minimus* are the two major malaria vectors in hilly forested regions of Thailand (Patipong and Yongchaitrakul, 2008). *An. dirus* ranks first in malaria vectorial capacity followed by *An. minimus* (Prasittisuk, 1994). These two species complement each other, maintaining malaria transmission in forest reservoirs and communities living in the forest fringes. Although *An. dirus* is sylvatic in nature and mainly exophilic and exophagic, it enters the house to feed on man and leaves soon after (Enayati *et al*, 2009).

Vector control is an important component of malaria control, especially in the continued absence of an effective vaccine, with the emergence of drug resistance and costly antimalarials (Karunamoorthi, 2011). The main objective of using a chemical insecticide is to prevent transmission of malaria parasites using indoor residual spraying and insecticide treated materials in the form of bed nets or curtains (Chandre *et al*, 1999). However, development of mosquito resistance to insecticides poses a major concern for malaria prevention and control. Vector resistance is a major challenge to malaria control efforts. A knowledge of vector resistance is a basic requirement for a malaria control program (Bortel *et al*, 2008). In this study we aimed to determine insecticide susceptibilities among *Anopheles dirus* from Chong Khab Village and *Anopheles minimus* from Ton Mamuang Village, Sai Yok District, Kanchanaburi Province, Thailand. Since this study is the first of its kind in those villages, it will provide baseline data for malaria prevention program planning.

MATERIALS AND METHODS

Mosquito collections

The mosquito samples were collected from two villages in Sai Yok District, Kanchanaburi Province, Thailand. The larvae of *An. dirus* were collected from Chong Khab Village by dipping method at breeding sites; adult *An. minimus* mosquitoes were collected from cattle bait in Ton Mamuang Village. The mosquitoes were identified morphologically using a taxonomy key (Rattanarithikul *et al*, 2006). The identified specimens were then transferred and reared at the laboratory of the Insecticide Research Unit, Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

Mosquito rearing

Wild caught female mosquitoes were reared in the laboratory at 28±2ºC and 70-80% humidity with artificial light 12 hours a day. Two hundred fifty-three *An. dirus* larvae and 520 adult female *An. minimus* mosquitoes were included in the study. Three to 5 day old female mosquitoes were fed on a hamster. After three days the engorged females were allowed to mate with 3-5 day old male mosquitoes. Three days after mating, the female mosquitoes were transferred to trays with water lined with filter paper for oviposition. The emerging F1 females were raised until they were 3-5 days old then used for insecticide testing.

Susceptibility testing

Standard WHO test kits were used for testing insecticide susceptibility among adult female mosquitoes (WHO, 1998). One hundred mosquitoes (four replicates of 25 mosquitoes each) were used for each concentration and control.

Both species of mosquitoes were exposed to 0.005, 0.0025, 0.00125, 0.000625, and 0.0003125% deltamethrin. After 1 hour of exposure, the mosquitoes were transferred to a holding tube provided with a cotton pad containing 10% sugar solution. Mortality was recorded 24 hours post-exposure. Lethal doses causing 50% and 90% mortality were calculated by probit analysis (Finney, 1971; Yadouleton *et al*, 2010). The resistance ratio was calculated based on the ratio of LD_{50} field/LD_{50} lab mosquitoes.

For time-mortality testing, both species were exposed to deltamethrin (0.05%), permethrin (0.75%) and malathion (5%) for various exposure times. Batches of 25 mosquitoes were introduced into each tube. Exposure times ranged from 0.5 to 15 minutes. At the end of each exposure time, the mosquitoes were transferred to a holding tube and provided with cotton pads with 10% sugar solution**.** Mortality was calculated 24 hours post-exposure; LT_{50} and LT_{90} values were determined. The resistance ratio was calculated based on the ratio of LT_{50} field/LT_{50} lab mosquitoes. The number of mosquitoes knocked down at 60 minutes was recorded and knockdown percentage (%KD) was calculated. Mortality at 24 hours was recorded.

Data analysis

The LD_{50} and LD_{90} values were calculated from dosage-mortality regression using probit analysis software. The LT_{50} and LT_{90} values were calculated from the time-mortality relationship (Finney, 1971; Raymond, 1985). Resistance was classified using WHO criteria: 1) susceptible when mortality was 98% or greater, 2) possible resistance when mortality was 80-97% and 3) resistance when the mortality was <80% (WHO, 1998). If control mortality was >5%, but <20%, a correction of the observed mortality was made by using the Abbott's formula (WHO, 1998).

