INSECTICIDE SUSCEPTIBILITY OF MOSQUITOES INVADING TSUNAMI-AFFECTED AREAS OF THAILAND

Narumon Komalamisra, Yuwadee Trongtokit, Kaewmala Palakul, Samrerng Prummongkol, Yudthana Samung, Chamnarn Apiwathnasorn, Theerawit Phanpoowong, Achara Asavanich and Somjai Leemingsawat

Insecticide Research Unit, Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Abstract. In order to control the mosquitoes invading tsunami-affected areas of Thailand, the insecticide susceptibility status of field larvae and mosquitoes (*Anopheles sundaicus* and *Culex sitiens*) was tested under laboratory conditions. Larval bioassay tests were conducted using the WHO standard method. Three larvicides: temephos, malathion, and plant extract (ethanolic extract of the Southeast Asian long pepper, *Piper retrofractum* Vahl), were used in the experiments. The results revealed that *Cx. sitiens* was more susceptible to temephos than malathion and the plant extract, with LC_{50} ranges of 0.0008-0.0014 mg/l, 0.0046-0.0078 mg/l, and 5.3180-10.1030 mg/l, respectively. *Cx. quinquefasciatus* showed greater tolerance to every tested larvicide than *Cx sitiens*. Adult bioassay tests using a WHO test kit and diagnostic doses of 5% malathion, 0.75% permethrin, 0.05% deltamethrin, and 4% DDT were also conducted. The results revealed that *Cx. sitiens* and *An. sundaicus* were susceptible to all tested insecticides. The LT_{50} of 5% malathion ranged between 25.7-26.0 minutes for *Cx. sitiens*, and 44.7 minutes for *An. sundaicus*. In addition, *Cx. quinquefasciatus* showed susceptibility to malathion, with LT_{50} of 19.7 minutes. However, it showed resistance to both pyrethroid insecticides, with LT_{50} of 33.1 minutes for 0.75% permethrin, and 19.6 minutes. for 0.05% deltamethrin; it showed low percentage mortality at 24 hour post-exposure, of 48 and 32%, respectively. In conclusion, every tested larvicide could be used for controlling *Cx. sitiens* larvae, even in brackish water, pyrethroid insecticides for adult *Cx. sitiens* and *An. sundaicus,* and malathion for all three species.

INTRODUCTION

The giant Asian tsunami struck six Provinces (Phang-nga, Phuket, Krabi, Trang, Satun, and Ranong) along Thailand's Andaman coast in the morning of December 26th, 2004. It caused extraordinary damage, loss of life, livelihoods and homes among the local people, including thousands of tourists, in the affected areas. Major environmental changes in areas almost two kilometers from the coastline have been observed. Several inland water areas became brackish due to flooding by sea water. Two months' post-tsunami, a team from the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University began a survey of the affected area. The preliminary random survey of 33 water sites in Takua Pa District, Phang-nga Province, revealed changes in salinity and pollution due to obstruction of drainage by soil residues and clay sediments from the sea. These new ponds became suitable breeding sites for *Anopheles sundaicus* and *Culex sitiens* mosquitoes. Adult mosquitoes were also observed in high densities.

Anopheles sundaicus is a malaria vector in coastal areas of Thailand and other countries. *Culex sitiens* causes annoyance and dermatitis; it can be a vector for Japanese encephalitis (JE), and has been studied for its potential as a filariasis carrier (Vythilingan *et al,* 1994, 2002). *Culex sitiens* is a possible secondary vector of Ross River (RR) virus in northern and southern Australia. Both species were found with considerable density in every random sampling site suitable for breeding. Although no epidemic of mosquito-borne diseases, such as malaria, JE, or filariasis has been reported in this area, the insecticide susceptibility of these two mosquito species must be known for effective control. Because of environmental changes and the lack of any report on the insecticide susceptibility of mosquitoes in the coastal area, this study was performed to assess the insecticide susceptibility status of field larvae and mosquitoes under laboratory conditions. The baseline results will be a guideline for the control of the brackish-water mosquitoes, *An. sundaicus* and *Cx. sitiens*, in Phang-nga Province or other similar tsunami-affected areas.

Correspondence: Narumon Komalamisra, Insecticide Research Unit, Department of Medicial Entomology, Faculty of Tropical Medicine, Mahidol University, 420/6 Ratchawithi Road, Bangkok 10400, Thailand.
E-mail: tmnkm@mahidol.ac.th

MATERIALS AND METHODS

Mosquitoes

This study was conducted in March 2005, 3 months post-tsunami. Mosquito larvae were collected from ponds in several locations in the tsunami-affected area of Phang-nga Province (Table1). Most of the collected larvae were *Cx. sitiens*, *Cx. quinquefasciatus* or *An. sundaicus*. Water from the breeding sites was also taken to the laboratory station at Phang-nga Thai Navy Base Hospital, to rear mosquito larvae and test the insecticides. Late 3rd to early 4th instar larvae were selected for bioassay; the rest were reared until emergence for adult susceptibility testing. Adult *An. sundaicus* mosquitoes were also collected from animal baits and colonized at the insectarium, Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University; their progeny were used for adulticide susceptibility testing.

