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

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Abstract. Tablet (40 mg a.i./tablet) and granular (2% a.i.) formulations of diflubenzuron, a chitin synthesis inhibitor, insect growth regulator, were evaluated for larvicidal efficacy against the larvae of *Aedes aegypti* (L.) in water-storage containers under field conditions in Thailand. Each formulation was applied to 200-l clay jars at 5 different dosages (0.02, 0.05, 0.1, 0.5 and 1 mg/l a.i.). The jars were covered with solid celocrete sheets and placed in the shade under a roof. Another experiment was also carried out using 3 different dosages (0.1, 0.5 and 1 mg/l) where half the water in each treated jar and the control was removed and refilled weekly. Each treatment was replicated four times. The treatments were challenged by adding 25 3rd instar larvae/jar weekly. Assessments were made of each treatment through emergence inhibition (%EI) by removing and counting pupal skins one week after larval addition. Using these assessment techniques, a high degree of larvicidal efficacy (96-100%EI) was achieved with 4 dosages (0.05, 0.1, 0.5 and 1 mg/l) of both (tablet and granular) formulations for a period of 23 weeks post-treatment. The efficacy of the lowest dosage (0.02 mg/l) of tablet and granular formulations lasted for 21 and 22 weeks post-treatment, respectively. Under the conditions of water removal and weekly refilling, a high degree of larvicidal efficacy (96-100%EI) at the 3 dosages was obtained with the tablet formulation 18 to 21 weeks post-treatment, whereas the efficacy of the granular formulation persisted 15 to 23 weeks post-treatment depending on the dosage. This study clearly demonstrates a high level of residual activity with both formulations of diflubenzuron against larvae of *Ae. aegypti* in water-storage containers. Considering environmental factors and water-use conditions, it is likely that dosages of 0.05 to 0.1 mg a.i./l are effective dosages providing long-lasting control for 3 to 4 months in the field.

INTRODUCTION

Aedes aegypti (L.) is generally recognized as the most important vector responsible for transmission of dengue viruses causing dengue fever, a major mosquito-borne disease. This mosquito species is widely spread throughout the world, including in tropical, subtropical and temperate regions. Chemical and microbial larvicides containing a variety of active ingredients, such as temephos, Bti and insect growth regulators (IGRs), have been developed and recommended for the control of *Ae. aegypti* larvae (Mulla *et al*, 2004; Thavara *et al*, 2004). The IGRs are diverse groups of

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synthetic chemical compounds which are highly effective against immature stages of mosquitoes and also other insects, but they possess a good margin of safety for most non-target organisms (Mulla, 1995). Their safety and environmental friendliness offer a potential advantage for their use as larvicides in vector control programs. The IGRs have a specific ability to interrupt the life cycle of insects by inhibiting the maturity of insects and keeping them from reaching the critical adult stage. The compounds having insect growth regulating properties belonging to the classes of benzamides, benzoylureas, carbamates, terpenoids, triazines, and other classes of chemicals (Mulla, 1995). IGRs are now available for testing against *Ae. aegypti*. Mulla and Darwazeh (1988), Mulla (1995), Mulla *et al* (2003), Su *et al* (2003), Martins and da Silva (2004) and Batra *et al* (2005) have reported studies on laboratory evaluation and field efficacy of a number of IGRs against mosquito larvae.

This study was initiated to evaluate residual efficacy of two formulations of diflubenzuron (tablets and granules) against larvae of *Ae. aegypti* in water-storage containers under field conditions in Thailand. Multiple dosages of each formulation were used and the treated jars and controls were challenged weekly with larvae for about 27 weeks.

MATERIALS AND METHODS

Study site

This study was carried out at a field research station for the evaluation of mosquitocidal products and other experimental agents for vector control, in Bang Bua Thong District, Nonthaburi Province, Thailand. A detailed description of the research facilities is given in Mulla *et al* (2003, 2004).

Materials and treatments

Diflubenzuron [1-4(chlorophenyl)-3-(2,6-difluorobenzoyl) urea] is a chitin synthesis inhibitor applied to water in order to control breeding of disease vectors. Two formulations of diflubenzuron: tablets (Bi-Larv T, 2 g in weight/tablet with 40 mg a.i./tablet) and granules (Bi-Larv G, 2% a.i.) were evaluated in this study. These formulations were provided by Crompton Corporation. Each formulation was applied to 200-l glazed-clay jars using 5 dosages (0.02, 0.05, 0.1, 0.5 and 1 mg/l a.i.) and each dosage consisted of 4 jars. To achieve these dosages, each jar of each particular dosage was treated with 1/10, 1/4, 1/2, $2^1/_2$, and 5 tablets of Bi-Larv T, respectively, whereas those treatments with Bi-Larv G were 0.2, 0.5, 1, 5, and 10 g, respectively. The 200-l glazed-clay jars used in this study are commonly used water-storage containers in Thailand. The jars were covered with solid celocrete sheets to prevent wind-borne debris from entering the jars and oviposition by wild mosquitoes and were placed in the shade under a roof. Another concurrent experiment was also carried out using 3 dosages (0.1, 0.5 and 1 mg/l) of both formulations, where half of the water in each treated jar and controls was removed and refilled weekly. The treatments, including controls were arranged in a block design and set in a row from east to west. The jars were treated after addition of the first cohort of larvae and the water loss was replaced weekly.

