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ความต้านทานของลูกน้ำยุงลาย *Aedes aegypti* Linnaeus (Diptera: Culicidae) ต่อสารทีมีฟอส

Resistance to temephos of *Aedes aegypti* Linnaeus larvae (Diptera: Culicidae)

วาสนา สอนเพ็ง (Wasana Sornpeng)[1]
สุภาณี พิมพ์สมาน (Supanee Pimsamarn)[2]
สมศักดิ์ อรรฆศิลป (Somsak Akksilp)[3]

บทคัดย่อ

ได้ทำการศึกษาความต้านทานของลูกน้ำยุงลาย (*Aedes aegypti* Linnaeus) ต่อสาร temephos โดยเตรียมสารละลาย temephos ที่ระดับความเข้มข้น 0.02 มิลลิกรัมต่อลิตร ทดสอบตามวิธีการที่เป็นมาตรฐานขององค์การอนามัยโลกกับลูกน้ำยุงลายที่เก็บจากเขตเทศบาลเมือง 7 จังหวัดในภาคตะวันออกเฉียงเหนือระหว่างเดือนเมษายนถึงเดือนมิถุนายน 2549 ผลการศึกษา พบว่า ลูกน้ำยุงลายสายพันธุ์อุบลราชธานี ศรีสะเกษ ยโสธร อำนาจเจริญ และกาฬสินธุ์มีความไวต่อสารเคมีในระดับสูง เมื่อนำลูกน้ำยุงลายระยะที่ 4 ทั้ง 7 สายพันธุ์ ทดสอบกับสารละลาย temephos ที่ระดับความเข้มข้นต่างๆ กัน เพื่อศึกษาค่า LC_{50} และ LC_{95} พบว่า ลูกน้ำยุงลายสายพันธุ์มุกดาหารมีค่า LC_{50} และ LC_{95} สูงที่สุดคือ 0.011_{54} และ 0.02_{96} มิลลิกรัมต่อลิตร ส่วนค่าความต้านทานต่อสาร temephos ของลูกน้ำยุงลายสายพันธุ์ภาคสนาม เปรียบเทียบกับลูกน้ำยุงลายสายพันธุ์มาตรฐาน พบว่าที่ระดับ LC_{50} และ LC_{95} ลูกน้ำยุงลายสายพันธุ์มุกดาหารมีค่าความต้านทานสูงที่สุด คือ 12.68 และ 11.93 เท่า จึงสามารถสรุปได้ว่า ลูกน้ำยุงลายสายพันธุ์มุกดาหารมีความต้านทานต่อสาร temephos ดังนั้นจึงควรทำการศึกษาติดตามการต้านทานต่อสาร temephos ที่ใช้ในการควบคุมลูกน้ำยุงลายอย่างต่อเนื่อง ผลการศึกษาข้างต้นสามารถใช้เป็นข้อมูลพื้นฐานในการพิจารณาเลือกใช้สารเคมีกำจัดแมลงให้เหมาะสมตามสภาพพื้นที่ อันจะนำไปสู่การจัดการควบคุมโรคไข้เลือดออกที่มีประสิทธิภาพต่อไป

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 areas 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 from April-June 2006. The fourth larval stage was tested in 0.02 mg/l temephos solution as recommended by the World Health Organization as a standard method. The results

[1]*นักศึกษาระดับบัณฑิตศึกษา สาขาวิชากีฏวิทยา คณะเกษตรศาสตร์ มหาวิทยาลัยขอนแก่น*
[2]*รองศาสตราจารย์ ภาควิชาพืชศาสตร์และทรัพยากรการเกษตร คณะเกษตรศาสตร์ มหาวิทยาลัยขอนแก่น*
[3]*รองอธิบดี กรมควบคุมโรค กระทรวงสาธารณสุข*

demonstrate that Ubon Ratchathani, Si Sa Ket, Yasothon, Amnat Charoen, and Kalasin *Ae. aegypti* strains had mortality indicating susceptibility. When the fourth larval stage was selected for bioassay test, the LC_{50} and LC_{95} 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 was 12.68 and 11.93 fold for LC_{50} and LC_{95} respectively. The conclusion of this study is that Mukdahan *Ae. aegypti* larva 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.

คำสำคัญ: ลูกน้ำยุงลาย, ความต้านทาน, ทีมีฟอส

Keywords: *Aedes* larvae, resistance, temephos,

Introduction

Dengue Hemorrhagic Fever 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 the transmission of this viral disease (Nogueira et al., 1999). *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 the late afternoon. Although vaccination would be an ideal method to control DHF, development of vaccines for dengue viruses is 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 pyrethroid used to control adult *Aedes* mosquitoes through mass spraying (Swaddiwadhipong et al., 1992). Temephos is an organophosphorus insecticide that has been used as a larvicide against *Ae. aegypti* in Thailand since 1967 (Jurjevskis and Stiles, 1978). The widespread use of insecticides has led to insecticide resistance in mosquitoes that will be another problem for the ability to control disease (Robert and Andre, 1994). 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 areas of northeast provinces where high numbers of dengue hemorrhagic fever cases have been reported. These populations had prior history of exposure to temephos. This study is to characterize the resistance to temephos of *Ae. aegypti* larvae in seven provinces and to determine whether selection of temephos will result in tolerance or resistance in these populations. Such knowledge is essential in defining

future control strategies against this medically important mosquito.

