Original Article *นิพนธ์ต้นฉบับ*

Insecticide Resistance of *Aedes aegypti* Linnaeus (Diptera: Culicidae) in the Northeast of Thailand

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Abstract *Aedes aegypti* Linnaeus is a carrier of dengue fever, which is a public health problem in the Northeast of Thailand. During epidemic, stresses were made on use of insecticides for control of the disease. A survey of chemicals used was carried out in eight provinces, namely Ubon Ratchathani, Si Sa Ket, Yasothon, Amnat Charoen, Mukdahan, Nakhon Phanom, Sakon Nakhon and Kalasin. The study indicated that one chemical used in the control of mosquito larvae was 1 % temephos. The most used chemical for control of adult mosquitoes by spraying was cypermethrin. From the study of the susceptibility of *Ae. aegypti* to chemically-impregnated paper using 0.05 % deltamethrin, 5 % malathion, and 1 % fenitrothion it was effective at a medium to high level. With regard to the susceptibility of *Ae. aegypti* to 0.75 % permethrin, the finding indicated that its effectiveness was at a low level. Based on the results of resistance ratio of *Ae. aegypti* comparing with susceptible strain, *Ae. aegypti* showed a resistance to deltamethrin and permethrin at a high level, with a resistance ratio of 10 fold greater. Regarding the resistance in all areas of *Ae. aegypti* to malathion and fenitrothion, the result was at a low to medium level. From a biochemical examination, the results demonstrated that monooxygenase and the esterase activity of *Ae. aegypti* had increased in all strains. Nevertheless, the glutathione-S-stransferase activity had increased in seven strains. Therefore, monooxygenase, esterase, and glutathione S-transferase activity were associated with mechanisms for creating pyrethroid-resistance in *Ae. aegypti*. Those were used for control of adult mosquitoes, which showed high resistance ratio to the chemicals in these areas. Consequently, if treatment using insecticides is still applied continuously, there is a possibility that those insects will become more resisting and, therefore hamper the control of mosquitoes in the future.

Key words: ***Aedes aegypti*, the Northeast of Thailand, insecticide, resistance**

Introduction

Dengue hemorrhagic fever is a contagious disease that is a public health concern in the Northeast of Thailand. At present, it is still a chronic and widespread problem. *Aedes aegypti* is a major carrier of dengue fever. Vector control by reducing *Ae. aegypti*

population is, at present, the only way to prevent the spreading of the disease. It is consisted of environmental management, biological, genetic and chemical control. However, insecticides have been frequently used for vector control programs. The Northeast of Thailand has mainly relied on the usage of organophosphate and synthetic pyrethroid. During endemic seasons, then deltamethrin and cypermethrin were the main synthetic pyrethroids used to control adult *Ae. aegypti* mosquitoes through mass spraying[1]. The spread of pyrethroid resistance has increased in controlling the vector-borne diseases worldwide. The increasing mosquito resistance is the primary concern of all control parties. Common insecticide resistance mechanisms include alteration of target sites and increased enzyme activities of non-specific esterase, glutathione S- transferase (GSTs) and P450 - mediated monooxygenases[2]. The major metabolic enzymes involved in resistance against pyrethroids in insects include P450 mediated monooxygenases, evaluated non-specific esterase, and reduced sensitivity of sodium ion channels along nerve axons[3]. Recently, several cases of field-associated resistance have been reported in *Ae. aegypti* against pyrethroid products. Resistance to pyrethroids including permethrin and deltamethrin in *Ae. aegypti* were documented in northeastern provinces of Thailand. An attempt was made to lay bare the determinants of the level of resistance of the *Ae. aegypti* to chemicals by susceptibility test and studying the changes in the level of enzymes that were related to the reduction and detoxification of chemical toxins of the *Ae. aegypti*.

Methodology

Survey of insecticides: Urban respondents from 8 sites were interviewed by using questionnaire in a cross-sectional survey on the type of insecticides to control larvae and adult *Ae. aegypti*. The key informants were the officials who are responsible for the control of contagious diseases at municipal officers, hospitals and district administration offices in these areas. The target sample in urban was respondents from 8 sites: Ubon-Ratchathani, Yasothon, Amnat Chareon, Si Sa Ket, Nakhon Panom, Mukdahan, Kalasin and Sakon Nakhon. The survey took place between May and July 2007.

