LARVICIDAL EFFICACY OF NEW FORMULATIONS OF TEMEPHOS IN NON-WOVEN SACHETS AGAINST LARVAE OF *AEDES AEGYPTI* (L.) (DIPTERA: CULICIDAE) IN WATER-STORAGE CONTAINERS

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Abstract. Three new formulations of temephos (LAVIFOS SG 1%, MOSQ SG 1% and AZAI-SS ZG 1%) were evaluated for larvicidal efficacy against larvae of *Aedes aegypti* (L.) in water-storage jars under field-simulated conditions. LAVIFOS SG 1% and MOSQ SG 1% are sand granule formulations, whereas AZAI-SS ZG 1% is zeolite granule formulation. Each formulation contained 1% temephos as an active ingredient. Each formulation was packed in a non-woven sachet at quantity of 20 g per sachet and placed in a 200-liter glazed clay jar to obtain a dosage of 1 mg/l (one sachet per jar). Each treatment and control (jar without larvicide) was replicated four times. A concurrent set of treatments and controls were carried out in parallel, but the water in each treated and control jars was removed and refilled weekly. All jars (treatment and control) were challenged weekly by adding 25 third-instar larvae per jar and assessment was made of larval mortality by counting pupal skins one week after the addition of larvae. The three formulations provided complete larvicidal efficacy (100%) for at least 24 weeks post-treatment (the length of this study). In the jars where all the water was removed and refilled weekly, LAVIFOS SG 1%, and MOSQ SG 1% provided complete larvicidal efficacy for at least 24 weeks post-treatment, whereas AZAI-SS ZG 1% showed complete larvicidal efficacy for 16 weeks post-treatment. AZAI-SS ZG 1% still demonstrated a high degree of larvicidal activity (93-99%) from 17 to 24 weeks post-treatment. The present study reveals an excellent residual efficacy of the three new formulations of temephos against larvae of *Aedes aegypti* in water-storage jars lasting for at least 16 to 24 weeks post-treatment. These new formulations will make the control of DHF vectors in Thailand more cost effective as they are removable and retrievable sachets that can be reused after cleaning the water-storage containers.

INTRODUCTION

Aedes aegypti (L.) is generally accepted as a primary vector of dengue viruses in tropical and subtropical regions (Halstead, 1966; Gubler and Casta-Valez, 1991; Thavara *et al*, 1996). One of the main strategies to control this vector is application of larvicides to control larval population of mosquitoes. Various larvicidal formulations that possess long-lasting residual activity have been developed, tested and used to control this mosquito vector. In Thailand, temephos sand granule formulation (1% active ingredient) has been used since the early 1970s in operational control programs at a dosage of 1 mg/l a.i. in water-

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storage containers (Bang and Tonn, 1969a,b; Bang *et al*, 1972). Until now, the temephos sand granule formulation (1%) is still an effective larvicide against *Ae. aegypti* larvae. In practice, temephos sand granules possess two negative features: they cause an objectionable odor and water turbidity (Thavara *et al*, 2001). These factors cause objection by household members regarding the use of temephos sand granules in their water-storage containers. As a result, a new formulation of temephos zeolite granules was recently developed to overcome these disadvantages. Mulla *et al* (2004) revealed the newly developed formulation of temephos zeolite granules lacked these undesirable characteristics and possessed high residual efficacy against *Ae. aegypti* larvae for more than six months under field-simulated conditions. This formulation also provided high larvicidal activity for at least three months in village-scale trials under normal water-use practices with high acceptance by villagers (Thavara *et al*, 2004). However, loss of granules during the process of cleaning water-storage containers is another problem with granular formulations of temephos larvicide found in the field (Thavara *et al*, 2004). As a result, Thavara *et al* (2005) initiated an experiment to show that temephos 1% sand granules enclosed and tied in a muslin cloth and placed in 200-liter water storage jars then transferred sequentially to new sets of jars four time successively provided high larvicidal efficacy (92-100% emergence inhibition) for about five months or longer. It was therefore necessary to develop a new effective formulation of temephos larvicide that is retrievable during the cleaning process, which is more practical and desirable. This study was carried out to evaluate the larvicidal efficacy of three new formulations of temephos larvicides packed in non-woven sachets against the larvae of *Ae. aegypti* in water-storage jars under field-simulated conditions. The results obtained from this study could provide information regarding a cost effective strategy for the control of *Ae. aegypti* larvae in Thailand and elsewhere.

