Efficacy and Longevity of a New Formulation of Temephos Larvicide Tested in Village-Scale Trials against *Aedes aegypti* Larvae in Water-Storage Containers

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**Abstract**

Field trials on the initial and long-term efficacy of a new formulation of temephos granules (1% on zeolite) applied at 1 ppm AI were conducted in water-storage containers against *Aedes aegypti* in 3 villages in the Kanchanaburi Province in Thailand. A total of 316 water-storage containers of various types and sizes were included in the study. In the initial survey, we found that some containers were positive for *Ae. aegypti* larvae, while others were devoid of larvae prior to the initiation of treatments. The containers were all numbered with paint and divided into 4 groups: with larvae and treated, without larvae and treated, with larvae untreated, and without larvae and untreated. Assessment of larval abundance was made 48 hrs post-treatment and monthly thereafter for 5 months. Containers with larvae and treated, exhibited almost complete absence of *Ae. aegypti* larvae for 2 months, but a small proportion became positive after 3 months. Most of these positive containers were devoid of zeolite granules which are visible in the containers. The number of positive containers increased in month 4 and 5, despite the fact that residues of temephos granules were present in some of the positive containers. The containers group without larvae (initially) and temephos treated, essentially were devoid of larvae for 2 months. After 3, 4 and 5 months about 6-23% of the containers became positive despite the fact that some had visible amounts of temephos granules. In the two control groups with larvae and no larvae initially, there was sustained and consistent production of larvae. Even in the group without initial larval population, the containers became positive for larvae one month after start of the experiment. The positivity rate increased as the trial proceeded. From these studies it can be concluded that a single application of temephos zeolite granules
at 1 ppm AI can provide highly satisfactory control of *Aedes aegypti* larvae in water-storage containers for period of at least 3 months in the field under normal water use practices.

**Keywords**

*Aedes aegypti*, mosquito control, village trials, temephos, zeolite granules

**Introduction**

Dengue haemorrhagic fever (DHF) has been reported in Thailand since the late 1950s (Hammon et al. 1960, Halstead 1966, Ungchusak and Kunasol 1988) and since then it has become one of the major public health problems of the country. The incidence of the disease has been increasing with cyclic outbreaks occurring every 2-3 years. Although the incidence of DHF has been increasing over the past decades, case fatality rate (CFR) has decreased from 10% in the late 1950s to about 0.7% in the late 1980s (Ungchusak and Kunasol 1988). The most severe outbreak of DHF occurred in 1987 with 174,285 reported cases and 1007 deaths (Gratz 1993) in Thailand. Since the turn of the 21st century, incidence of DHF has been high, estimated at 100,000 reported cases each year with CFR of about 1% or less. DHF is caused by dengue viruses (Hammon et al. 1960), which were isolated from *Aedes* mosquitoes in Thailand: *Aedes aegypti* (L.) and *Ae. albopictus* Skuse (Thavara et al. 1996). Scanlon (1965) reported that the first record of *Ae. aegypti* in Thailand was published by Theobald in 1907, and thereafter the mosquito was found in various places of the country as reported by several researchers. It is believed that *Ae. aegypti* now exists in almost every village of Thailand. It occurs in urban, suburban and rural areas of the country where ample developmental sites are present. At the present time there is no effective vaccine available for DHF, and the disease control therefore relies mainly on the control of mosquito vectors. The two main approaches used against *Ae. aegypti* control in Thailand are larviciding and adulticiding. Larval control by larvicide applications and source reduction of mosquito breeding sites is primarily measures relied upon and used routinely, whereas the adult control by space spraying of adulticides is usually carried out as emergency measures for suppressing vector populations during epidemic outbreaks of DHF. Abate (temephos SG 1%) was tested as a larvicide for the control of *Ae. aegypti* in Thailand in water-storage containers in early 1970s (Bang and Pant 1972, Bang et al. 1972), and since then temephos
sand granules have been used in DHF vector control program. The temephos sand granules showed good initial and residual larvicidal efficacy against *Ae. aegypti* larvae in water-storage containers; however, this formulation due to its unpleasant odor has faced major obstacles from the villagers for use in potable and daily-used water (Phanthumachinda et al. 1985, Thavara et al. 2001). The development of a temephos granular larvicide formulation lacking the unpleasant odor poses a challenge to researchers who deal with *Ae. aegypti* control. Mulla et al. (2004) evaluated efficacy of a newly developed temephos zeolite granules, against *Ae aegypti* larvae in Thailand, and found that the formulation possessed high initial and residual efficacy against the larvae for over 6 months under the experimental conditions. This formulation lacks the unpleasant odor when added to water-storage containers breeding *Ae. aegypti* and has the additional advantage of rendering water less turbid. The present study was carried out to evaluate the field efficacy of this new formulation of temephos larvicide in village trials against *Ae. aegypti* larvae in water-storage containers. A large number of water-storage containers were treated for the purpose of determining its efficacy under normal water-use conditions. It is hoped that this formulation will provide a new acceptable alternative for use of temephos by villagers. The acceptance of this larvicide for use in water-storage containers by villagers was then investigated.