The susceptibility of the *Ae. aegypti* to chemically-impregnated paper: Testing of susceptibility of the *Ae. aegypti* to chemicals was carried out, and results were interpreted, as recommended by the World Health Organization[4]. The mosquitoes used came from mosquito larvae that were gathered from the studied areas and then reared (generation F1) to adult. Fully-grown females aged 3 - 5 days old were fed on 5% sugar, which was given to them before testing, and then placed inside an exposure tube that was lined with chemically-impregnated paper. Each tube contained 25 mosquitoes and there were 4 tubes in all. The mosquitoes were left in the tubes for 15, 30, 60 and 120 minutes. They were then kept at a temperature of 25 ±2° Celsius. After the exposure time, the test mosquitoes were transferred to the holding tubes and cotton pad soaked in 10% sugar solution was provided. Their mortality was recorded after 24 hours.

- Interpretation of the results of the susceptibility of the *Ae. aegypti* to chemicals according to the World Health Organization manual[5] were as follows:

98-100 percent mortality indicated susceptibility,

80-97 percent mortality suggested the possibility of resistance that needed to be confirmed, and

<80 percent mortality suggested resistance.

- Bioassay data were pooled and LT_{50} was obtained by probit analysis[6].
- Resistance ratio (RR_{50}) was calculated by comparing LT_{50} with LT_{50} of susceptible strain.

$$\text{Resistance ratio, } RR_{50} = \frac{LT_{50} \text{ Resistance strain}}{LT_{50} \text{ Susceptible strain*}}$$

Note: * The susceptible strain (Bora Bora) from the Faculty of Tropical Medicine, Mahidol University.

Biochemical assay

Protein assay: The total protein content of individual *Ae. aegypti* mosquitoes was determined using a commercial protein assay system. Result were compared to a derived standard curve. The plates were read after 5 min using an Elisa plate reader at 570 nm wavelength.

Monooxygenase assay: The procedure described by Valule et al. (1999)[7] was followed with only minor modifications. Fresh individual mosquitoes were homogenized in 50 ml distilled water in a 1 ml plastic vial. Homogenates were diluted with an additional 150 µl distilled water. Twenty µl of each homogenate was transferred to a microplate followed by addition of 80 µl 0.0625 M potassium phosphate buffer (PPB) at pH 7.0. A solution of 0.01 g of 3,3,5,5/ - Tetramethyl Benzidine (TMBZ) in 5 ml methanol was prepared and a 0.25 M sodium acetate buffer (pH 5.0) was added. Two hundred µl of TMBZ solution was then added with the 100 µl of mosquito homogenate plus PPB in each well followed by 25 µl of 3% hydrogen peroxide. The plates were read after 5 and 10 min using an ELISA plate reader at 620 nm wavelength.

Esterase Assay: In the method of Peiris and Hemingway (1990)[8], the test could begin by adding 30 mM α-naphtyl acetate that had been previously prepared at a fixed quantity in each hole and leaving it at a temperature of 30˚ Celsius for 15 min. Following that, 0.1 % fast blue B-SDS was added and then a 20 µl sample of the mosquito that has been separated was added to each hole and it was left at room temperature, 25 ±2˚ Celsius. The test was then carried out using a micro plate reader and the absorbance value was read at 620 nm.

Glutathione -S-transferase assay: GST activity was assayed following by Habig et al. (1974)[9]. They were mixed at a ratio of 125 µl CDNB to 2.5 ml GSH. In the test, 20 µl of the prepared liquid was added to each hole. Following that, a 20 µl sample of the mosquito that has been separated was added to each hole and it was left at room temperature, 25 ±2˚ Celsius for 15 min. The test was then carried out using a micro plate reader and the absorbance value was read at 340 nm.

The analysis was carried out using ANOVA, which was to compare the protein contents and enzyme expression levels within and between population. All levels of statistical significance were determined at 0.5% confidence limit.

