

Resistance to Temephos of *Aedes aegypti* Linnaeus Larvae (Diptera: Culicidae)

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Abstract This study was conducted to measure the resistance to temephos of *Aedes aegypti* Linnaeus larvae in the northeast of Thailand. *Ae. aegypti* larvae were collected from urban of 7 provinces in the northeast of Thailand namely Ubon Ratchathani, Si Sa Ket, Yasothon, Amnat Charoen, Mukdahan, Nakhon Panom, Kalasin and Sakon Nakhon provinces during April-June 2006. The fourth larval stage were tested in 0.02 mg/l temephos solution as recommended by the World Health Organization as a standard method. The results demonstrated that Ubon Ratchathani, Si Sa Ket, Yasothon, Amnat Charoen, and Kalasin *Ae. aegypti* strains had mortality indicating susceptibility. When the fourth larval stage, were selected for bioassay test, the LC₅₀ and LC₉₅ of Mukdahan *Ae. aegypti* larvae strains were the highest, with values of 0.01154 and 0.0296 mg/l respectively. However, when compared with the WHO standard susceptible strain, the resistance of the Mukdahan strain were 12.68 and 11.93 fold for the LC₅₀ and LC₉₅ respectively. The conclusion of this study is that Mukdahan *Ae. aegypti* larvae strain can develop higher levels of resistance to temephos than other strains. The results obtained from this research study can be applied to other regions with the same problem with control of *Ae. aegypti* larvae as an important part of a Dengue Hemorrhagic Fever control program.

Key words: resistance, temephos, *Aedes aegypti*

Introduction

Dengue Hemorrhagic Fever-DHF has been a major problem in public health in Thailand since 1958. In the year 2002, a total of 93,131 cases of DHF, with 139 deaths was reported by the Division of Epidemiology, Ministry of Public Health. *Aedes aegypti* Linnaeus plays a crucial role in transmission of this

viral disease⁽¹⁾. *Ae. aegypti* is highly anthropophilic and thrives in close proximity to humans and often lives indoors. They usually feed during the day, once in mid morning and again in late afternoon. Although vaccination would be an ideal method to control DHF, development of vaccines for dengue viruses are in progress and their trials have been slow. Moreover

there are some difficulties in application of vaccine trials owing to the live only method in addition to clinical case management available to control DHF. Permanent control of *Ae. aegypti* must be by the destruction of the mosquito's breeding sites. However, for immediate local control of epidemic transmission of DHF, it is very important to carefully plan for vector control by using insecticides against larvae and adult mosquitoes.

During the endemic seasons, volunteers use temephos to kill larvae and deltamethrin is the main synthetic pyrethroids used to control adult *Aedes* mosquitoes through mass spraying⁽²⁾. Temephos is an organophosphorus insecticide that has been used as larvicide against *Ae. aegypti* in Thailand since 1967⁽³⁾. The widespread use of insecticides has led to insecticide resistance in mosquitoes that will be another problem for the ability to control disease⁽⁴⁾. Recently, several cases of field associated resistance have been reported in *Ae. aegypti* against temephos products. Studies of resistance to temephos in *Ae. aegypti* are undertaken to obtain information on susceptibility of the insect. The susceptibility condition test has been used for *Ae. aegypti* larva on field collected larvae from urban area in northeast provinces where high numbers of dengue haemorrhagic fever cases have been reported. These populations had prior history of exposure to temephos. This study was to characterize of the resistance to temephos in *Ae. aegypti* larvae in seven provinces and to determine whether selection of temephos would result in tolerance or resistance in those population. Such knowledge is essential in defining future control strategies against this medically important mosquito.

Methodology

1. Seven field populations of *Ae. aegypti* larvae were randomly sampled from water containers within

houses of villages in seven provinces in the northeast of Thailand from April-June 2006. All provinces were chosen because each area had continuously used temephos as the larvicide to control *Ae. aegypti* larva for a long time and they also had had recent DHF cases. The larvae of the reference population strain are *Ae. aegypti* Bora Bora strain (WHO susceptible strain) which was obtained from the Faculty of Tropical Medicine, Mahidol University.

2. Insecticides : The technical grade (90% purity) of temephos, an organophosphorus insecticide, was obtained from Cyanamid Co. Solutions were stored at 4°C.

3. Mass rearing of mosquitoes

The eggs laid by stock mosquitoes on filter paper were kept in the tray for 3-4 days and left to dry at room temperature in order to allow the eggs to develop and be ready to hatch when they were immersed in water.

The eggs were immersed in a plastic tray (30x30x6 cm³) containing about 1,500 ml of dechlorinated tap water. Larvae hatched within 24 hours after immersion. The adults emerged about two days after and were supplied with 10% sugar solution soaked in cotton wool.

