COMPARATIVE FIELD EFFICACY OF NEWLY DEVELOPED FORMULATIONS OF LARVICIDES AGAINST *AEDES AEGYPTI* (L.) (DIPTERA: CULICIDAE)

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Abstract. *Aedes aegypti* (L.) is known as vector of dengue and chikungunya fever. Larvicides are used to control this vector. We evaluated the efficacy of newly developed formulations of larvicides to control *Ae. aegypti* under field conditions for 24 weeks post single application. Mosdop P™ and Mosdop TB™ containing diflubenzuron (2% and 40 mg/tablet, respectively) as the active ingredient, were applied at a dosage of 0.1 mg a.i./l and Mosquit TB10™, Mosquit TB100™ and Temecal™ containing temephos (1%, 10% and 1%, respectively) as the active ingredient were applied at a dosage of 1 mg active ingredent (a.i.) to 200 liter water storage jars. Two water regimens were used in the jars: in one regimen the jar was kept full of water all the time and in the other regimen a full jar had half the volume removed and refilled weekly. The larvicidal efficacy was reported as the level of inhibition of emergence (IE%) calculated based on the pupal skins in the jars versus the original number of larvae added. Mosdop P™, Mosdop TB™, Mosquit TB10™, Mosquit TB100™ and Temecal™ showed complete larvicidal efficacy (100% IE) in the constantly full jars for 16, 17, 14, 20 and 13 weeks post-treatment, respectively; in the jars where half the volum of water was replaced weekly, the larvicides had complete larvicidal efficacy (100% IE) for 19, 20, 17, 24 and 15 weeks post-treatment, respectively. The five larvicide regimens evaluated in this study are effective for controlling *Ae. aegypti* larvae.

Keywords: larvicides, diflubenzuron, temephos, control, *Aedes aegypti*, Thailand

INTRODUCTION

Aedes aegypti (L.) is a vector of dengue fever and chikungunya infection in tropical and subtropical regions (Halstead, 1966; McIntosh and Gear, 1981). Efforts to control these diseases involve the use of various strategies directed against the primary vector and to a lesser degree against *Ae. albopictus* Skuse. Since no vaccine is currently available to prevent these diseases, vector control has

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been the major method to control these diseases. Chemical, biological and microbial control strategies have been used to control these vectors (Gould *et al*, 1971; Nathan, 1993; Marten *et al*, 1994). Chemical and microbial agents have been used to control the larvae of *Ae. aegypti* propagating in artificial containers in domestic and peridomestic situations (Gould *et al*, 1971; Bang *et al*, 1972; Thavara *et al*, 2004; Tan *et al*, 2012). The organophosphate larvicide temephos sand granules have been used to control *Ae. aegypti* in artificial containers in Thailand (Bang and Tonn 1969a,b; Bang *et al*, 1972) and have been used to control larvae in artificial containers at a concentration of 1 mg a.i./l of water for nearly 50 years in Thailand and elsewhere around the globe (Tavara *et al*, 2004). Until now, various formulations of temephos have been effective larvicides against *Ae. aegypti* larvae (Mulla *et al*, 2004; Thavara *et al*, 2004, 2005; Tawatsin *et al*, 2007a). However, the temephos sand granules have an unpleasant odor from the impregnation solvent (Thavara *et al*, 2001). Low to moderate levels of resistance to temphos have been reported in Thailand (Jirakanjanakit *et al*, 2007; Bisset *et al*, 2011; Mulyatno *et al*, 2012). In recent years, new insect growth regulators (IGRs) known as chitin synthesis inhibitors, such as diflubenzuron, novaluron and triflumuron, have been used to control a variety of insect pests, including mosquitoes (Mulla and Darwazeh, 1975; Mulla *et al*, 2003; Thavara *et al*, 2007; Tawatsin *et al*, 2007b; Martins *et al*, 2008; Belanito *et al*, 2009). Thus, IGRs may be effective for mosquito larvae control. In view of the potential emergence of resistance, it is desirable to evaluate these new agents against the larvae of *Ae. aegypti* in water-storage containers.

