タイにおけるネッタイシマカおよびネッタイイエカの殺虫剤抵抗性

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Original Article

Insecticide resistance of *Aedes aegypti* and *Culex quinquefasciatus* in Thailand

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In this study, nine strains of *Aedes aegypti* mosquitoes from different localities in Thailand were subjected to susceptibility tests against commonly used insecticides for vector control in Thailand. *Aedes aegypti* from different localities were strongly resistant to DDT and permethrin, except for one strain from Chiang Mai (Chiang Mai-P) that was found to be susceptible to permethrin. In contrast, most *Ae. aegypti* strains are susceptible to deltamethrin and alphacypermethrin, except that Bangkok and Nonthaburi strains showed incipient resistance to deltamethrin and alphacypermethrin, respectively. Various levels of malathion and propoxur susceptibility were also detected in *Ae. aegypti* strains; however, two strains of *Ae. aegypti* (Satun and Nonthaburi) were found to be completely susceptible to malathion. The insecticide susceptibility level in three strains of *Culex quinquefasciatus* was also investigated. The results indicated strong resistance to DDT and moderate resistance to permethrin. Two strains from Bangkok and Nonthaburi were found to be completely susceptible to malathion and propoxur. One strain from Pathum Thani showed incipient resistance to both compounds. We conclude that deltamethrin and alphacypermethrin are still effective insecticides for the dengue control program and malathion and propoxur may be alternative insecticides to control *Cx. quinquefasciatus*.

Keywords: insecticide, resistance, *Aedes aegypti*, *Culex quinquefasciatus*, Thailand, pyrethroids.

Introduction

Several countries continue to experience endemic and re-emerging dengue fever (DF) and dengue hemorrhagic fever (DHF).[1)] Annually, millions of people are estimated to be infected with dengue virus worldwide.[2,3)] In Thailand, outbreaks of DHF were first recognized in 1958 and subsequently the disease has expanded throughout the country.[4)] Approximately 40,000 cases of dengue with a mortality rate of 0.09 (per 100,000 people) were reported in 2007.[5)] In spite of continued vigilance in control methods, dengue cases remain a major health threat in Thailand. The reason is unclear but is possibly related to the increase of human and economic activities in urban and semi-urban zones. Moreover, traditional water storage practices increase the breeding sites for *Aedes aegypti*, a primary vector of DF and DHF in Thailand.[6,7)]

Aedes aegypti, a daytime biting mosquito, is highly anthropophilic, often resides in and near human dwellings and preferentially feeds on humans.[1,8,9)] This species has been found to be highly adapted to all man-made and natural environments and is a potential vector of dengue.[10–12)] The key to preventing dengue transmission is to reduce human-vector contact using synthetic chemicals.[13–17)] Several synthetic insecticides, including organochlorines, organophosphates, carbamates and synthetic pyrethroids, have been used in dengue control programs.[18)] DDT (organochlorine) was widely used to control *Ae. aegypti* after the first dengue epidemic in 1958.[19,20)] Deltamethrin (synthetic pyrethroids) is currently one of the most commonly used insecticides in public health programs and has been the mainstay for the emergency control of *Ae. aegypti* adults in Thailand since 1994.[18,21)] Temephos (organophosphate) is commonly used in water containers for the control of *Ae. aegypti* larvae. Ultra-low-volume (ULV) applications of fenitrothion and malathion are used during the peak period of adult *Aedes* populations, especially during the rainy season.[16)] Additionally, many synthetic pyrethroids, *i.e.* resmethrin, tetramethrin, permethrin, cyper-

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methrin, bifenthrin, and cyfluthrin, are commonly used by home owners to control household mosquitoes and other arthropod pests.[16,18,22,23] Years of routine contact with insecticides have led, in some cases, to high levels of resistance in disease vectors, especially *Ae. aegypti*.

The development of pesticide resistance by arthropods is a primary concerns for the management of human pest and disease control. A few published papers on insecticide resistance in *Ae. aegypti* population have been reported[15,17,20,24]; however, most recent studies failed to investigate the susceptibility status of insecticide resistance on a large scale. Without a clearer understanding of the dynamics between insecticides used and the susceptibility level of the mosquito population, successful vector control activities have not been developed. A better understanding of the insecticide susceptibility level will allow for greater efficiency in program design for targeting mosquito vectors. We report here the insecticide susceptibility level of *Ae. aegypti* populations from several collection sites across Thailand by using the standard World Health Organization (WHO) susceptibility method.[25] In addition, susceptibility of *Culex quinquefasciatus* to currently used insecticides was also investigated.