4. Bioassay procedures

The early fourth instar larvae of the field and Bora Bora strains were used for bioassay test. The procedures recommended by WHO⁽⁵⁾ was followed; 25 larvae in 249 ml of dechlorinated tap water with 1 ml of each concentration of temephos were tested. Mortality counts were made after 24 hours and mortality calculated by Abbott's formula⁽⁶⁾. Scores of mortality at different exposure concentration were used to further calculate lethal concentrations (LC₅₀ and LC₉₅). The results were analyzed for the lethal concentration by probit analysis⁽⁷⁾. Resistance ratio (RR₅₀) was calculated by comparing LC₅₀ and LC₉₅ of each population with Bora Bora susceptible strain.

5. Analysis

• The WHO (1963) recommendation on the following is still valid: 98-100 percent mortality indicates susceptibility, 80-97 percent mortality suggests the possibility of resistance that needs to be confirmed, <80 percent mortality suggests resistance.

- LC₅₀ Probit analysis⁽⁷⁾
- Resistance Ratio, RR

$$RR = \frac{LC \text{ Resistance strain}}{LC \text{ Susceptible strain}}$$

Results

The results of temephos bioassay on *Ae. aegypti* larvae from seven strains of each population are presented in Figure 1. The mortality of each strain on diagnostic concentration (0.02 mg/l) are shown. *Ae. aegypti* larvae that were collected from Ubon Ratchathani, Si Sa Ket, Yasothon, Amnat Charoen and Kalasin had mortalities indicating susceptibility. Then Mukdahan and Sakon Nakhon strains had mortalities suggesting the possibility of resistance that needs to be confirmed.

The LC₅₀ values of the field populations are presented in Table 1. The present study indicates that only *Ae. aegypti* larvae from Mukdahan had high resistance with 12.68 fold of the RR₅₀. Amnat Charoen strain had low resistance ratio with 4.37 fold of the RR₅₀ compared with the susceptible strain.

The LC₉₅ values of the field population are pre-

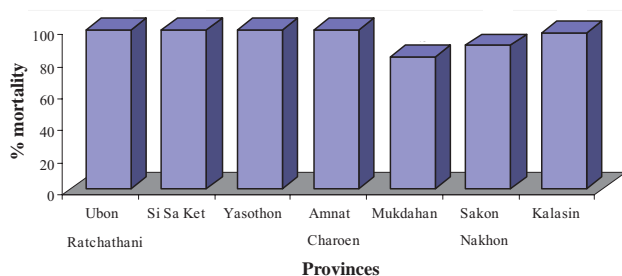


Fig. 1 The mortality of temephos bioassay on *Aedes aegypti* Linnaeus larvae from seven provinces

Table 1 LC₅₀ and RR₅₀ by susceptibility of *Aedes aegypti* Linnaeus larvae from each strain exposed to temephos for 24 hours

<i>Aedes aegypti</i> Linnaeus larvae strain	LC ₅₀ (mg/l)	RR ₅₀
Bora Bora*	0.00091	1
Ubon Ratchathani	0.00636	6.99
Si Sa Ket	0.00681	7.48
Yasothon	0.00496	5.45
Amnat Charoen	0.00396	4.37
Mukdahan	0.02960	12.68
Sakon Nakhon	0.02852	7.92
Kalasin	0.00402	4.42

*Susceptible strain from Mahidol University

Table 2 LC₉₅ and RR₉₅ by susceptibility of *Aedes aegypti* Linnaeus larvae from each strain exposed to temephos for 24 hours

<i>Aedes aegypti</i> Linnaeus larvae strain	LC ₉₅ (mg/l)	RR ₉₅
Bora Bora*	0.00248	1
Ubon Ratchathani	0.01629	6.57
Si Sa Ket	0.02480	10.00
Yasothon	0.01793	7.22
Amnat Charoen	0.01548	6.24
Mukdahan	0.02960	11.93
Sakon Nakhon	0.02852	11.50
Kalasin	0.01448	5.84

*Susceptible strain from Mahidol University

sented in Table 2. The present study indicates that only *Ae. aegypti* larvae from Mukdahan had high resistance ratio with 11.93 fold of the RR₉₅. Although the Kalasin strain had low resistance ratio the level of resistance was 5.84 fold the RR₉₅ compared with the susceptible strain.