Results

Survey of chemicals:

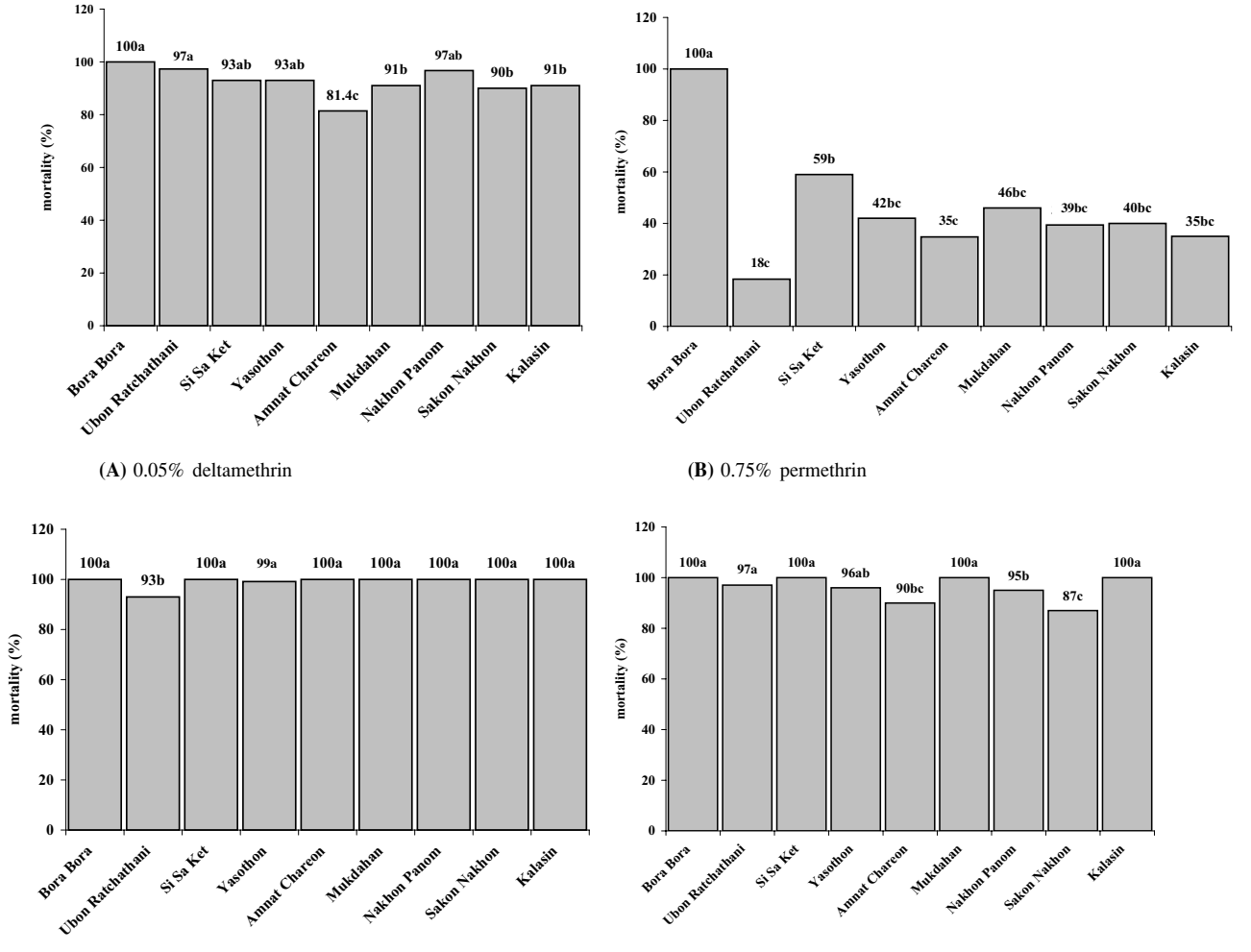
Most of insecticides used in the epidemic season stress were 1% temephos for larvicide and cypermethrin for adulticide.