MATERIALS AND METHODS

Study site

The experiment was carried out at a research station for the evaluation of larvicidal products and other experimental agents for vector control, Nonthaburi Province, Thailand. Description of the research facilities are given in Mulla *et al* (2004).

Materials and treatments

Three new formulations of temephos larvicide, namely: LAVIFOS SG 1%, MOSQ SG 1% and AZAI-SS ZG 1%, were evaluated against larvae of *Aedes aegypti* (L.) in water-storage jars under field-simulated conditions. LAVIFOS SG 1% and MOSQ SG 1% are sand granule formulations, whereas AZAI-SS ZG 1% is a zeolite granule formulation, each one contained 1% temephos as an active ingredient. These formulations were provided by Ikari Trading (Thailand), Bangkok, Thailand. Each formulation was placed in a non-woven sachet at a quantity of 20 g per sachet and placed in a 200-liter glazed clay jar to obtain a final dosage of 1 mg/l a.i. (one sachet per jar). Each treatment and control (jar without larvicide) was replicated four times. A concurrent set of treatments and controls was also carried out in parallel, but all of the water in each treated and control jar was removed and refilled weekly. The treatment and control jars were arranged in a block design and set in a row from east to west. All the jars were positioned in the shade under a roof and were covered with celocrete sheets to prevent windborne debris and oviposition by wild mosquitoes. The treatments and controls were challenged weekly with a fresh cohort of laboratory-reared third-instar larvae of *Ae. aegypti*, where a total of 25 larvae were added to each jar. About 0.5 g of ground mouse food was

added weekly per jar for larval food. The experiment was carried out for 24 weeks post-treatment.

Assessment of efficacy

Mortality occurred almost completely in the larval stages, not many surviving to lead to the adult stage. Pupal skins (indicating adult emergence) floating on the water surface, mostly at the meniscus level can be picked up easily with a dropper without disturbing the water in the jars. Adult emergence based on pupal skins was then used as an assessment parameter of the overall effectiveness of treatment. The rates of failure to emerge are shown in percentages, which were calculated on the basis of the total number of pupal skins compared to the initial number of larvae added.

Data analysis

Comparison of larvicidal efficacy among test larvicides was carried out employing the one-way analysis of variance (ANOVA) with Duncant's multiple range test. All differences were considered significant at $p \leq 0.05$.

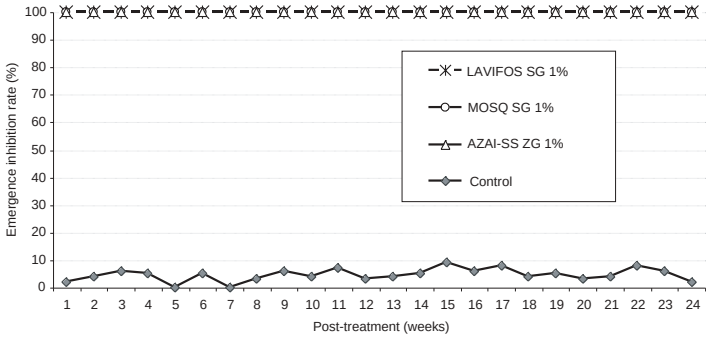

Fig 1–Larvicidal efficacy of three new formulations of temephos larvicides against *Ae. aegypti* larvae in 200-liter water-storage jars.

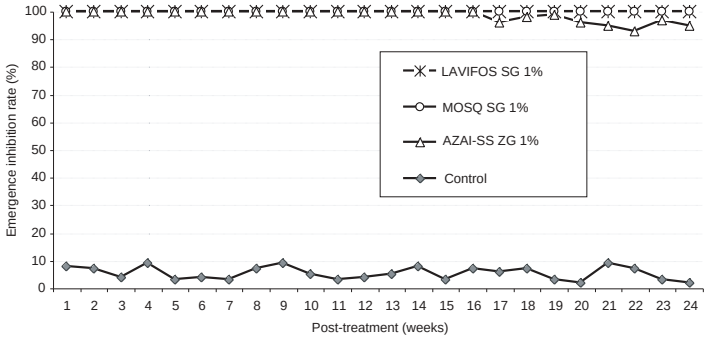

Fig 2–Larvicidal efficacy of three new formulations of temephos larvicides against *Ae. aegypti* larvae in 200-liter water-storage jars with the condition of all water removed and refilled weekly.