Bioassay test

Larval bioassay tests were conducted using the WHO standard method (WHO, 1996). Three larvicides: temephos, malathion, and plant extract (ethanolic extract of *Piper retrofractum* Vahl), at various concentrations, were used in the experiments. Two kinds of water were used, distilled water and breeding-site water. One ml of insecticide was added to 99 ml of water in a cup; 20 late-3rd to early-4th instar larvae were used in each cup. Larval mortality was recorded at 24 hours post-exposure.

Diagnostic susceptibility test

The insecticide susceptibility of the adult mosquitoes was tested using WHO test kits (WHO, 1981a). Female mosquitoes, 3-5 days old, were exposed to diagnostic dosages of 5% malathion, 0.75% permethrin, 0.05% deltamethrin, and 4% DDT. For each test, 3 test kit tubes, one control and 2 for treatment, were used. The control tube contained filter paper impregnated with carrier oil only, while the treatment tubes contained paper impregnated with diagnostic dosages of insecticide plus carrier oil. Twenty unfed female mosquitoes were exposed to the insecticide and the knockdown effect was observed at 5-minute intervals for 60 minutes. Mosquitoes were then transferred to clean holding containers and provided with cotton pad soaked with 10% sucrose solution. Mortality was recorded at 24 hours post-exposure. Each test was replicated 3 times. Resistance status was determined according to WHO criteria; populations were considered resistant if > 20% survived the diagnostic dose after 24 hours, compared with susceptible controls (WHO, 1981b).

Data analysis

The 50% lethal concentration (LC_{50}) and 95% lethal concentration (LC_{95}) values of the larvae, and 50% lethal time (LT_{50}) and 95% lethal time (LT_{95}) of the adults, were estimated using dosage-mortality regression probit analysis (Finney, 1971). Abbott's formula was used to correct the observed mortality in both adult and larva

Table 1
Mosquito larvae collection sites, 18-29 March 2005.

Location	Code	GPS coordinates	pH value		Water quality			DO value		Salt(ppt)	Predominant spp
			pH	Temp(°C)	Con(mS/cm)	TDS(g/l)	Temp(°C)	DO(%)	Temp(°C)		
Phang-nga Navy Base	Y3	8° 34' 11.8" N; 98° 13' 40" E	8.19	31.2	5.32	2.66	29.5	167.8	31.1	2.8	*Cx. sitiens*
Khao Lak	N2	8° 39' 54.7" N; 98° 15' 40.8" E	7.75	24.6	11.76	5.89	30.2	70.2	32	6.7	*Cx. sitiens*
Keuk-kak	N3	8° 41' 47.2" N; 98° 15' 28.6" E	8.26	31.7	21.1	10.54	32.3	124.3	31	12.6	*Cx. sitiens* 90%, *An. sundaicus* 10%
Keuk-kak	N4	8° 41' 47.2" N; 98° 15' 28.6" E	6.95	28.3	0.41	0.20	28.9	16	28.9	0.1	*Cx. quinquefasciatus*
Keuk-kak	N11	8° 42' 52.1" N; 98° 15' 5.2" E	7.69	31	7.28	3.64	31	80.7	31.1	4	*An. sundaicus*

Temp = Temperature, Con = Conductivity, TDS = Total dissolved solids, DO = Dissolved oxygen, Salt = Salinity

susceptibility tests (WHO, 1981a,b).

RESULTS

Field-collected *Cx. sitiens* and *Cx. quinquefasciatus* larvae were used for bioassay testing against 3 larvicides: temephos, malathion, and plant extract (ethanolic extract of *Piper retrofractum* Vahl). Due to the limited number that could be collected from the field, *An. sundaicus* larvae were tested only with the plant extract. The larvicides, in distilled water and water from breeding places, gave similar LC_{50} and LC_{95} values against *Cx. sitiens* and *Cx. quinquefasciatus* larvae (Tables 2, 3). Among the 3 larvicides, *Cx. sitiens* was more susceptible to temephos than malathion and the plant extract; LC_{50} ranged from 0.0008-0.0014 mg/l, 0.0046-0.0078 mg/l, and 5.3180-10.1030 mg/l, respectively (Table 3). For LC_{50}, *Cx. quinquefasciatus* showed 5 times more tolerance to temephos, 3.8 times more tolerance to malathion, and 3.25 times more tolerance to the plant extract than *Cx. sitiens* larvae.

Culex sitiens and *Cx. quinquefasciatus* were used for adult bioassay testing using 3 diagnostic doses--5% malathion, 0.75% permethrin, and 0.05% deltamethrin; additional 4% DDT was only used to test *An. sundaicus*. The result showed that *Cx. sitiens* and *An. sundaicus* were susceptible to all tested insecticides. Both species showed 100% mortality at 24 hours post-exposure (Table 4). The LT_{50} of 5% malathion ranged between 25.7-26.0 minutes for *Cx. sitiens*, and 44.7 minutes for *An. sundaicus*. The LT_{50} of 0.75% permethrin and 0.05% deltamethrin ranged between 8.2-10.2 minutes and 6.0-8.2 minutes, respectively, in *Cx. sitiens,* while in *An. sundaicus* it was 10.4 and 9.7 minutes, respectively. In addition *Cx. quinquefasciatus* was susceptible to malathion with LT_{50} of 19.7 minutes. However, it revealed resistance to both pyrethroid insecticides, with LT_{50} of 33.1 minutes. for 0.75% permethrin, and 19.6 minues. for 0.05% deltamethrin, and showed low-percentage mortality at 24 hours post-exposure, at 48 and 32%, respectively.