Susceptibility of *Ae. aegypti* to insecticides:

The results of the susceptibility to insecticides are presented in Figure 1. It was found from the assessment of the toxicity of chemically-impregnated paper, that the *Ae. aegypti* is susceptible to paper treated with 0.05% deltamethrin, 5% malathion, and 1% fenitrothion at a medium to high level with a susceptibility rate of 87 - 100 percent. Regarding the susceptibility with the chemical 0.75% permethrin, the level was low with a mortaliy rate of just 18 - 59 percent. The statistical difference was at a confidence level of 95 percent when compared with the Bora Bora strain.

Resistance Level of the *Ae. aegypti* to Insecticides:

In the study of the resistance level to insecti-



(A) 0.05% deltamethrin

(B) 0.75% permethrin

(C) 5% malathion

(D) 1% fenitrothion

**In every column that ends with the same letter, there is no statistical difference based on a comparison using the Duncan multiple range test at a confidence level of 95%*

Figure 1 The mortality of chemically-impregnated paper bioassay on *Aedes aegypti* Linnaeus from eight provinces

cides, it was found that the *Ae. aegypti* in every area showed a high resistance to deltamethrin and permethrin. *Ae. aegypti* from Si Sa Ket had the highest resistance ratio to 0.05% delta-methrin at 24.80 fold. Next in order were the *Ae. aegypti* from Mukdahan, Ubon Ratchathani, and Sakon Nakhon with resistance ratios of 23.96, 22.78, and 20.91 fold respectively. With regard to the study of the resistance rate to paper that had been chemically treated with 0.75% permethrin, the *Ae. aegypti* from Kalasin had the highest resistance ratio at 15.13 fold. Regarding the chemicals 5% malathion and 1% fenitrothion, the *Ae. aegypti* from all areas had resistance ratio that varied from low to medium. (Table 1)

Biochemical Study:

From the results of testing for the amount of proteins and enzymes in *Ae. aegypti* in all 8 provinces. The investigations demonstrated that the amount of protein, monooxygenase, and esterase enzyme had showed a tendency to increase when compared with

Table 1 Resistance ratios of the 8 strains of *Aedes aegypti* Linnaeus to four types of chemically-impregnated paper in the Northeast of Thailand

	Chemical							
	Deltamethrin 0.5%		Permethrin 0.75%		Malathion 5%		Fenitrothion 1%	
Aedes aegypti Linnaeus strains	LT_{50}* (minute)	RR_{50}**	LT_{50}* (minute)	RR_{50}**	LT_{50}* (minute)	RR_{50}**	LT_{50}* (minute)	RR_{50}**
Bora Bora	1.0821	1	6.3388	1	2.7756	1	5.9366	1
Ubon Ratchathani	24.6451	22.78	74.2632	11.72	10.0989	3.64	9.1136	1.54
Si Sa Ket	26.8370	24.80	64.6004	10.19	13.8454	4.99	27.6615	4.66
Yasothon	20.4058	18.86	64.3992	10.16	6.7857	2.44	11.5399	1.94
Amnat Charoen	21.9124	20.25	67.0799	10.58	21.6527	7.80	25.5521	4.30
Mukdahan	25.9294	23.96	80.6522	12.72	17.8287	6.42	25.1353	4.23
Nakhon Phanom	21.5179	19.88	68.3064	10.78	14.3295	5.16	21.7699	3.67
Sakon Nakhon	22.6224	20.91	70.8879	11.18	16.5196	5.95	28.9672	4.88
Kalasin	15.9024	13.95	95.8781	15.13	12.4080	4.47	19.6806	3.32

**The LT_{50} value of the Aedes aegypti Linnaeus to chemically-impregnated paper.*
***The resistance ratio (RR_{50}) = LT_{50} field strain / LT_{50} susceptible strain.*

the Bora Bora strain. Nevertheless, the glutathione-transferase activity had increasing in seven strains.