MATERIALS AND METHODS

We evaluated four newly developed formulations of larvicides containing diflubenzuron (2% and 40 mg/tablet) and temephos (1% and 10%) as active ingredients and compared them with temephos (1% sand granules), a conventional larvicide used in Thailand, under field conditions. The materials were tested in identical containers (200 liter water storage jars). The test was conducted for 24 weeks using 3rd instar larvae of *Ae. aegypti*.

The study was conducted in a semi-rural part of Bang Bua Thong District, Nonthaburi Province, Thailand. The procedure followed Mulla *et al* (2004) and the guidelines for laboratory and field testing of mosquito larvicides (WHO, 2005). Glazed earthen 200 liter water storage jars, the most common water storage containers used in Thailand were placed on a concrete slab (6 meters wide and 24 meters long) covered with a roof (3 meters high) but open on the sides. The jars were in open shade at all times. Fitted aluminum lids covered the mouths of the jars at all times except while filling, emptying, adding larvae, or counting larvae or pupal skins (about 4-5 hours per week). The covers precluded light entry and prevented deposition of debris, oviposition by wild mosquitoes and invasion by predacious macro-invertebrates. Half a gram of ground larval food was added to each container along with 25 laboratory bred third instar *Ae. aegypti* mosquito larvae each week during the study along with water to replace evaporative water loss.

Formulations and treatments

Five formulations of larvicides were evaluated: Mosdop P™ (diflubenzuron 2% w/w, powder formulation), Mosdop

TB™ (diflubenzuron 40 mg/tablet, tablet formulation, weight 300 mg/tablet), Mosquit TB10™ (temephos 1% w/w, tablet formulation, weight 320 mg/tablet), Mosquit TB100™ (temephos 10% w/w, tablet formulation, weight 800 mg/tablet) and Temecal™ (temephos 1% w/w, sand granule formulation). These larvicides were produced and provided by Project Field Co, Ltd, Bangkok, Thailand. The first four formulations were new formulations developed by Project Field Co, Ltd and the fifth formulation was the current commercial larvicide already registered with the Food and Drug Administration (FDA) of Thailand. The larvicides containing diflubenzuron (Mosdop P™ and Mosdop TB™) were tested at a concentration of 0.1 mg a.i./l and the larvicides containing temephos were tested at a concentration of 1 mg a.i./l. All larvicides were applied directly to water without stirring. Control jars (without larvicide) were also evaluated in parallel. Two water regimens were used for the jars: in the first regimen the jar was kept full all the time and none of the water was replaced; in the second regimen, half the water (100 liters) was replaced weekly. In the jars where half the water was replaced weekly, the water was removed with a submersible water pump lowered into the mid-depth of the jar and then the water was pumped out. All treatments and controls were replicated 5 jars and challenged with cohorts of 25 of the 3rd instar larvae of *Ae. aegypti* (laboratory reared) at weekly intervals after treatment for 24 weeks.

Assessment of efficacy

The number of pupal skins indicating successful emergence into adults was counted every 7 days. The larvicidal efficacy was reported as the level of inhibition of emergence (%IE) calculated:

$$\%IE = 100 - [(PS/TL)x100],$$

where PS = pupal skins found in the jar, and TL = total larvae added to the jar.

The mortality rate in the control group was not included in this calculation, since the mortality rate in the control jars was low, less than 5%.

RESULTS

The two larvicides containing diflubenzuron, Mosdop P™ and Mosdop TB™, at a concentration of 0.1 mg a.i./l, gave complete larvicidal efficacy (100% IE) against *Ae. aegypti* larvae in the constantly full jars for 16 and 17 weeks post-treatment, respectively (Fig 1). Beyond these periods, both larvicides still exhibited a high degree of larvicidal efficacy until the end of the study (24 weeks) giving an overall %IE of 98.4-99.2 and 90.4-99.2, respectively.