DISCUSSION

Similar results were achieved when mosquito larvae were tested against larvicides in distilled water and water from breeding places. Therefore, chemical larvicides, temephos and malathion, or the tested natural

Table2
Susceptibility of *Cx. sitiens*, *Cx. quinquefasciatus* and *An. sundaicus* to larvicides in distilled water.

Mosquito	Site	Temephos (mg/l)		Malathion (mg/l)		Plant extract (mg/l)	
		LC50	LC95	LC50	LC95	LC50	LC95
Cx. sitiens	Y3	0.0008	0.0033	0.0046	0.0159	5.3180	15.1763
	N2	0.0008	0.0014	0.0047	0.0092	6.4128	11.4956
	N3	0.0014	0.0023	0.0078	0.0140	10.1030	18.3430
Cx. quinquefasciatus	N4	0.0053	0.0120	0.0296	0.0754	32.8465	72.8714
An. sundaicus						28.6567	220.7451

LC50 = 50% lethal concentration; LC95 = 95% lethal concentration

Table 3
Susceptibility of *Cx. sitiens*, *Cx. quinquefasciatus* and *An. sundaicus* to larvicides in water from breeding sites.

Mosquito	Site	Temephos (mg/l)		Malathion (mg/l)		Plant extract (mg/l)	
		LC50	LC95	LC50	LC95	LC50	LC95
Cx. sitiens	Y3	0.0006	0.0015	0.0061	0.0149	5.7496	27.0249
	N2	0.0008	0.0017	0.0056	0.0121	5.6106	8.7346
	N3	0.0013	0.0026	0.0078	0.0140	8.2418	15.5928
Cx. quinquefasciatus	N4	0.0047	0.0105	0.0259	0.0536	43.1937	106.4588
An. sundaicus	N11					11.0060	55.4529

LC50 = 50% lethal concentration; LC95 = 95% lethal concentration

Table 4
Susceptibility of *Cx. sitiens*, *Cx. quinquefasciatus* and *An. sundaicus* to diagnostic doses of adulticide.

Mosquito	Site	5% malathion			0.75% permethrin			0.05% deltamethrin			4% DDT		
		LT_{50} (min)	LT_{95} (min)	% mortality	LT_{50} (min)	LT_{95} (min)	% mortality	LT_{50} (min)	LT_{95} (min)	% mortality	LT_{50} (min)	LT_{95} (min)	% mortality
Cx. sitiens	Y3	26.0	40.4	100	10.2	16.1	100	8.0	13.0	100			
	N2	25.8	45.0	100	9.7	16.0	100	8.2	14.5	100			
	N3	25.7	47.3	100	8.2	15.5	100	6.0	9.0	100			
Cx. quinquefasciatus	N4	19.7	31.2	100	33.1	111.8	48	19.6	74.4	32			
An. sundaicus (cow)	Bang Sak	44.7	91.8	100	10.4	25.2	100	9.7	22.0	100	26.3	47.7	100

LT50 = 50% lethal time; LT95 = 95% lethal time

plant larvicide for controlling freshwater mosquito larvae can also be used effectively for controlling *Cx. sitiens*. Using the recommended diagnostic dose from WHO (1992) to determine insecticide resistance status, both *Culex* species were susceptible to malathion. The LC_{95} against *Cx. sitiens* ranged between 0.0092-0.0159 mg/l and 0.0754 mg/l in *Cx. quinquefasciatus,* which were < 0.125 mg/l (the diagnostic dose of malathion against *Cx. sitiens* and *Cx. quinquefasciatus*). On the other hand, the diagnostic dose of temephos against *Cx. quinquefasciatus* was 0.002 mg/l. *Cx. quinquefasciatus* showed tolerance to temephos, with an LC_{95} of 0.012 mg/l, about 6 X the diagnostic dose. However, *Cx. sitiens* was still susceptible to temephos, with an LC_{95} range of 0.0014-0.0033 mg/l.

Control of adult *Cx. sitiens* and *An. sundaicus* by malathion, permethrin and deltamethrin was possible. However, *Cx. quinquefasciatus* revealed resistance to permethrin and deltamethrin, but not malathion. In addition, *An. sundaicus* in Phang-nga, on the western coast of Thailand, remains susceptible to DDT.

In conclusion, every tested larvicide could be used to control *Cx. sitiens* larvae, even in brackish water. Pyrethroid insecticides can be used to control adult *Cx. sitiens* and *An. sundaicus,* and malathion can be used to control all three species.

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