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EFFICACY AND LONGEVITY OF A NEW FORMULATION OF TEMEPHOS LARVICIDE TESTED IN VILLAGE-SCALE TRIALS AGAINST LARVAL *AEDES AEGYPTI* 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 active ingredient (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 larval *Ae. aegypti,* whereas others were devoid of larvae before the initiation of treatments. The containers all were 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 h after treatment and monthly thereafter for 5 months. Containers with larvae and that were treated exhibited almost complete absence of larval *Ae. aegypti* 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 months 4 and 5, despite the fact that residues of temephos granules were present in some of the larvae-positive containers. The containers initially without larvae and treated with temephos 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 2 control groups initially with and without larvae, sustained and consistent production of larvae occurred. Even in the group initially without a larval population, the containers became positive for larvae 1 month after the start of the experiment and the positivity rate increased as the trial progressed. From these studies, the conclusion can be made that a single application of temephos zeolite granules at 1 ppm AI can provide highly satisfactory control of larval *Ae. aegypti* in water-storage containers for at least 3 months in the field under normal water-use practices.

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

INTRODUCTION

Dengue hemorrhagic 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, the 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 1,007 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 a CFR of about 1% or less. Dengue hemorrhagic fever is caused by dengue viruses (Hammon et al. 1960), which were isolated from *Aedes* mosquitoes in Thailand, including *Aedes aegypti* (L.) and *Ae. albopictus* Skuse (Thavara et al. 1996). Scanlon (1965) reported that the 1st 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. *Aedes aegypti* is now believed to exist in almost every village of Thailand. The species occurs in urban, suburban, and rural areas of the country where ample developmental sites are present. At the present time no effective vaccine is available for DHF, and therefore control of the disease relies mainly on the control of mosquito vectors. The 2 main approaches used for control of *Ae. aegypti* in Thailand are larviciding and adulticiding. Larval control by larvicidal applications and source reduction of mosquito breeding sites are primarily relied upon and used routinely, whereas the adult control by space spraying of adulticides usually is carried out as an emergency measure for suppressing vector populations during epidemic outbreaks of DHF. Abate (temephos sand granules 1%) was tested as a larvicide for the control of *Ae. aegypti* in Thailand in water-storage containers in the early 1970s (Bang and Pant 1972, Bang et al. 1972), and since then temephos sand granules have been used in DHF vector control programs. The temephos sand granules showed good initial and residual larvicidal efficacy against larval *Ae. aegypti* in water-storage containers; however, because of its unpleasant odor, this formulation has faced major obstacles from the villagers for use in potable and daily-use water (Phanthumachinda et al. 1985, Thavara et al. 2001). The development of a temephos granular

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larvicide formulation lacking the unpleasant odor poses a challenge to researchers who deal with control of *Ae. aegypti*. Mulla et al. (2004) evaluated efficacy of a newly developed temephos zeolite granule formulation against larval *Ae. aegypti* in Thailand, and found that the formulation possessed high initial and residual efficacy against the larvae for more than 6 months under experimental conditions in simulated field tests. This formulation lacks the unpleasant odor when added to water-storage containers where *Ae. aegypti* breeds 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 larval *Ae. aegypti* in water-storage containers. A large number of water-storage containers were treated for the purpose of determining the efficacy of this formulation 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 also was investigated.

MATERIALS AND METHOD

Test material: The new temephos zeolite formulation AZAI-SS (manufactured 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 liters of water, yielding 1 ppm of temephos active ingredient in each container, a concentration that currently is used in the national control program for *Ae. aegypti* in Thailand.

Study sites: Field evaluation studies were carried out in 3 villages (Sahakornnikom, Ongthi, and Hindad) of the Thongphaphum District of Kanchanaburi Province, Thailand. This district lies in western Thailand and is about 240 km from Bangkok, and the test villages are approximately 5–10 km away from each other. The 3 villages were chosen as the study sites because of the historical background of absence of use of any chemical larvicides 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 the estimate of prevalence of larval *Ae. aegypti* in water-storage containers. Most houses are single-storied residences, with 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 liters), large cement jars (1,500–2,000 liters), plastic pails and metal drums (100–200 liters), and concrete tanks in bathrooms (50–1,000 liters), all of which support production of *Ae. aegypti*.