The three larvicides containing temephos, Mosquit TB10™, Mosquit TB100™ and Temecal™, in the constantly full jars at a concentration of 1 mg a.i./l, gave complete larvicidal efficacy (100% IE) for 14, 20 and 13 weeks post-treatment, respectively (Fig 1). After these periods, Mosquit TB100™ gave relatively high levels of %IE of 93.6-99.2 until the end of the study. The Mosquit TB10™ and Temecal™ gave lower levels of %IE of 72.8-83.2 and 68.0-97.6, respectively. The %IE in the control jars (without larvicides) was usually less than 5 (range %IE 1.6-8.8) for the duration of the study (Fig 1).

In the water replaced jars, Mosdop P™ and Mosdop TB™ at a concentration of 0.1 mg a.i./l yielded complete larvicidal efficacy (100 %IE) for 19 and 20 weeks post-treatment, respectively (Fig 2). After these periods the %IE of both these larvicides were 66.4-95.2 and 97.6-99.2, respectively.

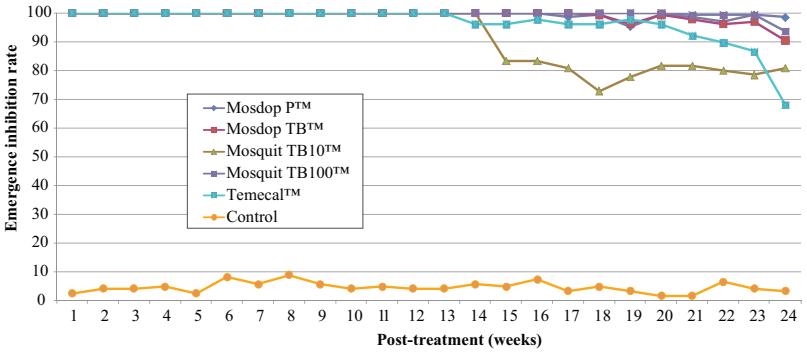

Fig 1–Larvicidal efficacy of five formulations of larvicides against *Aedes aegypti* larvae in 200 liter water-storage jars.

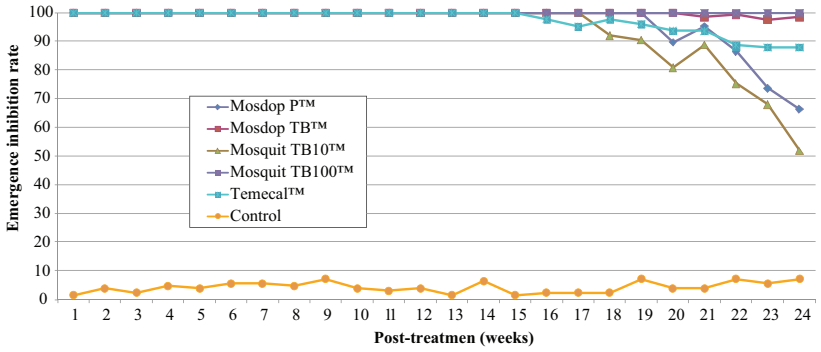

Fig 2–Larvicidal efficacy of five formulations of larvicides against *Aedes aegypti* larvae in 200 liter water-sorage jars with 100 liters of water exchange weekly.

In the temphos containing larvicides at a concentration 1 mg a.i./l, Mosquit TB100™ gave excellent larvicidal efficacy (100 %IE) for the duration of the study (24 weeks). Mosquit TB10™ and Temecal™ gave complete larvicidal efficacy (100%IE) for 17 and 15 weeks post-treatment, respectively (Fig 2). Beyond these periods Mosquit TB10™ and Temecal™ gave %IE of 52-92 and 88-97.6, respectively. The inhibition of emergence in the control jars was low, (%IE 1.6-7.2) (Fig 2).