**Materials and methods**

**Test material**

The new temephos zeolite formulation AZAI-SS (provided by Ikari Trading Co., Ltd. Bangkok, Thailand) was evaluated for larvicidal efficacy and longevity under normal village conditions in this study. This product contains temephos 1% (w/w) and the inert mineral clinoptilolite (zeolite) 99% (w/w). This product was used at the dosage of 10 g per 100 L of water, yielding 1 ppm of temephos AI in each container, a concentration that is currently used in national *Aedes aegypti* control program in Thailand.

**Study sites**

Field evaluation studies were carried out in 3 villages namely Sahakornnikom, Ongthi and Hindad of the Thong Pha Phum District of Kanchanaburi Province, Thailand. This district lies in western region of Thailand.
and is about 240 Km from Bangkok, and each village is approximately 5-10 Km away from each other. The 3 villages were chosen as the study sites due to the historical background of absence of any chemical larvicides used in water-storage containers for at least 1 year. A total of 103 houses (30, 31 and 42 from each village, respectively) in the district were selected for this evaluation which was based on prevalence of *Ae. aegypti* larvae in water-storage containers. Most houses are single-storey residences, each having several water-storage containers of various types and capacities placed both inside and outside the houses. The containers included glazed clay jars (50-200 L), mega cement jars (1,500-2,000 L), plastic pails and metal drums (100-200 L) and concrete tanks in bathrooms (50-1,000 L), all of which support *Ae. aegypti* production.

**Field evaluation procedures**

To gather baseline data, visual larval surveys were carried out in the study sites to estimate prevalence of *Ae. aegypti* larvae in water-storage containers prior to the initiation of the study. Visual assessments were made by experienced entomologists and survey team. The number of larvae in each container was estimated roughly and scored in the following categories: 0, 1+, 2+, 3+ and 4+, where the number of larvae estimated in each container were 0, 1-10, 11-30, 31-100 and >100, respectively. Based on larval presence or absence, the water-storage containers in this study were categorized into 4 groups: T-1 containers with larvae and treated, T-2 containers without larvae and treated, C-1 containers with larvae and not treated, and C-2 containers without larvae and not treated. T1 and T2 constituted treatments with temephos zeolite granules while C-1 and C-2 were controls. Each container was estimated for water capacity in order to administer the product at the designated concentration of 1 ppm of temephos. For example, glazed clay jars (200 L) and mega concrete jars (1,500 L) were treated with 20 g and 150 g of the product, respectively. All of the 4 groups (T-1, T-2, C-1 and C-2) of water-storage containers were assigned randomly to the various treatments in most houses. It was noted that most houses in this study contained all 4 groups of containers in the same house, whereas the rest had at least 2 groups on the same premises. Prior to treatment, each water-storage container was inspected for larvae, scored and larval presence and abundance recorded and treated (T-1 and T-2 groups only). In addition, each container was numbered and marked with permanent-color
paint spray on its side for subsequent follow-up surveys. This method of marking guaranteed that the same containers are inspected each time in the future assessment of larval absence or presence. Treatments were made only once in T-1 and T-2 groups of containers after the first survey. Assessments were carried out by larval inspection, scoring, and recording results at 48 hours post-treatment and at monthly intervals thereafter. Assessment of efficacy and control was carried out by inspecting the containers and categorizing the larval abundance for 5 months. Attitude of residents for larvicide application was investigated by interviewing one member of each treated house.