Field evaluation procedures: To gather baseline data, visual larval surveys were carried out in the study sites to estimate prevalence of larval *Ae. aegypti* in water-storage containers before the initiation of the study. Visual assessments were made by experienced entomologists and the 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. The treatments with temephos zeolite granules were denoted as T-1 and T-2, whereas C-1 and C-2 were controls. The capacity of each container was estimated in order to administer the product at the designated concentration of 1 ppm of temephos based on the total volume. For example, glazed clay jars (200 liters) and large cement jars (1,500 liters) 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. Most houses in this study were noted to contain all 4 groups of containers in the same house, whereas the others had at least 2 groups on the same premises. Before treatment, each water-storage container was inspected for larvae, scored, larval presence and abundance were recorded, and the container was 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 would be 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 1st survey. Assessments were carried out by larval inspection, scoring, and recording the results at 48 h after 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 and acceptability of larvicide application were investigated by interviewing 1 member of each house where containers were treated.

RESULTS

At the outset, 357 containers were inspected on the 1st visit when the study was initiated. The numbers 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 de-

Fig. 1. Typical cement (large) and earthen (small) jars used in the study area for water use and storage. The 2 jars to the right are large (2,000 liters) and the 3 jars to the left are small (150 liters). The large jars are used for long-term water storage, whereas the small jars are refilled from the large ones for daily water use. Note the trough for carrying rainwater from the roof gutter into the large jars.

creased during the course of this study because all the water was used in some containers and they were dry, some containers were turned upside down, some were broken, and some had disappeared or were 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-month period after treatment. The containers included in this study were of different types and sizes, but the majority of them were glazed clay jars, large cement jars (Fig. 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 glazed clay jars, especially those 200 liters in capacity with and without larval infestations, were the most commonly used water-storage containers in this study. Several researchers in Thailand also have reported similar results relating to the type of water-storage containers used and infested with larval *Aedes* (Kittayapong and Strickman 1993, Jamulitrat et al. 1998, Thavara et al. 2001). In many areas of Thailand, we have noted that glazed clay jars are commonly used as water-storage containers for drinking as well as daily-use water. Therefore, glazed clay

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 in Kanchanaburi Province, Thailand.

Group and larval occurrence	No. containers	Type of container (%)				
		Glazed clay jars	Large cement jars	Concrete tanks	Metal drums	Plastic pails
T-1, positive and treated	129	56.5	15.5	16.3	7.8	3.9
T-2, negative and treated	61	63.9	1.6	11.5	19.7	3.3
C-1, positive with no treatment	62	50.0	14.5	4.9	29.0	1.6
C-2, negative with no treatment	64	62.5	17.2	10.9	7.8	1.6

Table 2. Frequency of containers (% positive) in 4 groups of containers (with or without larvae initially) treated or not treated with temephos zeolite (1%) granules at 1 mg of active ingredient per liter in Kanchanaburi Province, Thailand.