DISCUSSION

Diflubenzuron, an IGR acting as a chitin synthesis inhibitor, was discovered and evaluated against mosquito larva in the 1970s (Mulla and Darwazeh, 1975). This compound is chemically known as benzoylurea, derived from urea types of compounds. To date, many compounds of benzoylurea have been developed for insect control, but diflubenzuron is the compound most studied for mosquito control. It acts as a larvicide, causing larvae to die during ecdysis from one instar to the next. Two formulations (WP 25% and GR 2%) were extensively studied against many species of mosquitoes (Mulla and Darwazeh, 1975, 1976) and aquatic nuisance midges (Mulla *et al*, 1976) and found to be extremely efficacious. Other formulations of diflubenzuron have also became available. We studied GR 2% and 2% tablet (40 mg a.i./tablet) formulations of diflubenzuron at 3-5 dosages against *Ae. aegypti* in 200 liter clay water storage jars in Thailand (Thavara *et al*, 2007), and found both formulations equally effective against *Ae. aegypti* larvae, with a 90% IE at 22-24 weeks post-treatment at concentrations of 0.05 and 0.1 mg a.i./l in full jars. In refilled jars, the longevity of efficacy was lower at the lower concentration but gave excellent control for 16-24 weeks.

The 9th WHOPES (WHO, 2006) working group reviewed the efficacy of diflubenzuron WP (25%), DT (2%) and GR (2%) formulations. WHOPES arrived at a recommendation to use diflubenzuron, DT (2%) and GR 2% formulations to control container-breeding mosquitoes such as *Ae. aegypti*. The recommended concentrations were 0.02 to 0.25 mg a.i./l, providing a longevity of control with at least 90 %IE of 2-4 months under variable field situations. It was recommended higher concentrations be used in containers with high organic content and having exposure to sunlight. In the present study, Mosdop P™ and Mosdop TB™ had 100%IE for 19-20 weeks post-treatment in the water exchange regimen. This is excellent efficacy and longevity; adequate for DHF and chikungunya control programs. The larvicidal efficacy of the tablet formulation (Mosdop TB™) were greater than the powder formulation (Mosdop P™) in both water regimens. Operationally, it is easier to use the tablet formulation. Tablets sink to the bottom of the jar and are not visible to the water users. A portion of powder or granule formulations may float on the surface or be visible at the bottom of the jar and may be objectionable to water users.

Temephos was discovered in the 1950s by American Cyanamid Co, Ltd. It was evaluated in Thailand (Bang and Tonn, 1969a,b; Bang *et al*, 1972) and found effective for controling of *Ae. aegypti* larvae in water storage jars. As a result of these findings, temephos sand granules (1%) were used in dengue vector control programs during 1970-1972, and it continues to be used. Ministry of Public Health personnel distribute temephos sand granules (1%) in 20 g quantities to homeowners to treat 200 liters water storage containers as well as other types of containers. In recent years, improved sand and zeolite granule formulations have become available. The three formulations of temephos larvicide: LAVIFOS™ SG1% (sand granules), MOSQ™ SG1% (sand granules) and AZAI-SS™ ZG1% (zeolite granules), formulated and packed in non-woven sachets provided excellent results (92-100% larval mortality) for controling of *Ae. aegypti* larvae in water-storage jars for at least 24 weeks when all the water was removed and refilled weekly (Tawatsin *et al*, 2007a). In this study, Mosquit TB100™ (tablet formulation) also gave complete larvicidal efficacy (100 %IE) against *Ae. aegypti* larvae for at least 24 weeks under the water exchange regimen. It is possible the efficacy of this formulation could last longer if the study were extended.

Temephos is released slowly from the formulations over a long period of time because temephos has low solubility in water (about 0.03 mg/l at 25ºC) and once released in adequate quantities, it remains in the jars and provides larvicidal efficacy for 3-6 weeks after removal of the granule packets depending on the extent released (Thavara *et al*, 2005). Low dosages of temephos (0.1-0.25 mg a.i./l), 1/10-1/4 the currently recommended dosage used in the larval control program, have been found effective against *Ae. aegypti* larvae for up to five months (Thavara *et al*, 2005). This means it might be possible to reduce the currently used dosage used to treat water-storage containers.

Some formulations of temephos sand granules have an unpleasant odor, but Mosquit TB10™ and Mosquit TB100™, two new formulations containing temephos, showed no signs of bad odor making them more readily acceptable to users.