Materials and methods

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 a 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 cm^3) containing about 1,500 ml of declorinated 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 procedure recommended by WHO (1963) was followed; 25 larvae in 249 ml of declorinated 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 (Abbott, 1925). Scores of mortality at different exposure concentration were used to further calculate lethal concentrations (LC_{50} and LC_{95}). The results were analyzed for the lethal concentration (LC_{50} and LC_{95}) by probit analysis (Finney, 1971). Resistance ratio (RR_{50}) was calculated by comparing LC_{50} and LC_{95} of each population with Bora Bora susceptible strain.

5. Analysis

- The WHO (1963) recommendation on the following is still valid:

98-100% mortality indicates susceptibility

80-97% mortality suggests the possibility of resistance that needs to be confirmed

<80% mortality suggests resistance

- LC_{50} Probit analysis (Finney, 1971)
- Resistance Ratio, RR

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

Results

The results of temephos bioassay on *Ae. aegypti* larvae from seven strains of each population are presented in Figure 1. The mortalities 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 LC_{50} 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 times the RR_{50}.

The Amnat Charoen strain had low resistance ratio with 4.37 times the RR_{50} compared with the susceptible strain.

The LC_{95} values of the field population are presented in Table 2. The present study indicates that only *Ae. aegypti* larva from Mukdahan had high resistance ratio with 11.93 times the RR_{95}. However, the Kalasin strain had low resistance ratio, the level of resistance being 5.84 times the RR_{95} 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 from 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 LC_{50} and LC_{95} of Mukdahan and Sakon Nakhon strains were the highest, with values of 0.01154, 0.0296 and 0.01448, 0.01548 mg/l respectively. Other strains showed low level of resistance to temephos except Mukdahan and Sakon Nakhon. However, when compared with the WHO standard susceptibility strain, the resistance of the Mukdahan strain was 12.68 and 11.93 times the LC_{50} and LC_{95}. Sakon Nakhon strains were 7.92 and 11.5 times the LC_{50} and LC_{95}, The conclusion of this study is that, Mukdahan and Sakon Nakhon strains can develop higher levels 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 is 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 individuals in a normal population of the same species (WHO, 1975). The presence of resistance in the natural population is probably due to the impact from insecticides used for mosquito control and agricultural practices (Paeporn et al., 2004). Detection of resistance will help public health personnel 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 require switching to an alternative method, or to insecticides for disease control. The results obtained from this research study can be applied to other regions with the same problems with control of *Ae. aegypti* larvae as an important part of the Dengue Hemorrhagic Fever control program.

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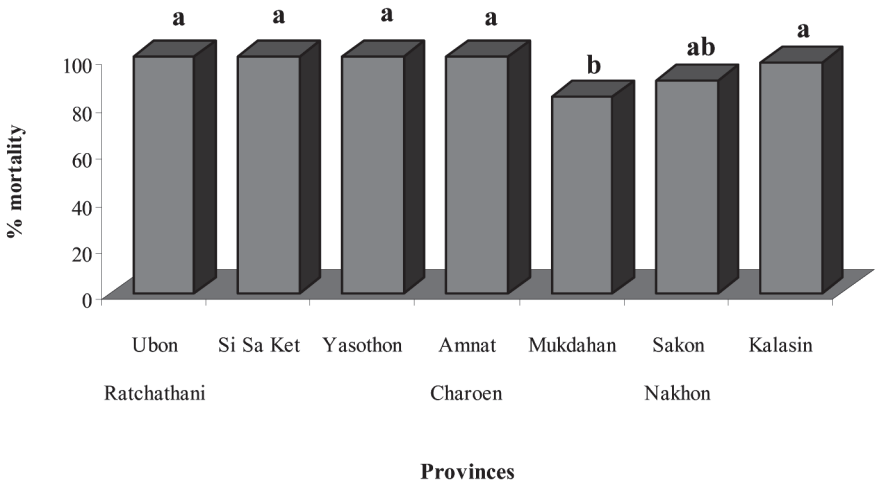

Figure 1. The mortality of temephos bioassay on *Aedes aegypti* Linnaeus larvae from seven provinces

Table 1. LC_{50} and RR_{50} by susceptibility of *Aedes aegypti* Linnaeus larva from each strain exposed to temephos for 24 hours.

***Aedes aegypti* 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	0.01154	12.68
Sakon Nakhon	0.00721	7.92
Kalasin	0.00402	4.42

*Susceptible strain from Mahidol University

Table 2. LC_{95} and RR_{95} by susceptibility of *Aedes aegypti* Linnaeus larvae from each strain exposed to temephos for 24 hours.

***Aedes aegypti* 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	0.01154	11.93
Sakon Nakhon	0.02960	11.50
Kalasin	.01448	5.84

*Susceptible strain from Mahidol University