Assessment of efficacy

The treatments were challenged weekly with a fresh cohort of laboratory reared larvae, where 25 larvae (third instars) of *Ae. aegypti* transferred in water in cups, were added per jar. About 1 g of ground mouse food was added per jar for the larvae. Larval mortality at the start and later adult emergence, evaluated by counting pupal skins, were assessed. It was noted that almost complete mortality occurred in the larval stage, not surviving to adult emergence. Assessment of pupal skins provide a good measure of the yield of emerging adults. Pupal skins were always found in expected numbers in control

jars, but very few if any in the treated jars, except toward the end of the experiment. Pupal skins float on surface, mostly at the meniscus level and can be picked up with a syringe without disturbing the water. The syringed pupal skins were placed in water in white plastic trays and counted. The magnitude of inhibition of emergence was presented in percentage (%EI), which was calculated on the basis of the number of pupal skins (indicating adult emergence) compared to the initial number of larvae added.

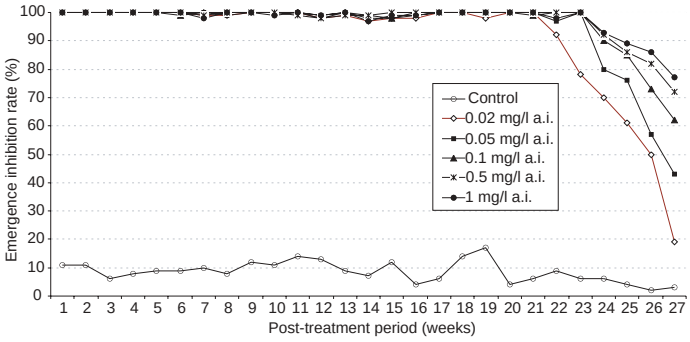

Fig 1–Residual efficacy (% emergence inhibition) of diflubenzuron tablet (40 mg a.i./tablet) at various dosages (mg/l a.i.) in water-storage jars (200 l), kept full without exchange of water.

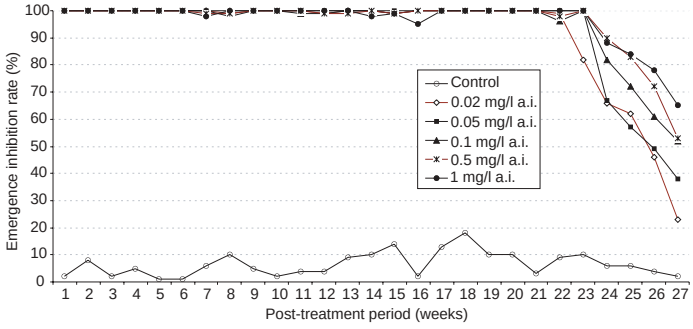

Fig 2–Residual efficacy (% emergence inhibition) of diflubenzuron granules (2% a.i.) at various dosages (mg/l a.i.) in water-storage jars (200 l), kept full without exchange of water.

RESULTS

The residual efficacy of diflubenzuron (tablet formulation) at five different dosages (0.02, 0.05, 0.1, 0.5 and 1 mg/l active ingredient) against larvae of *Ae. aegypti* in constantly full jars is presented in Fig 1. As can be seen, the lowest dosage (0.02 mg/l) of the tablet formulation provided excellent efficacy with a high emergence inhibition rate (96-100%EI) for 21 weeks post-treatment, after which its efficacy declined continuously over the remaining period, reaching about 50%EI at 26 weeks post-treatment, and dropped to about 19%EI by the end of the experiment (27 weeks post-treatment). In contrast, the higher dosages (0.05, 0.1, 0.5 and 1 mg/l) of the tablet formulation demonstrated somewhat longer efficacy for 23-25 weeks post-treatment. After that, the efficacy of these dosages also declined, but the efficacy remained higher than the dosage of 0.02 mg/l during the same period. By the end of this experiment (27 weeks post-treatment), the efficacy of the diflubenzuron tablet at dosages of 0.05, 0.1, 0.5 and 1 mg/l were 43, 62, 72 and 77%EI, respectively. The emergence inhibition rate of the control group in this study was usually low, except in some weeks when it was more than 10%.