Materials and Methods

1. Study sites

Aedes aegypti larvae and pupae were collected from containers located in and around houses in eleven different provinces of Thailand (Fig. 1). Additionally, *Cx. quinquefasciatus* larvae were collected as immature stages (larvae/pupae) from stagnant and organically polluted water in and around houses in three different provinces (Fig. 1). GPS coordinates and a brief description of the locations are provided herein (Table 1).

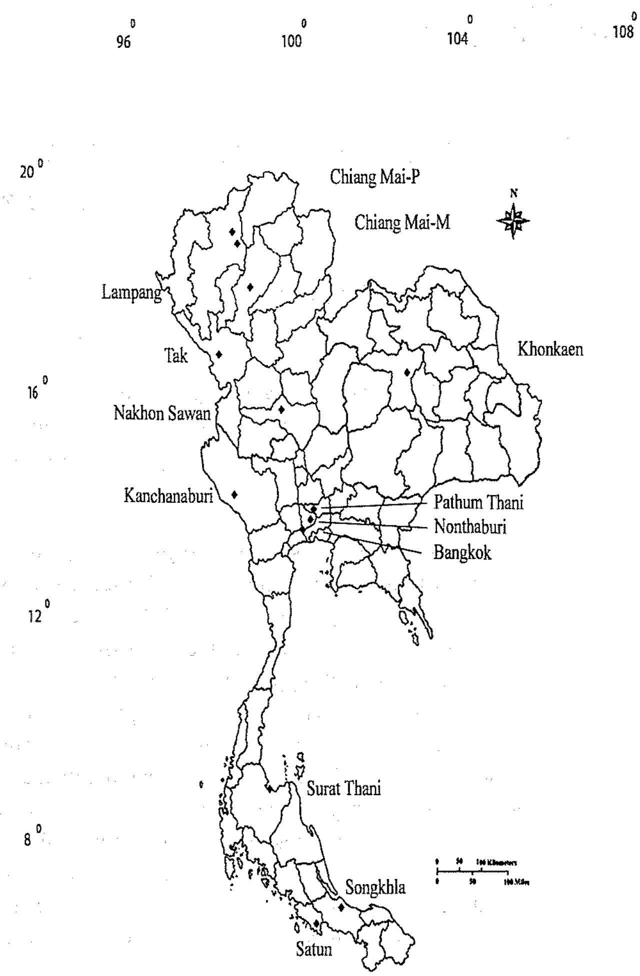

Fig. 1. *Aedes aegypti* and *Culex quinquefasciatus* collection sites in various parts of Thailand.

2. Mosquito strain

Mosquito larvae and pupae were collected at each site, brought back to the insectary at Kasetsart University, and reared to the adult stage. Adult mosquitoes identified as *Ae. aegypti* were introduced into a mosquito cage and permitted guinea-pig blood on the fourth day after emergence. All mosquito strains were colonized under laboratory-controlled conditions at 25°C and 65–80% RH in the Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand.[24] For *Cx. quinquefasiatus*, species identification was based on both larval and adult characters.[26,27] All mosquitoes were reared under the same laboratory conditions described above. Only F1 and F2 were used in the tests.

3. Insecticides

Six insecticides were used in susceptibility testing including, Alpha-cypermethrin [(*RS*)-α-cyano-3-phenoxybenzyl (1*R*,3*R*;1*S*,3*S*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] (BASF, USA, purity 95%), Deltamethrin [(*S*)-α-cyano-3-phenoxybenzyl (1*R*,3*R*)-3-(2,3-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate (BASF, USA, purity 99%), Permethrin [3-phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] (Ladda Company, Bangkok, Thailand, purity 92%), DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane] (Sigma-Aldrich, purity 98%), Malathion [diethyl (dimethoxythiophosphorylthio)succinate] (Ladda Company, Bangkok, Thailand, purity 95%), and Propoxur [2-isopropoxyphenyl methylcarbamate] (Bayer Environmental Science, Bangkok, Thailand, purity 97%).