**Results**

At the outset, a total of 357 containers were inspected on the first visit when the study was initiated. The number of containers assigned to T-1, T-2, C-1 and C-2 groups were 147, 70, 70 and 70, respectively. However, the numbers of inspected containers decreased during the course of this study because in some containers all water was used up and were dried up, turned upside down, some were broken and some had disappeared or used for other purposes. As a result of this attrition, only 316 containers (i.e. 129, 61, 62 and 64 for T-1, T-2, C-1 and C-2, respectively) were thoroughly and repeatedly inspected and included in the study for larval absence or presence, and data gathering during the 5 months period post-treatment. The containers included in this study were of different types and sizes, but the majority of them were glazed clay jars, mega cement jars (Figure 1), concrete tanks and metal drums (Table 1). Overall the plastic pails represented the smallest proportion of the containers in the villages included in this study. It was noted that the glazed clay jars, especially 200-L in capacity with and without larval infestations were the most commonly used water-storage containers in this study. Several researchers in Thailand also reported similar results relating to the type of water-storage containers used and infested with Aedes larvae (Kittayapong and Strickman 1993, Jamulitrat et al. 1998, Thavara et al. 2001). In many areas of Thailand, we have noted that the glazed clay jars are commonly used as water-storage containers for drinking as well as daily-use water. The glazed clay and mega cement jars are therefore important target containers, which should be focused on for treatment when larval control programs against DHF vectors are carried out.
Table 1. Types and proportion of water-storage containers in the 4 designated groups (total 316 containers) that were inspected for data gathering in 3 villages, Kanchanaburi, Thailand.

<table>
<thead>
<tr>
<th>Group and larval occurrence</th>
<th>No. of containers</th>
<th>Glazed clay jars (%)</th>
<th>Mega cement jars (%)</th>
<th>Concrete tanks (%)</th>
<th>Metal drums (%)</th>
<th>Plastic pails (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-1 +ve treated</td>
<td>129</td>
<td>56.5</td>
<td>15.5</td>
<td>16.3</td>
<td>7.8</td>
<td>3.9</td>
</tr>
<tr>
<td>T-2 -ve treated</td>
<td>61</td>
<td>63.9</td>
<td>1.6</td>
<td>11.5</td>
<td>19.7</td>
<td>3.3</td>
</tr>
<tr>
<td>C-1 +ve no treatment</td>
<td>62</td>
<td>50.0</td>
<td>14.5</td>
<td>4.9</td>
<td>29.0</td>
<td>1.6</td>
</tr>
<tr>
<td>C-2 -ve no treatment</td>
<td>64</td>
<td>62.5</td>
<td>17.2</td>
<td>10.9</td>
<td>7.8</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Post-treatment larval prevalence in the containers that were positive for larvae at the start and treated with temephos zeolite granules (T-1 group) is shown in Table 2. All of the treated containers had no larvae at 48 hours, 1 month and 2 months post-treatment with the exception of 1 container (plastic pail) becoming positive with few larvae 2 months post-treatment. The one treated container that became positive for larvae two months after treatment had no visible traces of temephos zeolite granules, apparently the container had been drained, cleaned and refilled. The temephos zeolite granules leave bright green residues at the bottom and if present were easily visible in each container. At the 3-month observation the numbers of larva-free containers were 78% but declined some more during the experimental period. By the end of the 4 and 5-month period larval free containers dropped to 71% with 29% positive for larvae. Heavy larval infestation (category 2, 3, 4) was noted in 18 to 24% of the containers 3 to 5 months after treatment. The proportion of treated jars becoming positive for larvae (in all categories 1, 2, 3, 4) ranged from 22 to 29% after 3, 4 and 5 months post-treatment. It was noted that no granular material was visible in most of the containers that had become positive for larvae during the 3 months post-treatment. We noted that these containers were emptied, cleaned and refilled with new water, and the treatment materials were entirely washed out during the cleaning process. These results imply that the treatment could be effective for larval control for longer periods had the material not been washed out. In the last two assessments (4 and 5 months post-treatment), some containers that had visible amounts of granules were also positive for larvae. This indicates that the formulation even though not washed out in those containers had lost activity by the 4th and 5th months.
probably due to heavy water use and refilling resulting in dilution. This scenario implies that temephos zeolite granules are highly efficacious under normal water use conditions for about 3 months. One other important thing we noted was that all of the containers having material and becoming positive for larvae were always those on the outside and exposed to sunlight most of the day, or they had received windborne organic debris, dry grass or plant roofing materials. It is possible that temperature, organic debris and UV light from sunlight degraded the active compound rather rapidly. Coupled with these factors, rapid water use, draining and refilling are practices which get rid of the granules and thus shorten the residual efficacy of larvicidal formulations. It is likely that larvicidal formulations will last longer in mega jars (Figure 1) which store water for longer periods. These jars are drained and washed infrequently, water is drained off through a faucet set 10-15 cm above the bottom and water is added to these jars from the top.