Larval prevalence category[1]	Distribution of containers (%)[2] for larval abundance categories before and after treatment (months)						
	Pretreatment	48 h	1 month	2 months	3 months	4 months	5 months
	Treated-1 (129 containers), positive for larvae initially and treated						
0	0	100	100	99.2	77.5	70.5	70.5
1+	6.2	0	0	0.8	4.7	5.4	9.3
2+	19.4	0	0	0	3.1	7.8	3.9
3+	48.8	0	0	0	3.9	2.3	10.1
4+	25.6	0	0	0	10.8	14.0	6.2
	Treated-2 (61 containers), negative for larvae initially and treated						
0	100	100	96.7	93.5	83.6	77	91.8
1+	0	0	3.3	1.6	4.9	3.3	3.3
2+	0	0	0	3.3	3.3	8.2	0
3+	0	0	0	0	4.9	3.3	1.6
4+	0	0	0	1.6	3.3	8.2	3.3
	Control-1 (62 containers), positive for larvae initially and not treated						
0	0	0	37.1	29.0	29.1	32.3	40.4
1+	6.4	6.4	12.9	19.4	17.7	17.7	14.5
2+	22.6	22.6	11.3	9.7	12.9	12.9	17.7
3+	35.5	35.5	8.1	12.9	12.9	14.5	16.1
4+	35.5	35.5	30.6	29.0	27.4	22.6	11.3
	Control-2 (64 containers), negative for larvae initially and not treated						
0	100	100	78	62.5	45.3	59.3	64.1
1+	0	0	9.4	7.8	20.3	17.2	10.9
2+	0	0	4.7	17.2	14.1	9.4	7.8
3+	0	0	4.7	4.7	7.8	4.7	17.2
4+	0	0	3.2	7.8	12.5	9.4	0

[1] 0, without larvae; +, with larvae at different densities: 0 = 0; 1 = 1–10; 2 = 11–30; 3 = 31–100; and 4 = >100 larvae/container.
[2] Percent distribution is based on all larval prevalence categories.

and large cement jars are important target containers that should be focused on for treatment when larval control programs against DHF vectors are carried out.

Posttreatment 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. In pretreatment, all larvae-positive containers except those in category 1+ had a high rate of positivity of 19.4–48.8%. All of the treated containers had no larvae at 48 h, 1 month, and 2 months after treatment with the exception of 1 container (plastic pail) becoming positive with a few larvae 2 months after treatment. The 1 treated container that became positive for larvae 2 months after treatment had no visible traces of temephos zeolite granules. The container apparently had been drained, cleaned, and refilled. The temephos zeolite granules leave bright green residues at the bottom and if present are easily visible in each container. At the 3-month observation, the proportion of larvae-free containers that initially were positive was 77.5% but declined further during the experimental period (all larval abundance categories). By the end of the 4- and 5-month period larvae-free containers (initially positive) still constituted 70.5%, with 29.5% positive for larvae. Heavy larval infestation (categories 2, 3, and 4) was noted in 18–24% of the containers 3–5 months after treatment. The proportion of treated jars becoming positive for larvae (in all categories 1, 2, 3, and 4) ranged from 22 to 29.5% after 3, 4, and 5 months after treatment. No granular material was visible in most of the containers that had become positive for larvae during the 3 months after treatment. We noted that these containers were emptied, cleaned, and refilled with new water, and the treatment material was washed out during the cleaning process. These results imply that temephos treatment could be effective for larval control for longer periods had the material not been washed out. However, in the last 2 assessments (4 and 5 months after treatment), some containers that had visible amounts of granules also were positive for larvae. This indicates that the formulation, even though not washed out completely in those containers, had lost activity by the 4th and 5th months, probably because of 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 still having treatment material and becoming positive for larvae were always those outdoors that were exposed to sunlight most of the day, or that had received windborne organic debris, dry grass, or plant roofing materials. Temperature, organic debris, and ultraviolet light from sunlight possibly degraded the active compound rather rapidly. Coupled with these factors, rapid water use, draining, and refilling are practices that remove the granules and thus shorten the residual efficacy of larvicidal formulations. Larvicidal formulations likely will last longer in large jars (Fig. 1) that 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.

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 determine how long temephos zeolite granules at operational dose would prevent larval appearance in the larvae-negative containers. Examination of the results revealed that no container became positive for larvae at 48 h after treatment and only 2 containers were found with low numbers of larvae 1 month after treatment. At 2 months after treatment, the number of positive containers (all larval abundance categories) amounted to only 6.5%, increasing to 23% at month 4 and going down to 8% positive 5 months after treatment. No applied granules were discernible in the containers becoming positive 3 months after treatment, but the granules were found in some larvae-positive containers 4 and 5 months after treatment. It also was noted that some of the containers that had visible amounts of granules became positive for larvae during the 4- and 5-month posttreatment period. This study further shows that the 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 the T-1 group, which lasted for about 3 months. It is apparent that jars that are negative for larvae at the start, although becoming positive in time, are not heavy producers of larvae.