4. Insecticide impregnated paper

Test paper ($12\times15\,cm^2$) of insecticides at operational field concentrations were prepared according to the WHO protocol.[28,29]

5. Insecticide susceptibility test

The susceptibility of each strain to six insecticides at operational field concentrations of 0.05% alphacypermethrin 0.05% deltamethrin, 0.25% permethrin, 4% DDT, 0.8% malathion and 0.1% propoxur was assessed by exposing

Table 1. Description and GPS coordinates of field sites where collections were made in 2006–2007

Mosquito/Site	Village[a]	District	GPS coordinates
Aedes aegypti			
Chiang Mai-P	Pang Mai Deang	Mae Teang	19°14′N 98° 82′E
Chiang Mai-M	—	Muang	18°47′N 99° 00′E
Kanchanaburi	Pu Teuy	Sai Yok	14°20′N 98° 59′E
Khonkaen	Non Ton	Muang	16°25′ N 102°50′E
Nonthaburi	Tha Sai	Muang	13°53′N 100°29′E
Songkhla	Bon Wua	Muang	7°11′N 100°35′E
Satun	Pi-marn	Muang	6°37′N 100°03′E
Surat Thani	Muang	Muang	9°08′N 99°20′E
Bangkok	Lad Yao	Chatuchak	13°50′N 100°34′E
Lampang	—	Muang	18°17′N 99°29′E
Nakhon Sawan	—	Muang	15°42′N 100°08′E
Tak	Mae Kasa	Mae Sot	16°42′N 98°14′E
Culex quinquefasciatus			
Nonthaburi	Muang	Muang	13°50′N 100°31′E
Bangkok	Phom Pabsatupai	Phom Pabsatupai	13°44′N 100°29′E
Pathum Thani	—	Lad Lumkeaw	14°02′N 100°24′E

[a] — Not available

unfed 3–4 day-old female mosquitoes to a single 'diagnostic' dosage on insecticide-treated test papers as recommended by WHO and following standard testing procedures and exposure times.[25,29] After exposure, mosquitoes in tested and control groups were carefully transferred to separate clean holding containers and mortality was recorded 24 h after exposure. Each test was replicated four times (25 mosquitoes/replicate).

6. *Data analysis*

Interpretation of results of the bioassay test were determined according to WHO criteria.[25] If mortality exceeded 20% in the control, the whole test was rejected. If mortality in the control was above 5%, the result of the treated samples was corrected using Abbott's formula.[30]

Results

The results of susceptibility tests with a single diagnostic concentration of alphacypermethrin (0.05%), deltamethrin (0.05%), permethrin (0.25%), DDT (4%), malathion (0.8%) and propoxur (0.1%) for different *Ae. aegypti* strains are given in Tables 2 and 3.

Based on WHO recommendations, the results of susceptibility tests can be divided into three categories. Mosquitoes are susceptible to insecticides if the percent mortality ranged from 98 to 100%, there was a possibility of incipient insecticide resistance if mortality varied from 80 to 97%, and they were insecticide resistant if percent mortality was $<80\%$.[25] In the present study, no mortality was recorded in the untreated control over a 24 h holding period for all paired tests. With synthetic pyrethroids, various levels of physiological resistance to permethrin were seen in all strains (2.0–72.7% mortality), except one strain from rural area of Chiang Mai (98.0% mortality) and USDA standard susceptible test strain

Table 2. Percent mortalities of *Aedes aegypti* strains after exposure to diagnostic concentration of three synthetic pyrethroids

Strains	Mortality (%)[a]		
	Alphacypermethrin	Deltamethrin	Permethrin
Chiang Mai-P	100	100	98.0±1.1
Chiang Mai-M	—	—	61.2±1.6
Kanchanaburi	98.0±1.2	100	9.0±3.4
Khonkaen	100	100	38.4±9.7
Nonthaburi	85.8±4.1	100	5.0±1.9
Songkhla	91.0±1.9	98.0±1.5	72.7±3.2
Satun	98.0±2.2	100	65.4±6.5
Surat Thani	—	—	61.0±2.5
Bangkok	—	87.0±0.5	9.0±1.1
Lampang	—	—	8.0±2.0
Nakhon Sawan	—	—	12.0±3.4
Tak	—	100	2.0±1.4
USDA[b]	100	100	100

[a] — Not applicable
[b] USDA Beltsville, Florida USA strain

Table 3. Percent mortalities of *Aedes aegypti* strains after exposure to diagnostic concentration of malathion, propoxur and DDT

