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 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 cm\(^3\)) 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 (LC\(_{50}\) and LC\(_{95}\)). The results were analyzed for the lethal concentration by probit analysis\(^7\). 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 percent mortality indicates susceptibility, 80-97 percent mortality suggests the possibility of resistance that needs to be confirmed, <80 percent mortality suggests resistance.
- \( \text{LC}_{50} \) Probit analysis \(^{(7)}\)
- Resistance Ratio, RR
  \[ \text{RR} = \frac{\text{LC Resistance strain}}{\text{LC Susceptible strain}} \]

Results

The results of temephos bioassay on *Ae. aegypti* larvae from seven strains of each population are presented in Figure 1. The mortality of each strain on diagnostic concentration (0.02 mg/l) are shown. *Ae. aegypti* larvae that were collected from Ubon Ratathani, 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 \( \text{LC}_{50} \) 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 fold of the \( \text{RR}_{50} \). Amnat Charoen strain had low resistance ratio with 4.37 fold of the \( \text{RR}_{50} \) compared with the susceptible strain.

The \( \text{LC}_{95} \) values of the field population are presented in Table 2. The present study indicates that only *Ae. aegypti* larvae from Mukdahan had high resistance ratio with 11.93 fold of the \( \text{RR}_{95} \). Although the Kalasin strain had low resistance ratio the level of resistance was 5.84 fold the \( \text{RR}_{95} \) compared with the susceptible strain.

Discussion

This study was conducted to measure the level

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**Table 1** \( \text{LC}_{50} \) and \( \text{RR}_{50} \) by susceptibility of *Aedes aegypti* Linnaeus larvae from each strain exposed to temephos for 24 hours

<table>
<thead>
<tr>
<th><em>Aedes aegypti</em> Linnaeus</th>
<th>( \text{LC}_{50} ) (mg/l)</th>
<th>( \text{RR}_{50} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>larvae strain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bora Bora*</td>
<td>0.00091</td>
<td>1</td>
</tr>
<tr>
<td>Ubon Ratathani</td>
<td>0.00636</td>
<td>6.99</td>
</tr>
<tr>
<td>Si Sa Ket</td>
<td>0.00681</td>
<td>7.48</td>
</tr>
<tr>
<td>Yasothon</td>
<td>0.00496</td>
<td>5.45</td>
</tr>
<tr>
<td>Amnat Charoen</td>
<td>0.00396</td>
<td>4.37</td>
</tr>
<tr>
<td>Mukdahan</td>
<td>0.02960</td>
<td>12.68</td>
</tr>
<tr>
<td>Sakon Nakhon</td>
<td>0.02852</td>
<td>7.92</td>
</tr>
<tr>
<td>Kalasin</td>
<td>0.00402</td>
<td>4.42</td>
</tr>
</tbody>
</table>

*Susceptible strain from Mahidol University*

**Table 2** \( \text{LC}_{95} \) and \( \text{RR}_{95} \) by susceptibility of *Aedes aegypti* Linnaeus larvae from each strain exposed to temephos for 24 hours

<table>
<thead>
<tr>
<th><em>Aedes aegypti</em> Linnaeus</th>
<th>( \text{LC}_{95} ) (mg/l)</th>
<th>( \text{RR}_{95} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>larvae strain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bora Bora*</td>
<td>0.00248</td>
<td>1</td>
</tr>
<tr>
<td>Ubon Ratathani</td>
<td>0.01629</td>
<td>6.57</td>
</tr>
<tr>
<td>Si Sa Ket</td>
<td>0.02480</td>
<td>10.00</td>
</tr>
<tr>
<td>Yasothon</td>
<td>0.01793</td>
<td>7.22</td>
</tr>
<tr>
<td>Amnat Charoen</td>
<td>0.01548</td>
<td>6.24</td>
</tr>
<tr>
<td>Mukdahan</td>
<td>0.02960</td>
<td>11.93</td>
</tr>
<tr>
<td>Sakon Nakhon</td>
<td>0.02852</td>
<td>11.50</td>
</tr>
<tr>
<td>Kalasin</td>
<td>0.01448</td>
<td>5.84</td>
</tr>
</tbody>
</table>

*Susceptible strain from Mahidol University*
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 LC$_{50}$ of Mukdahan was the highest, with value of 0.01154 and LC$_{50}$ 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 LC$_{50}$ 12.68 fold and LC$_{95}$ 11.93 fold. Sakon Nakhon strains were LC$_{50}$ 7.92 fold and LC$_{95}$ 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 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 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.

References

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

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654

นั้นยื่นขอ ความค้นหาของอุกศัตรู Aedes aegypti Linnaeus (Diptera: Culicidae) ต่อสารที่มีผล

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อุบะราชานิ

วารสารวิทยาศาสตร์สุขภาพ 2552; 18:650-4.

ได้ทำการศึกษาความค้นหาของอุกศัตรู Aedes aegypti Linnaeus) ต่อสาร temephos โดย

เตรียมสารละลาย temephos ที่ระดับความเข้มข้น 0.02 มิลลิกรัมต่อตัว ทดสอบความรับรู้ที่เป็นมาสรูป

ของอุกศัตรูน้ำตกไทยถึงจำเข้าถึงหลายเมื่อ 7 จุดหวังในภาคตะวันออกเฉียงเหนือ

ระหว่างเดือนเมษายนเดือนมิถุนายน 2549 ผลการศึกษา พบว่า ลูกน้ำยุงลายผสมที่อุบะราชานิ ศึกษาระบบ

ออกמכון อ่านแปลง และผลสัมพันธ์มีความใกล้เคียงเสถียรมีในระดับสูง หมู่น้ำยุงลายระยะที่ 4 ถึง 7 สาย

พันธุ์ ทดสอบกับสารละลาย temephos ที่ระดับความเข้มข้นต่ำ ๆ กัน เพื่อศึกษาที่ LC50 และ LC50 พบว่า

ลูกน้ำยุงลายผสมที่มีความเข้มข้น LC50 และ LC50 สูงที่สุดคือ 0.01154 และ 0.0296 มิลลิกรัมต่อตัว ด้วย

ความค้นหาของสาร temephos ของลูกน้ำยุงลายผสมที่มีการลงมัน เกี่ยวข้องกับลูกน้ำยุงลายผสมที่มี

ผลกระทบ พบว่า ที่ระดับ LC50 และ LC50 ลูกน้ำยุงลายผสมมีความค้นหาที่สุด คือ 12.68

และ 11.93 เท่า จึงสามารถสรุปได้ว่า ลูกน้ำยุงลายผสมมีความค้นหาที่ต่ำสุดต่อสาร temephos ดังนี้

เนื่องจากสารบนเทคนิคการควบคุมกลุ่มต่อสาร temephos ที่ใช้ในการควบคุมลูกน้ำยุงลายของต้องเนื่อง แต่

การศึกษาข้างต้นนี้สามารถใช้เป็นข้อมูลพื้นฐานในการพิจารณาเลือกใช้สารเคมีที่จัดแฉกรังให้เหมาะสมตาม

สถานพื้นที่ อันจะนำไปสู่การจัดการควบคุมโรคได้ผลดีขึ้นที่มีประสิทธิภาพต่อไป

ผู้ที่เขียน: ความค้นหา, หันเนียร, ยุงลาย