Figure 1. Typical cement and earthen jars used in the study area for water use and storage. Two of the jars to the right are large (2000 L), while 3 jars to the left are small (150 L). The mega jars used for long-term water storage, while the small ones are refilled from the large ones for daily water use. Note the trough for carrying rain water from the roof gutter into the mega jars.
The larval prevalence in the containers that were negative for larvae at the start and were treated with temephos zeolite granules (T-2 group) is presented in Table 2. The purpose of this treatment was to see as to how long temephos zeolite granules at operational dose would prevent larval appearance in the larval-negative containers. The results reveal that no container became positive for larvae at 48 hours post-treatment and only 2 containers were found with low number of larvae 1 month post-treatment. At two months after treatment the number of positive containers amounted to only 6%, increasing to 23% at month 4 and going down to 8% positive 5 months post-treatment. No applied granules were visually seen in the positive containers 3 months post-treatment, but the granules were found in some larval-positive containers on 4- and 5-months post-treatment. It was also noted that some of the containers that had visible amounts of granules became positive for larvae during the 4- and 5-month post-treatment period. This study shows that temephos zeolite formulation is effective in preventing larval occurrence for at least 3 months after application in most of the containers where the material still remained. This experiment lends support to the longevity of temephos zeolite granules tested in T-1 group, which lasted for about 3 months.

In contrast to the treated containers (see Tables 2), containers with larvae at the start and not treated supported sustained and constant presence of larvae. The larval prevalence in these containers (C-1 group) is shown in Table 2. This group of containers was used as the control group to see how larval positivity will progress over time. These containers, 62 in total, experienced natural fluctuations in larval prevalence during the study period. It was found that the numbers of containers positive for larvae at any evaluation inspection fluctuated between 60 and 71% positivity for larvae. This experiment documented that larval populations of *Ae. aegypti* without intervention are always prevailing in most of these containers. The abundance and constant presence of larvae in productive containers provide a sound basis for comparison of larval populations with the treated containers.

Larval prevalence in the containers that were negative for larvae at the start and also were not treated with temephos (C-2 group) is presented in Table 2. The data shows that some of the larval negative containers do become positive for larvae in due time. The data show that the number of containers becoming positive for larvae increased dramatically from 0% at the first 48-hr evaluation to 22% (one month) and to 37% (2 months), reaching the peak of 55% positive
at the fourth evaluation (3 months post-treatment), and then declining to 41% (4 month) and to 36% at the last evaluation (5 months post-treatment). These trends reveal that larval population of *Ae. aegypti* appeared naturally in the initially negative containers and prevailed at relatively high frequency over the course of this study.