RESULTS

The LD_{50} values for field collected and laboratory strains of *An. dirus* were 0.00077% and 0.0006%, respectively. A slight increase in tolerance was observed in the field collected population compared to the laboratory strain (RR_{50}=1.26) (Table 1).

Exposure of *An. dirus* field strain mosquitoes to deltamethin 0.05%, permethrin 0.75% and malathion 5% for 60 minutes resulted in LT_{50} values of 1.20, 3.16 and 10.07 minutes, respectively (Table 2); there were slightly high tolerances of 1.79, 1.68 and 1.38 times, respectively, compared to the laboratory strain. *An. dirus* field strain had 2.63 times more tolerance to permethrin (LT_{50} permethrin/LT_{50} deltamethrin) and 8.39 times more tolerance to malathion (LT_{50} malathion/LT_{50} deltarmethrin) when compared to deltamethrin (Table 2). *An. dirus* specimens were fully susceptible to all three insecticides tested, with 100% mortality 24 hours post-exposure (Table 3).

LD_{50} and LD_{90} of deltamethrin against *An. minimus* field and laboratory strains were 0.00066% and 0.00055%, respectively, which is a 1.2 fold increase in tolerance compared to laboratory susceptible mosquitoes (Table 1).

The LT_{50} values for delamethrin (0.05%), permethrins (0.75%) and malathion (5%) against *An. minimus* were 0.48, 1.92 and 5.94 minutes for field strain and 0.47, 1.29 and 4.84 minutes for laboratory strain mosquitoes, respectively (Table 4). There was a slightly higher tolerance to deltamethrin, permethrin and malathion (1.02, 1.48 and 1.22 times) when compared to the laboratory susceptible mosquitoes. *An. minimus* field strain was 4 times more

Table 1
Deltamethrin susceptibility of field and laboratory strains of *Anopheles dirus* and *Anopheles minimus* mosquitoes.

Species	No. tested	LD_{50}	95% CI	RR_{50}	LD_{90}	95% CI	RR_{90}	Slope
An. dirus (field)	500	0.00077	0.0003-0.0019	1.26	0.00744	0.0012-0.1842	2.43	1.29±0.37
An. dirus (lab)	500	0.00061	0.0004-0.0006		0.00306	0.0028-0.0053		1.99±0.18
An. minimus (field)	500	0.00066	0.0005-0.0008	1.2	0.00290	0.0038-0.0105	1.18	1.77±0.20
An. minimus (lab)	500	0.00055	0.0004-0.0006		0.00244	0.0028-0.0053		1.99±0.18

Table 2
Median lethal times (LT_{50}) and resistance ratios (RR) of field and laboratory strains of *Anopheles dirus* to 0.05% deltamethrin, 0.75% permethrin and 5% malathion.

Insecticide	Field				Lab				RR_{50}
	No. tested	LT_{50} (min)	95%CI	Slope	No. tested	LT_{50} (min)	95%CI	Slope	
0.05% Deltamethrin	100	1.20	0.6073-2.3606	4.17±1.08	100	0.67	1.7925-51.7487	1.87±0.25	1.79
0.75% Permethrin	100	3.16	2.1299-4.6475	1.84±0.31	100	1.88	0.8025-4.3094	1.40±0.23	1.68
5% Malathion	100	10.07	9.4234-10.7788	4.67±0.50	100	7.28	19.3520-28.6831	9.40±1.98	1.38

Table 3
Insecticide susceptibilities of *Anopheles dirus* and *An. minimus* to deltamethrin, permethrin and malathion.

Species	0.05% Deltamethrin		0.75% Permethrin		5% Malathion	
	% KD at 60 min	% mortality 24 hours	% KD at 60 min	% mortality 24 hours	% KD at 60 min	% mortality 24 hours
An. dirus (field)	100%	100%	98%	100%	99%	100%
An. dirus (lab)	100%	100%	100%	100%	100%	100%
An. minimus (field)	100%	100%	98%	100%	99%	100%
An. minimus (lab)	100%	100%	100%	100%	100%	100%

KD, knock down

Table 4
Median lethal times (LT_{50}) and resistance ratios (RR) of field and laboratory strains of *Anopheles minimus* to 0.05% deltamethrin, 0.75% permethrin and 5% malathion.