Discussion

The mosquito is a carrier of dengue hemorrhagic fever and has been a health problem in the Northeast of Thailand for more than 50 years. The control of the disease in the epidemic season stresses the use of chemicals to control the mosquito at the larval stage as well as for adult insects. The chemical used to control larvae was 1% temephos. However, temephos has been a convenient insecticide because of its low oral toxicity in mammals (8,600 mg/kg in male rats), and it is one of the few insecticides recommended for potable water[10]. Also, it has been difficult to withdraw the recommendation for using temephos to control container breeding of mosquitoes. In the part of the chemical that was mostly used to spray adult mosquitoes was cypermethrin, although this pyrethroid was recently started to be used in the areas to control the dengue vector.

The present study on the susceptibility was used adult mosquito exposure to chemically-impregnated paper. It was shown that *Ae. aegypti* is susceptible to 0.05% deltamethrin, 5% malathion, and 1% fenitrothion at a medium to high level, and the susceptibility rate achieved was 80 - 100 percent. Regarding the level of susceptibility to paper chemically-impregnated with 0.75% permethrin, it was recorded as low for all species with a susceptibility rate of 18 - 59. From the results of the study, the level of resistance of the *Ae. aegypti* in all eight provinces in comparison with that of the Bora Bora strains was high with respect to deltamethrin and permethrin[11]. The resistant ratio was found to be more than 10 fold[12]. Regarding the chemicals malathion and fenitrothion, the *Ae. aegypti* had a resistance ratio of low to medium. The study associated with Sathantriphop[13] who

Table 2 Levels of proteins and enzymes for all 8 strains of *Aedes aegypti* Linnaeus in the Northeast of Thailand when compared with susceptible strain

Strains (n=40 mosquitoes for each)	Total Protein Mean, SD (mg protein/ml per mosquito (n))	MFOs (nmol product/min /mg protein)	α Esterase (nmol α naphthol/ min/ mg protein	GSTs (nmol CDNB/min/ mg protein)
Bora Bora	0.6569, 0.0919 c*	0.0561, 0.3552 c	0.9175, 0.6176 c	0.9701, 0.7335 c
Ubon Ratchathani	0.6891, 0.1129 bc	0.0697, 0.0446 a	1.4553, 0.0844 a	1.1076, 0.0357 b
Si Sa Ket	0.7143, 0.1386 b	0.0573, 0.0139 a	1.5420, 0.0498 a	1.1241, 0.0348 b
Yasothon	0.7774, 0.1008 b	0.1331, 0.0927 bc	1.3223, 0.1346 b	1.2661, 0.0249 b
Amnat Charoen	0.8252, 0.0952 ab	0.1285, 0.1174 bc	1.4279, 0.2225 a	1.2642, 0.0481 b
Mukdahan	0.8604, 0.1340 a	0.3846, 0.1913 a	1.3962, 0.1315 b	1.4897, 0.0843 a
Nakhon Phanom	0.8613, 0.1254 a	0.2257, 0.1623 bc	1.3409, 0.1234 b	1.4941, 0.0614 a
Sakon Nakhon	0.8236, 0.1311 ab	0.1184, 0.0957 b	1.3970, 0.0815 b	0.9577, 0.0741 c
Kalasin	0.6747, 0.0936 bc	0.1054, 0.0646 b	1.3357, 0.1307 b	1.9832, 0.0824 c

**In every column that ends with the same letter, there is no statistical difference from a comparison using the Duncan multiple range test at a confidence level of 95%.*

studied resistance to various insecticides from four groups in a field strain *Ae aegypti,* which is a main dengue vector in the Baan Suan community, Nonthaburi province, Thailand was also tested with deltamethrin, permethrin and fenitrothion. The result demonstrated that mosquitoes were clearly resistant to permethrin and deltamethrin, but were 100 percent susceptible to fenitrothion.