Discussion

This study was conducted to measure the level

of resistance to temephos of *Ae. aegypti* larvae in the northeast of Thailand. *Ae. aegypti* larvae were collected from Ubon Ratchathani, Si Sa Ket, Yasothon, Amnat Charoen, Mukdahan, Kalasin and Sakon Nakhon provinces during April-June 2006. The fourth larval stage was tested in 0.02 mg/l temephos solution as recommended by the World Health Organization standard method. The mortality of each strain was measured. *Ae. aegypti* larvae collected from Ubon Ratchathani, Si Sa Ket, Yasothon, Amnat Charoen and Kalasin had mortality indicating susceptibility. Mukdahan and Sakon Nakhon *Ae. aegypti* larvae strains had mortality suggesting the possibility of resistance that needs to be confirmed. When the fourth instar larvae were selected for bioassay test, the LC₅₀ of Mukdahan was the highest, with value of 0.01154 and LC₅₀ of Sakon Nakhon was the highest with value of 0.0296. Other strains show low level of the resistance to temephos except those of Mukdahan and Sakon Nakhon. However, when compare with WHO standard susceptibility strain, the resistance of the Mukdahan strain had LC₅₀ 12.68 fold and LC₉₅ 11.93 fold. Sakon Nakhon strains were LC₅₀ 7.92 fold and LC₉₅ 11.5 fold. The conclusion of this study indicates that, Mukdahan and Sakon Nakhon strains can develop higher level of resistance to temephos than the other strains. In Thailand, the recommended dosage of temephos sand granules applied to domestic stored water is 1 g/10 liters water which is equivalent to 1 mg/l of active ingredient since 1967. Today temephos is an organophosphorus insecticide that has been used as larvicide against *Ae. aegypti*. Resistance is defined as the acquired ability of an insect population to tolerate doses of insecticide which can kill the majority of individual in a normal population of the same species⁽⁸⁾. The presence of resistance in the natural population is probably due to the impacts from insecticides used for mosquito control and agricultural practices⁽⁹⁾. Detection of resistance will help public health person-

nel to formulate appropriate steps to counter reductions in effectiveness of the control effort that may be accompanied with emerging problems of insecticide resistance. Furthermore, cross resistance or resistance as a result of agricultural uses of insecticides may expedite switching to an alternative method or insecticides for disease control. The results obtained from this research study can be applied to other regions with the same problem on control of *Ae. aegypti* larvae as an important part of the Dengue Hemorrhagic Fever control program.

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บทคัดย่อ ความต้านทานของลูกน้ำยุงลาย *Aedes aegypti* Linnaeus (Diptera: Culicidae) ต่อสารที่มีฟอสวาสนา สอนแท็ง*, สุภาณี พิมพ์สมาน*, สมศักดิ์ อรรถศิลป์**

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วารสารวิชาการสาธารณสุข 2552; 18:650-4.

ได้ทำการศึกษาความต้านทานของลูกน้ำยุงลาย (*Aedes aegypti* Linnaeus) ต่อสาร temephos โดยเตรียมสารละลาย temephos ที่ระดับความเข้มข้น 0.02 มิลลิกรัมต่อลิตร ทดสอบตามวิธีการที่เป็นมาตรฐานขององค์การอนามัยโลกกับลูกน้ำยุงลายที่เก็บจากเขตเทศบาลเมือง 7 จังหวัดในภาคตะวันออกเฉียงเหนือ ระหว่างเดือนเมษายนถึงเดือนมิถุนายน 2549 ผลการศึกษา พบว่า ลูกน้ำยุงลายสายพันธุ์อุบลราชธานี ศรีสะเกษ ยโสธร อำนาจเจริญ และกาฬสินธุ์มีความไวต่อสารเคมีในระดับสูง เมื่อนำลูกน้ำยุงลายระยะที่ 4 ทั้ง 7 สายพันธุ์ ทดสอบกับสารละลาย temephos ที่ระดับความเข้มข้นต่าง ๆ กัน เพื่อศึกษาค่า LC_{50} และ LC_{95} พบว่า ลูกน้ำยุงลายสายพันธุ์มุกดาหารมีค่า LC_{50} และ LC_{95} สูงที่สุดคือ 0.01154 และ 0.0296 มิลลิกรัมต่อลิตร ส่วนค่าความต้านทานต่อสาร temephos ของลูกน้ำยุงลายสายพันธุ์ภาคสนาม เปรียบเทียบกับลูกน้ำยุงลายสายพันธุ์มาตรฐาน พบว่า ที่ระดับ LC_{50} และ LC_{95} ลูกน้ำยุงลายสายพันธุ์มุกดาหารมีค่าความต้านทานสูงสุด คือ 12.68 และ 11.93 เท่า จึงสามารถสรุปได้ว่า ลูกน้ำยุงลายสายพันธุ์มุกดาหารมีความต้านทานต่อสาร temephos ดังนั้นจึงควรทำการศึกษาติดตามการต้านทานต่อสาร temephos ที่ใช้ในการควบคุมลูกน้ำยุงลายอย่างต่อเนื่อง ผลการศึกษาข้างต้นสามารถใช้เป็นข้อมูลพื้นฐานในการพิจารณาเลือกใช้สารเคมีกำจัดแมลงให้เหมาะสมตามสภาพพื้นที่ อันจะนำไปสู่การจัดการควบคุมโรคไข้เลือดออกที่มีประสิทธิภาพต่อไป

คำสำคัญ: ความต้านทาน, ที่มีฟอส, ยุงลาย