Insecticide	Field mosquitoes				Lab mosquitoes				RR_{50}
	No. tested	LT_{50} (min)	95%CI	Slope	No. tested	LT_{50} (min)	95%CI	Slope	
0.05% Deltamethrin	100	0.48	0.2325-0.7000	1.07±0.22	100	0.47	0.0599-3.5116	1.69±0.71	1.02
0.75% Permethrin	100	1.92	1.0830-3.3934	1.78±0.33	100	1.29	1.1025-1.5053	2.13±0.19	1.48
5% Malathion	100	5.94	5.0725-6.9043	8.90±1.75	100	4.84	4.2429-5.2302	5.75±0.94	1.22

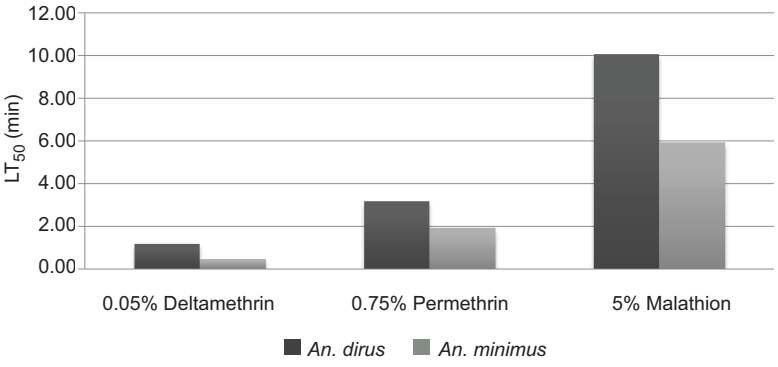

Fig 1–Efficacy of three insecticides against *An. dirus* and *An. minimus.*

tolerant to permethrin (LT_{50} permethrin / LT_{50} deltamethrin) and 12.37 times more tolerant to malathion (LT_{50} malathion / LT_{50} deltamethrin) compared to deltamethrin. However, *An. minimus* mosquitoes were fully susceptible to all three insecticides with 100% mortality 24 hours post-exposure (Table 3).

An. dirus field strain was 1.16 times more tolerant to deltamethrin than *An. minimus* field strain based on LD_{50} values (Table 1). When exposed to deltamethrin, permethrin and malathion, *An. dirus* and *An. minimus* field mosquitoes had the same patterns of response to the three insecticides, based on LT_{50} values, although the LT_{50} values were slightly higher in the *An. dirus* population (Fig 1). Both species had the highest tolerance to malathion followed by permethrin and then deltametrin.

DISCUSSION

The slight decreases in susceptibility based on LD_{50} values among field strains were not significant. A slight increase in resistance usually results from continued selection of a population of insects that do not have specific genes for resistance to that particular insecticide or its chemical groups. The slightly higher tolerance of *An. dirus* could be due to its larger size compared to *An. minimus* (WHO, 1975). The two anopheline species showed the same patterns of response to the three insecticides, based on LT_{50} values, although the LT_{50} values were slightly higher with *An. dirus* (Fig 1). The highest LT_{50} values were seen in both species against malathion (10.7 minutes against *An. dirus* and 8.27 minutes against *An. minimus*). These could be due to the low knock down effect of malathion, increasing the knockdown time (Lee *et al,* 1997). The slightly higher LT_{50} values with permethrin and deltamethrin may be due to their high excito-irritant effects which may reduce tarsal contact with treated surfaces, thus lengthening the knock down time. *An. minimus* had lower LT_{50} values for all three insecticides compared to *An. dirus.* The main reason could be the small size of *An. minimus.* Usually smaller sized species have lower LT_{50}, LC_{50} and LD_{50} values (Brown and Pal, 1971). However, both species were susceptible to all three insecticides tested with 100% mortality at 24 hours post-exposure. If adult mosquitoes have a 4 fold higher resistance than the susceptible strain, the population is considered resistant (Brown and Pal, 1971). However, in this study, the highest resistance ratio observed was only 1.79, showing the insecticides were still effective.

In conclusion, our study shows deltamethrin was the most effective insecticide tested, followed by permethrin and then

malathion. The effectiveness of insecticides should be continuously monitored to guide decision making for the most appropriate insecticide for malaria vector control.

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