In contrast to the treated containers (see Table 2), containers that had larvae at the start and were 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 determine how larval positivity without treatment will progress over time. These containers, 62 in total, experienced natural fluctuations in larval prevalence during the study period. It was noted that the numbers of containers positive for larvae at any evaluation inspection fluctuated between 60 and 71% (all abundance categories). This experiment documented that larval populations of *Ae. aegypti* without intervention always are prevailing at a constant level 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 were not treated with temephos (C-2 group) is presented in Table 2. Examination of the data shows that some of the larvae-negative containers do become positive for larvae with time. Examination of the data shows that the number of containers becoming positive for larvae increased from 0% at the 1st 48-h evaluation to 22% (at 1 month) and to 37.5% (at 2 months), reaching the peak of 55% positive at the 4th evaluation (3 months after treatment), and then declining to 41% (at 4 months) and to 36% at the last evaluation (5 months after treatment). These trends reveal that larval populations of *Ae. aegypti* appeared naturally in the initially negative containers and prevailed at relatively high frequency and abundance over the course of this study.

Acceptability

To gauge the level of resistance to or acceptance of the use of temephos granules in domestic water-storage containers, we interviewed 96 residents in the test area in Kanchanaburi. Of these, 89% had not used any larvicide in their water containers in the near past, even though the material was supplied by the local health authorities. These individuals objected to the currently used temephos sand granules (1%) in water containers because of the unpleasant odor of the formulation and because of the increase in water turbidity that they associated with the use of the formulation. However, a small percentage (11%) used temephos granules, but only occasionally, again being reluctant to use the treatment because of odor, water turbidity, and safety considerations. When questioned regarding the use of temephos zeolite granules lacking odor and causing no 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 that have odor also were 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, 3 major problems exist 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. First, the current larvicide used for control of larval *Ae. aegypti* in Thailand is temephos sand granules (1%), which provides a high degree of control, but possesses an unpleasant odor when applied to water and renders water more turbid. These drawbacks are not accepted by many residents and they usually refuse to use the larvicide, as reported by Phanthumachinda et al. (1985) and Thavara et al. (2001). Second, with regard to water-consumption styles of villagers, the people were found to always keep water for drinking and daily use in various kinds of containers, such as jars, tanks, drums, pails, and so on, with capacities ranging from 50 to 2,000 liters. The main sources of water are from rain, wells, canals, rivers, and piped-water supply. In most rural areas of Thailand, people store water in containers because of drought, especially in the dry season, but in many urbanized areas having a piped-water supply, people still keep water in their water-storage containers because of an irregular water supply and because of traditional styles of water usage, with most people preferring rainwater over other supplies. The vast numbers of water-storage containers in use constitute major breeding sources of *Ae. aegypti.* From our observations, many containers (mostly ≤200 liters in capacity) were noted to be frequently washed, cleaned, and refilled with new water, resulting in loss of 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 subsequently 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 (1% sand and 1% zeolite granules) at the rate of 1 ppm AI with 3 water regimens (full, full but half removed and refilled weekly, and half 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 sunlight out. Apparently, normal water-use practices as noted in the trial villages culminate in decreased residual activity. A number of other 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 control of *Ae. aegypti,* deliver quantities insufficient for treatment of all larval sources. As a rule, a family receives only 20–40 g of larvicide once or twice a year from the local health station. This amount is grossly insufficient to treat all water-storage containers, because each house has at least 4 or 5 containers of various capacities (50–2,000 liters). We noted that some families declined from using the larvicide provided in sachets because they did not have sufficient quantities of larvicide. Because of the large number of water-storage containers in Thailand, which require 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 residents in the country each year, it is desirable to supervise the 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 larval *Ae. aegypti* in water-storage containers is the most appropriate and effective measure. Implementation of 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 large jars used for long-term water storage.

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