Strains	Mortality (%)[a]		
	Malathion	Propoxur	DDT
Chiang Mai-P	98.0±1.6	—	37.2±5.5
Chiang Mai-M	88.1±1.5	93.0±1.4	3.0±2.4
Kanchanaburi	97.0±1.9	—	2.0±2.0
Khonkaen	77.8±2.5	—	3.0±1.0
Nonthaburi	100	—	0
Songkhla	99.0±1.0	—	0
Satun	100	—	0
Lampang	84.1±1.3	87.1±0.5	0
Tak	2.2±1.4	85.0±1.5	0
Surat Thani	36.3±2.1	52.2±0.4	0
Bangkok	88.1±1.7	93.2±1.5	3.1±0.4
Nakhon Sawan	68.2±1.4	73.5±2.2	0
Pathum Thani	—	—	0
USDA[b]	100	—	100

[a] — Not applicable
[b] USDA Beltsville, Florida USA strain

(100.0% mortality). Four strains, Chiang Mai (Chiang Mai-P) (100.0% mortality), Khonkaen (100.0% mortality), Kanchanaburi (98.0% mortality), Satun (98.0% mortality) were susceptible to alphacypermethirn, except that a trend of tolerance/resistance was recognized in Nonthaburi (85.8% mortality) and Songkhla (91.0% mortality). The susceptibility to alphacypermethrin in *Ae. aegypti* from Chiang Mai-M, Surat Thani, Bangkok, Lampang, Nakhon Sawan, and Tak could not be identified because of an insufficient number of mosquitoes to test. All remaining test strains were susceptible to deltamethrin (98.0–100.0% mortality), except for the Bangkok strain (87.0% mortality).

Aedes aegypti from different localities demonstrated various levels of tolerance/resistance to malathion and propoxur. A high level of resistance to malathion was seen in Tak (2.2% mortality) and Surat Thani (36.3% mortality) strains whereas two strains from Satun and Nonthaburi were susceptible (100.0% mortality) to malathion. In addition, a USDA laboratory strain was found to be susceptible to malathion (100.0% mortality). The susceptibility to propoxur of *Ae. aegypti* from several localities could not be identified because of an insufficient number of mosquitoes. With DDT, strong resistance was observed in all strains, except the standard susceptible USDA strain (100.0% mortality).

Insecticide susceptibility tests with a single dose of DDT (4%), permethrin (0.25%), malathion (0.8%) and propoxur (0.1%) were performed on three strains of wild-caught *Cx. quinquefasciatus* and the results are presented in Table 4. *Culex quinquefasciatus* from Nonthaburi, Bangkok and Pathum Thani showed a high level of DDT resistance (2.2-8.3% mortality), and also exhibited moderate resistance to permethrin (67.4–72.1% mortality). Tolerance/resistance to permethrin was recognized in Bangkok (80.6% mortality). With malathion and propoxur, the possibility of incipient insecticide resistance was observed in the Pathum Thani strain, whereas two strains from Nonthaburi and Bangkok were susceptible.

Discussion

Arthropod borne diseases are an ever increasing cause of death and suffering worldwide.[31] Thailand is endemic for several vector borne diseases, including malaria, DF and DHF, Japanese encephalitis and lymphatic filariasis.[16] Significant growth in the human population combined with demographic movement to urban residential areas and increased tourism-based facilities have led to tremendous deforestation, irrigation and urbanization. Changes in the surrounding environment due to global warming have favored conditions for increasing mosquito vector transmission of diseases.[18,32] Despite research progress, a completely effective dengue vaccine is not yet available. The prevention of disease transmission has relied mainly on the reduction of human-vector contact using chemical compounds.

The insecticide susceptibility level of mosquitoes is considered one of the major factors influencing the success of vector control. For years, chemical companies have been developing synthetic chemicals, especially synthetic pyrethroids. These synthetic pyrethroids have demonstrated great promise for mosquito vector control because of their low toxicity to humans and great potency at low doses quickly immobilizing and killing insects[33,34]; however, overtime, resistance to these synthetic compounds has been recorded in several species of arthropods, including *Ae. aegypti* and *Cx. quinquefasciatus*.[15,17,18,24,32] In this study, it was clearly seen that most field collected *Ae. aegypti* and *Cx. quinquefasciatus* strains demonstrated comparatively high levels of resistance to permethrin. Permethrin is a common compound that is regularly used in Thai households for pest control.[20,35] Recently, Jirakanchanakit[17] and Ponlawat[20] reported that several strains of *Ae.*