The most common larvicides used to control *Ae. aegypti* larvae in Thailand con-

tain temephos because they are effective and relatively cheap compared to other larvicidals. Many larvicides containing temephos have excellent efficacy against *Ae. aegypti* larvae in water-storage jars for several months (Mulla *et al*, 2004, Thavara *et al*, 2004; Tawatsin *et al*, 2007a). However, there is a trend in the appearance of resistance to temephos among *Ae. aegypti* populations in Thailand (Jirakanjanakit *et al*, 2007). Alternative larvicides with different modes of action, such as IGRs (diflubenzuron, novaluron) or microbial agents (Bti, spinosad) should also be used to prevent or manage temephos resistance.

In conclusion, the present study reveals excellent larvicidal efficacies for the four studied formulations containing either diflubenzuron (Mosdop P™ and Mosdop TB™) or temephos (Mosquit TB10™ and Mosquit TB100™) as active ingredients against the larvae of *Ae. aegypti* in water storage jars for up to 24 weeks post-treatment. Mosdop TB™ (at a concentration of 0.1 mg a.i./l) and Mosquit TB10™ and Mosquit TB100™ (at a concentration of 1 mg a.i./l) can be used effectively against *Ae. aegypti* larvae in 200 liters water storage jars. These new formulations can strengthen the larval control program in Thailand.

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REFERENCES

Bang YH, Tonn RJ. Evaluation of 1% Abate (OMS-786) sand granules for the control of *Aedes ageypti* larvae in potable water. *WHO/VBC/69.* 1969a; 121: 1-10.

Bang YH, Tonn RJ. Residual life of Abate (OMS-786) sand granules in water containers under water usage practice. *WHO/VBC/69.* 1969b; 125: 1-10.

Bang YH, Tonn RJ, Jatanasen S. Pilot studies of Abate as larvicide for control of *Aedes aegypti* in Bangkok, Thailand. *Southeast Asian J Trop Med Public Health* 1972; 3: 106-15.

Belinato TA, Martins AJ, Lima JB, Lima-Camara TN, Peixoto AA, Valle D. Effect of the chitin synthesis inhibitor triflumuron on the development, viability and reproductive of *Aedes ageypti*. *Mem Inst Oswaldo Cruz* 2009; 104: 43-7.

Bisset JA, Rodriguez MM, Ricardo Y, *et al*. Temephos resistance and esterase activity in the mosquito *Aedes aegypti* in Havana, Cuba increased dramatically between 2006 and 2008. *Med Vet Entomol* 2011; 25: 233-9.

Gould DJ, Mount GA, Scanlon JE, Sullivan MF, Winter PE. Dengue control on an island in the Gulf of Thailand. I. Results of an *Aedes aegypti* control program. *Am J Trop Med Hyg* 1971; 5: 705-14.

Halstead SB. Mosquito-borne haemorrhagic fevers of South and South East Asia. *Bull World Health Organ* 1966; 35: 3-15.

Jirakanjanakit N, Saengtharatip S, Rongnoparut P, Duchon S, Bellec C, Yoksan S. Trend of

temephos resistance in *Aedes* (Stegomyia) mosquitoes in Thailand during 2003-2005. *Environ Entomol* 2007; 36: 506-11.

Marten GG, Borjas G, Cush M, Fernandez E, Reid JW. Control of larval *Aedes aegypti* (Diptera: Culicidae) by cyclopoid copepods in peridomestic breeding containers. *J Med Entomol*1994; 31: 36-44.

Martins AJ, Belinato TA, Lima JB, Valle D. Chitin synthesis inhibitor effect on *Aedes aegypti* populations susceptible and resistant to organophosphate temephos. *Pest Manag Sci* 2008; 64: 676-80.

McIntosh BM, Gear JHS. Arboviral zoonoses in southern Africa: Chikungunya fever. In: Steele JH, Beran GW, eds. Section B: Viral zoonoses. CRC Handbook Series in Zoonoses. Boca Raton, FL: CRC Press, 1981: 217-20.

Mulla MS, Darwazeh HA. Activity and longevity of insect growth regulators against mosquitoes. *J Econ Entomol* 1975; 68: 791-4.