Table 2. Larval prevalence (% positive) in 4 groups of containers (with or without larvae initially) treated or not treated with temephos zeolite (1%) granules at 1 mg/L AI, Kanchanaburi, Thailand.

<table>
<thead>
<tr>
<th>Prevalence score*</th>
<th>Larval presence and abundance in containers positive (%) post-treatment (month)</th>
<th>T-1 (120 containers), positive for larvae initially and treated</th>
<th>T-2 (61 containers), negative for larvae initially and treated</th>
<th>C-1 (62 containers), positive for larvae initially and not treated</th>
<th>C-2 (64 containers), negative for larvae initially and not treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-treatment 48 hrs 1 month 2 month 3 month 4 month 5 month</td>
<td>Pre-treatment 48 hrs 1 month 2 month 3 month 4 month 5 month</td>
<td>Pre-treatment 48 hrs 1 month 2 month 3 month 4 month 5 month</td>
<td>Pre-treatment 48 hrs 1 month 2 month 3 month 4 month 5 month</td>
<td>Pre-treatment 48 hrs 1 month 2 month 3 month 4 month 5 month</td>
</tr>
<tr>
<td>0</td>
<td>0 100 100 99.2 77.5 70.5 70.5</td>
<td>100 100 96.7 93.5 83.6 77 91.8</td>
<td>0 100 100 78 62.5 45.3 59.3 64.1</td>
<td>0 100 100 78 62.5 45.3 59.3 64.1</td>
<td>0 100 100 78 62.5 45.3 59.3 64.1</td>
</tr>
<tr>
<td>1+</td>
<td>6.2 0 0 0.8 4.7 5.4 9.3</td>
<td>0 0 3.3 1.6 4.9 3.3 3.3</td>
<td>6.4 6.4 12.9 19.4 17.7 17.7 14.5</td>
<td>6.4 6.4 12.9 19.4 17.7 17.7 14.5</td>
<td>6.4 6.4 12.9 19.4 17.7 17.7 14.5</td>
</tr>
<tr>
<td>2+</td>
<td>19.4 0 0 0 3.1 7.8 3.9</td>
<td>0 0 0 3.3 3.3 8.2 0</td>
<td>22.6 22.6 11.3 9.7 12.9 12.9 17.7</td>
<td>22.6 22.6 11.3 9.7 12.9 12.9 17.7</td>
<td>22.6 22.6 11.3 9.7 12.9 12.9 17.7</td>
</tr>
<tr>
<td>3+</td>
<td>48.8 0 0 0 3.9 2.3 10.1</td>
<td>0 0 0 0 3.3 1.6 3.3</td>
<td>35.5 35.5 8.1 12.9 12.9 14.5 16.1</td>
<td>35.5 35.5 8.1 12.9 12.9 14.5 16.1</td>
<td>35.5 35.5 8.1 12.9 12.9 14.5 16.1</td>
</tr>
<tr>
<td>4+</td>
<td>25.6 0 0 0 10.8 14.0 6.2</td>
<td>0 0 0 0 3.3 8.2 0</td>
<td>35.5 35.5 30.6 29 27.4 22.6 11.3</td>
<td>35.5 35.5 30.6 29 27.4 22.6 11.3</td>
<td>35.5 35.5 30.6 29 27.4 22.6 11.3</td>
</tr>
</tbody>
</table>

* 0 without larvae, + with larvae at different densities: 0 = 0; 1 = 1-10; 2 = 11-30; 3 = 31-100; and 4 = >100 larvae/container
Acceptability