Table 4. Percent mortalities of three strains of *Culex quinquefasciatus* after exposure to diagnostic concentrations of DDT, permethrin, malathion and propoxur

Strains	Mortality (%)			
	DDT	Permethrin	Malathion	Propoxur
Nonthaburi	8.1±1.5	72.1±0.4	100	100
Bangkok	2.2±2.4	80.6±1.7	100	100
Pathum Thani	8.3±1.4	67.4±2.4	84.5±1.3	81.6±2.8

aegypti across Thailand were resistant to permethrin. The reason for this is related to household products for pest control and impregnated bed nets for mosquito control.[18] In contrast, most *Ae. aegypti* strains have been found susceptible to deltamethrin, suggesting that this compound is still effective in control programs during dengue outbreaks; however, a trend of incipient resistance was detected in one strain of *Ae. aegypti* from Bangkok. This result was in agreement with Yaicharoen[36] who reported the development of physiological resistance to deltamethrin in several strains of *Ae. aegypti* from the Bangkok area. The reason for the possible deltamethrin resistance in the Bangkok strain is unclear but might be due to the tremendous use of insecticide products in the Bangkok area, one of the most important dengue outbreak areas. We know that ultra-low-volume (ULV) applications of deltamethrin have been used to attempt to interrupt mosquito virus transmission after reports of dengue cases. In addition, the *Ae. aegypti* mosquito appears to stay inside houses where they are more likely to be exposed to insecticide during ULV application. Resistance to deltamethrin could be related to the host seeking behavior of female mosquitoes as they may spend more time in insecticide treated areas close to their preferred hosts.[18]

Surprisingly, incipient resistance to alphacypermethrin was also detected in *Ae. aegypti* from Nonthaburi, and Songkhla where deltamethrin remains the mainstay of the dengue vector control program. In addition to deltamethrin, several synthetic pyrethroids are available in the market in the form of household products that are currently used in homes and may be one of the significant reasons for insecticide resistance in mosquitoes. Alphacypermethrin incipient resistance may have been arisen from previous synthetic pyrethroid usage in the area. Cross-resistance as a consequence of unintentional or extensive use of the same or related groups of compounds in mosquito populations has been reported elsewhere.[12,18,21]

A high level of physiological resistance to DDT in *Cx. quinquefasiatus* could be related to the previous use of DDT in agriculture and public health.[18] Although DDT has been completely stopped for public health use since 2000, frequent indoor residual spraying for over 40 years may have resulted in the development of resistant genes which contribute to physiological resistance in a certain mosquito population. Resistance to permethrin in *Cx. quinquefasciatus* could also be related to the tremendous use of this compound in Thai households for pest control in the form of aerosols, mosquito coils, or mats. This increasing use of permethrin in agricultural practices may have played a role in the development of physiological resistance to this compound in *Cx. quinquefasciatus*; however, several *Cx. quinqefasciatus* strains are still susceptible to malathion and propoxur, suggesting that these compounds may still be effective in controlling *Cx. quinqefasciatus* in Thailand.

In summary, insecticide resistance should be monitored and evaluated as early as possible and should be conducted over a wide geographical range to include as many known vector species as possible. In addition, this monitoring should be part of an insecticide evaluation program aimed at the success of disease control activity. Early detection of operationally unacceptable levels of resistance can prompt public health authorities to take appropriate steps to counter the potential reduced control efforts. In addition, control program should remain aware of cross-resistance to the same or related synthetic compounds against mosquito populations and agricultural pests.