From the results of the study by biochemical assay, that for the amount of proteins and enzymes in the *Ae. aegypti* from all 8 provinces using a micro plate reader, it was found that the amount of protein in all strains had increased when compared with that of the Bora Bora strain. One cause of the resistance, that has built up in the mosquitoes, is the ability to reduce the toxicity of the chemical. From the results of the study by biochemical assay, all strains of the *Ae. aegypti* had an increase level of protein which confirms that the *Ae. aegypti* has further developed its resistance to chemicals. Resistance to insecticides usually appears to be unstable, associated with a genetic cause in the absence of selection[14,15]. This has occurred due to other resistance creating mechanisms apart from the increase of enzymes that detoxify the aforementioned insecticidal toxins. Regular monitoring of the mosquitoes' susceptibility to the most widely used insecticides is necessary to ensure the appropriate choice of chemicals. Introduction of inappropriate insecticides without a proper understanding of the prevailing resistance mechanisms may lead to enhancement of vector resistance and disease control failure. Moreover early detection and knowledge on the resistance status and the underlying mechanisms in mosquitoes are essential for effective long-term control of the vector.

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บทคัดย่อ **ความต้านทานของยุงลาย (*Aedes aegypti* Linnaeus) ต่อสารฆ่าแมลงในภาคตะวันออกเฉียงเหนือ**

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วารสารวิชาการสาธารณสุข **2553; 19:182-9.**

ยุงลายเป็นพาหะนำโรคไข้เลือดออกที่เป็นปัญหาสาธารณสุขในพื้นที่ภาคตะวันออก-เฉียงเหนือ การควบคุมโรคในฤดูกาลระบาดจึงเน้นหนักมาตรการใช้สารเคมี ได้สำรวจสารเคมีในพื้นที่ 8 จังหวัดได้แก่ อุบลราชธานี ศรีสะเกษ ยโสธร อำนาจเจริญ มุกดาหาร นครพนม สกลนคร และกาฬสินธุ์ พบว่า สารเคมีที่ใช้ควบคุมในระยะลูกน้ำ คือ 1% ทีมีฟอส สารเคมีส่วนใหญ่ที่ใช้ในการพ่นควบคุมยุงลายตัวเต็มวัยคือ ไซเพอร์มิทริน การศึกษาความไวของยุงลายต่อกระดาษชุบสารเคมี 0.05% เดลต้ามิทริน 5% มาลาไธออน และ 1% เฟนิโตรไธออน พบว่ายุงลายมีความไวอยู่ในระดับปานกลางถึงสูง ส่วนระดับความไวต่อกระดาษชุบสารเคมี 0.75% เพอร์มิทริน อยู่ในระดับต่ำ ผลการศึกษาค่าระดับความต้านทานของยุงลายเปรียบเทียบกับสายพันธุ์มาตรฐาน พบว่า ยุงลายแสดงความต้านทานต่อสารเคมีเดลต้ามิทรินและเพอร์มิทรินในระดับสูง โดยมีค่าระดับความต้านทานมากกว่า 10 เท่า ส่วนสารเคมีมาลาไธออนและเฟนิโตรไธออนยุงลายจากทุกพื้นที่มีค่าระดับความต้านทานในระดับต่ำถึงปานกลาง จากการตรวจสอบทางชีวเคมี พบว่า ยุงลายทุกสายพันธ์มีระดับเอนไซม์โมโนออกซิจีเนส เอสเทอเรส เพิ่มขึ้นแต่ระดับเอนไซม์ กลูตาไธโอน เอส ทรานสเฟอเรส เพิ่มขึ้น 7 สายพันธุ์ ดังนั้นจึงมีแนวโน้มว่าทั้งเอนไซม์โมโนออกซีจีเนส เอสเทอเรส และกลูตาไธโอนเอสทรานเฟอเรส มีส่วนเกี่ยวข้องกับการสร้างความต้านทานของยุงลายต่อสารเคมีในกลุ่มไพรีทรอยด์สังเคราะห์ โดยสารกลุ่มดังกล่าวใช้ควบคุมยุงลายในระยะตัวเต็มวัย ซึ่งแสดงความสัมพันธ์กับค่าระดับความต้านทาน ดังนั้นหากมีการใช้สารเคมีดังกล่าวควบคุมยุงติดต่อกันเป็นเวลานาน สามารถชักนำให้ยุงลายสร้างความต้านทาน ซึ่งเป็นปัญหาต่อการควบคุมยุงในอนาคตได้

คำสำคัญ: **ความต้านทาน, ภาคตะวันออกเฉียงเหนือ, ยุงลาย, สารฆ่าแมลง**