RESULTS

The larvicidal efficacy of LAVIFOS SG 1%, MOSQ SG 1% and AZAI-SS ZG 1% applied at 1 sachet per jar (1 mg/l a.i.) against *Ae. aegypti* larvae in constantly full jars is presented in Fig 1. The three formulations provided complete larvicidal efficacy against *Ae. aegypti* for at least 24 weeks post-treatment. In comparison, the mortality rates of larvae in control jars (without larvicides) remained low at about 0-9% (Fig 1).

In jars where the water was removed and refilled weekly, LAVIFOS SG 1% and MOSQ SG 1% provided complete larvicidal efficacy for at least 24 weeks post-treatment, whereas AZAI-SS ZG 1% showed complete larvicidal efficacy for 16 weeks post-treatment (Fig 2). However, the AZAI-SS ZG 1% still demonstrated a high degree of larvicidal activity (93-99%) during the period from 17 to 24 weeks post-treatment. There was no significant differece in larvicidal efficacy among the three formulations during the course of this study ($p > 0.05$). The mortality rate of larvae in control jars in this experiment was less than 10% (Fig 2).

DISCUSSION

The residual efficacy of temephos (1%) sand granules in early studies (Bang and Tonn,

1969a,b) and temephos (1%) zeolite granules in recent trials (Thavara *et al*, 2004) was reported to be about three months against *Ae. aegypti* larvae. Based on these field studies, effectiveness over two to three months in water-storage containers can be expected under normal water-use practices. This residual activity is desirable for larval control of DHF vectors. However, many factors, especially water use practices, such as adding, removing, draining, and cleaning, will affect the residual efficacy of the larvicides used, even long-lasting formulations may lose efficacy due to dilution or removal of water. Thavara *et al* (2004) found small sizes of water-storage containers (50-200 liters) are usually used for daily consumption of water and are frequently cleaned by homeowners, whereas larger containers (200-2,500 liters) used for long-term storage are only cleaned occasionally. During cleaning, the loose sand or zeolite granules of temephos larvicide are washed out and eventually lost. Thus, newly developed formulations of temephos larvicides packed in sachets, as used in this study, provide an advantage over loose temephos granules in the containers. These formulations will certainly minimize the waste of larvicide and extend the capacity of the control program against *Ae. aegypti* larvae as the larvicides are retrievable and reuseable in the same water-storage containers or in other untreated ones.

As pointed out by Thavara *et al* (2005), temephos was released slowly from sand granules over a long period of time and once it was released in adequate quantities, it remained in the treated containers and yielded excellent control of *Ae. aegypti* larvae for a period of several weeks. Temephos sand granules enclosed and tied in muslin cloth and placed in 200-liter water storage jars and then transferred sequentially to new sets of jars four time successively still provided high larvicidal efficacy (92-100% larval mortality) even at the low dosage of 0.05 mg/l a.i. for five months or longer (Thavara *et al*, 2005). This observation provided the idea to develop new formulations of larvicide that are retrievable, and more practical. Three new formulations of temephos larvicides in this study, LAVIFOS SG 1%, MOSQ SG 1% and AZAI-SS ZG 1%, were then formulated and packed in non-woven sachets that were very thin and allowed release of active ingredient. The three new formulations demonstrated excellent control of *Ae. aegypti* larvae for at least six months without water replacement. However, villagers usually wash their water-storage containers used for daily consumption of water. It was assumed that people use water at about 200 liters per week and then cleaned their water-storage containers. On this basis, the experiment involved removing all the water in the treated jars and refilling weekly. It is clear that all three larvicidal formulations tested provide excellent results (92-100% larval mortality) for the control of *Ae. aegypti* larvae in water-storage jars for at least six months when all water was removed and refilled weekly. This implies that temephos was released slowly and continuously from the sachets during the course of this study. It is possible the three new formulations of temephos larvicide could last longer than six months if the experiment were extended.

In conclusion, the present study reveals an excellent residual efficacy of the three new formulations of temephos placed in non-woven sachets against larvae of *Ae. aegypti* in water-storage jars lasting for 16-24 weeks post-treatment depending upon water use practices. These new formulations could make the larval control program of DHF vectors in Thailand more cost effective as they are removable and retrievable sachets that can be reused after cleaning the water-storage containers.

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