In order to gauge the level of resistance or acceptability to the use of temephos granules in domestic water-storage containers by the residents, we interviewed 96 residents in the test area in Kanchanaburi. Of these, 89% did not use any larvicide in their water containers, even though, it was supplied by the local health authorities. These individuals objected to the use of currently used temephos sand granules (1%) in water containers, due to unpleasant odor of the formulation and secondly because of increase in water turbidity which they associated with the formulation. A small percentage (11%), however, used temephos granules but only occasionally, again being reluctant to use the treatment due to odor, water turbidity and safety considerations. When questioned regarding the use of temephos zeolite granules with lack of odor and water turbidity, the respondents indicated their willingness to employ safe and odorless formulations in their water supplies. Such objections to the use of temephos sand granules which has odor were also reported by Phanthumachinda et al. (1985) and Thavara et al. (2001). During the current experiment, the residents acknowledged marked reduction in the abundance and biting activity of adult mosquitoes and they showed eagerness to start treatments if a safe and odorless product is made available to them.

Discussion

At present, there are 3 major problems regarding the use of larvicides for the control of *Ae. aegypti* in water-storage containers in Thailand and other dengue-endemic areas. These are larvicide formulation characteristics, water-consumption styles of the dwellers and insufficient provision of larvicide formulation by government agencies. Firstly, the current larvicide used for *Ae. aegypti* larval control in Thailand is temephos sand granules (1%) which provides high degree of control, but it possesses unpleasant odor when applied to water and renders water more turbid. These drawbacks are not accepted by many dwellers and they usually refuse to use the larvicide as reported by Phanthumachinda et al. (1985) and Thavara et al. (2001). Secondly, with regard to water-consumption styles of villagers, it is found that the people always keep water for drinking and daily use in various kinds of containers, such as jars, tanks, drums, pails, etc., with capacity ranging from 50 to 2,000 L. The main sources of water are from rain, wells, canals, rivers and piped-water supply.
In most rural areas of Thailand people stock water because of drought, especially in dry season but in many urbanized areas having piped-water supply, people still keep water in their water-storage containers for 2 reasons: irregular water supply and traditional styles of water usage, most people preferring rain water over other supplies. The vast numbers of water-storage containers in use constitute major breeding sources of *Ae. aegypti*. From our observations, it was noted that many containers (mostly < 200 L in capacity) were frequently washed and cleaned up and refilled with new water, losing the granular materials applied. As shown in Table 2 some containers treated with larvicidal granules later were noted to be devoid of applied material and became positive for mosquito larvae. This practice of washing, cleaning and refilling reduces the residual efficacy of treatments. Under controlled experimental conditions, Mulla et al. (2004) demonstrated that 2 temephos formulations (sand and zeolite granules) at the rate of 1 ppm AI with 3 water regimens, full, full 1/2 removed and refilled and 1/2 full were equal in efficacy yielding almost 100% control of *Ae. aegypti* for over 6 months. In this experiment the applied materials remained in jars for the duration of the experiment. Moreover, the water-storage jars in that experiment were covered and located in shade under a roof, keeping light out. Apparently normal water-use practices as noted in the trial villages culminate in decreased residual activity. A number of environmental factors also influence residual activity in the containers.

Finally, we have noted that governmental agencies responsible for distributing larvicidal materials to the public for *Ae. aegypti* control, deliver quantities insufficient for treatment of all larval sources. As a rule a family receives only 20-40 grams of larvicide once or twice a year from the local health station. This amount is grossly insufficient to treat all water-storage containers, as there are at least 4-5 containers having various capacities (50-2,000 L) in each house. Not having sufficient quantities of larvicide, we noted that some families declined from using the larvicide provided in sachets. Due to the large number of water-storage containers in Thailand, requiring a large amount of larvicide, financial resources are inadequate to supply the needed quantities. Because of the limited government budget to provide larvicides to most of the country each year, it is desirable to supervise larval control program and to identify key infested containers to be treated routinely in a community based vector control program. At the present time, application of larvicides to
control Ae. aegypti larvae in water-storage containers is the most appropriate and effective measure. Implementing integrated control technology employing larvicides, larvivorous fish (Wu et al. 1987, Wang et al. 2000) and mosquito proof covers will yield sustainable control especially in mega jars used for long-term water-storage.

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