Mulla MS, Darwazeh HA. The IGR Dimilin and its formulations against mosquitoes. *J Econ Entomol* 1976; 69: 309-12.

Mulla MS, Kramer WL, Barnard DR. Insect growth regulators for control of chironomid midges in residential-recreational lakes. *J Econ Entomol* 1976; 69: 285-91.

Mulla MS, Thavara U, Tawatsin A, Chompoosri J, Zaim M, Su T. Laboratory and field evaluation of novaluron, a new acylurea insect growth regulator, against *Aedes aegypti* (Diptera: Culicidae). *J Vector Ecol* 2003; 28: 241-54.

Mulla MS, Thavara U, Tawatsin A, Chompoosri J. Procedures for the evaluation of field efficacy of slow-release formulations of larvicides against *Aedes aegypti* in water-storage containers. *J Am Mosq Control Assoc* 2004; 20: 64-73.

Mulyatno KC, Yamanaka A, Ngadino, Konishi E. Resistance of *Aedes aegypti* (L.) larvae to temephos in Surabaya, Indonesia. *Southeast Asian J Trop Med Public Health* 2012; 43: 29-33.

Nathan MB. Critical review of *Aedes aegypti* control programs in the Caribbean and selected neighboring countries. *J Am Mosq Control Assoc* 1993; 9: 1-7.

Tan AWA, Loke SR, Benjamin S, Lee HL, Chooi KH, Sofinan-Azirun M. Spray application of *Bacillus thuringiensis israelensis* (Bti strain AM65-52) against *Aedes aegypti* (L.) and *Ae. albopictus* Skuse populations and impact on dengue transmission in a dengue endemic residential site in Malaysia. *Southeast Asian J Trop Med Public Health* 2012; 43: 296-310.

Tawatsin A, Thavara U, Chompoosri J, Bhakdeenuan P, Asavadachanukorn P. Larvicidal efficacy of new formulations of temephos in non-woven sachets against larvae of *Aedes aegypti* (L.) (Diptera: Culicidae) in water storage containers. *Southeast Asian J Trop Med Public Health* 2007a; 38: 641-5.

Tawatsin A, Thavara U, Bhakdeenuan P, *et al*. Field evaluation of novaluron, a chitin synthesis larvicide, against mosquito larvae in polluted water in urban areas of Bangkok, Thailand. *Southeast Asian J Trop Med Public Health* 2007b; 38: 434-441.

Thavara U, Tawatsin A, Chansang C, *et al*. Larval occurrence, oviposition behavior and biting activity of potential mosquito vectors of dengue on Samui Island, Thailand. *J Vector Ecol* 2001; 26: 172-80.

Thavara U, Tawatsin A, Kong-ngamsuk W, Mulla MS. Efficacy and longevity of a new formulation of temephos larvicide tested in village-scale trials against larval *Aedes aegypti* in water storage containers. *J Am Mosq Control Assoc* 2004; 20: 176-82.

Thavara U, Tawatsin A, Srithommarat R, Zaim M, Mulla MS. Sequential release and residual activity of temephos applied as sand granules to water-storage jars for the control of *Aedes aegypti* larvae (Diptera: Culicidae). *J Vector Ecol* 2005; 30: 62-72.

Thavara U, Tawatsin A, Chansang C, Asavadachanukorn P, Zaim M, Mulla MS. Simulated field evaluation of the efficacy of two formulations of diflubenzuron, a

chitin synthesis inhibitor against larvae of *Aedes aegypti* (L.) (Diptera: Culicidae) in water-storage containers. *Southeast Asian J Trop Med Public Health* 2007; 38: 269-75.

World Health Organization (WHO). Guidelines for laboratory and field testing of mosquito larvicides. *WHO/CDS/WHOPES/GCDPP/2005.13*. 2005.

World Health Organization (WHO). Report of the 9th WHOPES working group meeting, WHO/HQ, Geneva, 5-9 December 2005. Review of: Dimilin GR and DT, Vectobac DT, Aqua K-Othrine, Aqua Reslin Super. *WHO/CDS/NTD/WHOPES/2006.2*. 2006.