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References

1) D. J. Gubler (ed.): "Dengue and dengue haemorrhagic fever," CAB International, 1997.
2) D. J. Gubler: *Clin. Microbiol. Rev.* **11**, 480–496 (1998).
3) M. Jacobs: *Trans. R. Soc. Trop. Hyg.* **94**, 7–8 (2000).
4) P. M.Sheparrd, W. W. Macdonal, R. J. Tonn and B. Grab: *J. Animal Ecol.* **38**, 661–702. (1969).
5) http://dhf.ddc.moph.go.th
6) P. Kittayapong and D. Strickman: *J. Med. Entomol.* **30**, 601–606 (1993).
7) C. Chansang and P. Kittayapong: *Am. J. Trop. Med. Hyg.* **77**, 897–902. (2007).
8) S. R. Christophers: "*Aedes aegypti* (L.), the Yellow Fever Mosquito," Cambridge University Press, London, 1960.
9) M. Yasuno and R. J. Tonn: *Wld. Hlth. Org. Bull.* **43**, 319–325 (1970).
10) F. Rodhain and F. Rosen (eds.): "Dengue and Dengue haemorrhagic fever," CAB International, 1997.
11) M. Vazeille, L. Rosen, L. Mousson and A. Failloux: *Am. J. Trop. Med. Hyg.* **68**, 203–208 (2003).
12) T. Chareonviriyaphap, A. Prabaripai, M. J. Bang and B. Aum-Aong: *J. Med. Entomol.* **40**, 876–881 (2003).
13) T. Chareonviriyaphap, D. R. Robert, R. G. Andre, H. H. Harland, S. Manguin and M. J. Bang: *J. Am. Mosq. Control. Assos.* **13**, 171–183 (1997).
14) D. R. Robert, L. L. Laughlin, P. Hshei and U. Letgers: *Emerg. Infect. Dis.* **3**, 295–302 (1997).
15) P. Somboon, L. Prapanthadara and W. Suwankerd: *Southeast Asia J. Trop. Med. Public Health* **34**, 87–93 (2003).
16) Ministry of Public Health, Thailand: "Vector Borne Disease Annual Report. 2002–2003," (in Thai).
17) N. Jirakanjanakit, P. Rongnopharut, S. Saengtharatip, T. Chareonviriyaphap, S. Dunchon and L. Bellec: *J. Econ. Entomol.* **100**, 545–550 (2007).
18) T. Chareonviriyaphap, B. Aum-Aong and S. Ratanatham: *Southeast Asian J. Trap. Med. Public Health* **30**, 130–141 (1999).
19) S. Jatanasen: *Bull. World Health Organ.* **35**, 91–91 (1966).
20) A. Ponlawat, G. Jeffrey, G. Scott and L. C. Harrington: *J. Med. Entomol.* **42**, 821–825 (2005).
21) M. Kongmee, A. Prabaripai, P. Akratankul, M. J. Bang and T.

Chareonviriyaphap: *J. Med. Entomol.* **4**, 1055–1063 (2004).
22) P. Paeporn, K. Suphapatthom, S. Boonyabancha and P. Phan-Urai: *Bull. Dept. Med. Sci.* **38**, 37–43 (1996).
23) P. Paeporn, K. Suphapatthom, S. Sathantriphop, M. Mukkhum and S. Sangkitporn: *Dengue Bull.* **29**, 210–213 (2005).
24) S. Sathatriphop, P. Paeporn and K. Suphapatthom: *Trop. Biom.* **23**, 97–101 (2006).
25) W.H.O.: WHO/CDS/CPC/MAL/98.12. 1998. Geneva.
26) S. Sirivanakarn: *Contrib. Am. Entomol. Inst.* **12**, 1–272 (1976).
27) R. Reuben, S. C. Tewari, J. Hiriyan J and J. Akiyama: *Mosq. Syst.* **26**, 76–96 (1994).
28) W.H.O.: WHO/CDS/WHOPES/98.10. 1998. Geneva.
29) W.H.O.: WHO/CDS/NTD/WHOPES/GCDPP/2006.3. 2006 Geneva.
30) W. S. Abbot: *J. Econ. Entomol.* **18**, 265–267 (1925).
31) W.H.O.: http://www.who.int/whr/2007/en/index.html
32) S. Sathatriphop, C. Ketavan., A. Prabaripai, S. Visetson, M.J. Bang, P. Akratananakul and T. Chareonviriyaphap: *J. Vec. Ecol.* **31**, 266–274 (2006).
33) M. Prasittisuk: Thailand Mahidol University. 1994. (Ph. D. Thesis).
34) J. P. Grieco, N. L. Archee, T. Chareonviriyaphap, W. Suwankerd, K. Chauha and D. R. Roberts: http://www.plosone.org. 2007.
35) P. Paeporn, S. Sathatriphop, P. Wattanachai, P. Ya-umphan and K. Suphapatthom: *J. Health Sci.* **13**, 327–332 (2004).
36) R. Yaicharoen, R. Kiatfengtoo, T. Chareonviriyaphap and P. Rongnoparut: *J. Vec. Ecology* **30**, 144–150 (2005).