Fig 2 shows the residual efficacy of diflubenzuron (granular formulation) at five dosages: 0.02, 0.05, 0.1, 0.5 and 1 mg/l a.i. against the larvae of *Ae. aegypti* in constantly full jars. A high degree of larvicidal efficacy at four dosages (0.05, 0.1, 0.5 and 1 mg/l) of this formulation lasted for 23 weeks post-treatment, similar to that of the tablet formulation. The longevity of efficacy at the lowest dosage (0.02 mg/l) of diflubenzuron granular formulation (22 weeks) was slightly longer than that of the tablet formulation (21 weeks). It is evident that the residual patterns of efficacy of diflubenzuron granular formulation were almost the same as those of the tablet formulation. However, when both formulations reached-

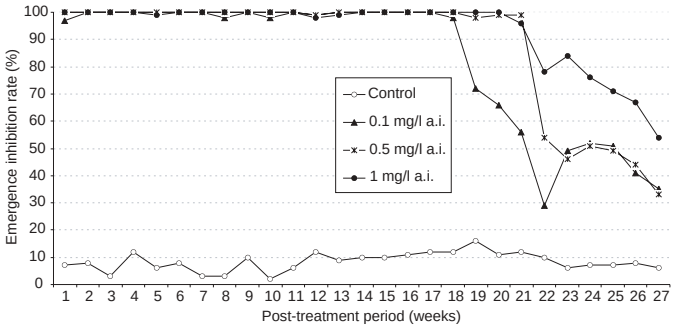

Fig 3–Residual efficacy (% emergence inhibition) of diflubenzuron tablet (40 mg a.i./ tablet) at various dosages (mg/l a.i.) in water-storage jars (200 l), 1/2 water volume removed and refilled weekly.

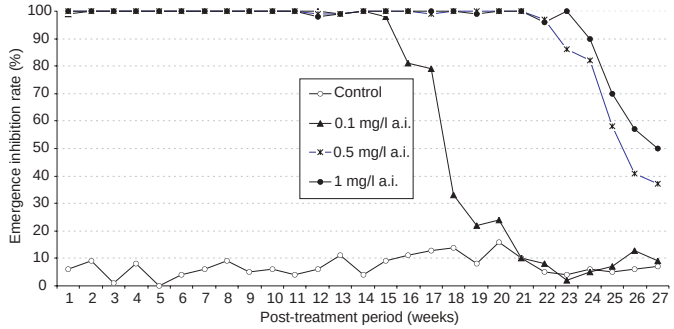

Fig 4–Residual efficacy (%emergence inhibition) of diflubenzuron (granules, 2% a.i.) at various dosages (mg/l a.i.) in water-storage jars (200 l), 1/2 water volume removed and refilled weekly.

their maximum period of excellent efficacy, the efficacy of the granular formulation group declined somewhat more rapidly than that of the tablet formulation group. At the end of this study (27 weeks post-treatment), the efficacy of diflubenzuron granules at the dosages of 0.02, 0.05, 0.1, 0.5 and 1 mg/l a.i. were 23, 38, 52, 53 and 65%EI, respectively.

Under the condition of exchange of half the volume of water (100 l) weekly, the residual efficacy of diflubenzuron (tablet formulation) at three dosages (0.1, 0.5 and 1 mg/l a.i.) against the larvae of *Ae. aegypti* is presented in Fig 3. The high degree of emergence inhibition rate of diflubenzuron tablet at the dosage of 0.1 mg/l a.i. lasted for about 18 weeks after treatment, after which its efficacy decreased rapidly and fluctuated between 29 and 52%EI during the last six weeks of this experiment. Under the same conditions of water removal and refilling weekly, the higher dosages (0.5 and 1 mg/l a.i.) of the diflubenzuron tablet exhibited a longer period of excellent efficacy for 21 weeks post-treatment. After reaching the maximum period of excellent efficacy at 21 weeks post-treatment, the efficacy of the diflubenzuron tablet at a dosage of 0.5 mg/l a.i. dropped rapidly to about 54%EI by the 22nd week, and then fluctuated between 51 and 33%EI during the last five weeks of this experiment. In contrast, the efficacy of the diflubenzuron tablet at the highest dosage (1 mg/l a.i.) declined gradually over the test period from 96%EI at the 21st week post-treatment to about 54%EI at the 27th week post-treatment.

Fig 4 reveals the residual efficacy of diflubenzuron (granular formulation) at three dosages (0.1, 0.5 and 1 mg/l a.i.) against larvae of *Ae. aegypti* under the conditions of half the volume of water (100 l) removal and refilling weekly. The lowest dosage (0.1 mg/l a.i.) showed a high degree of emergence inhibition rate for a period of 15 weeks post-treatment. Its efficacy decreased to about 80%EI during the period between the 16th and 17th week, then dropped sharply to about 33%EI by the 18th week post-treatment, remaining lower than 24%EI until the end of experiment. On the contrary, a longer efficacy was noted for dosages of 0.5 and 1 mg/l for a period of 22 and 23 weeks post-treatment, respectively. After these periods, the efficacy of both dosages declined gradually over the test period. At the end of this study (27 weeks post-treatment), the emergence inhibition rate of the diflubenzuron granules at the dosages of 0.5 and 1 mg/l were 37 and 50%EI, respectively.

DISCUSSION

Diflubenzuron is an IGR which inhibits chitin synthesis of mosquito larvae during ecdysis, affecting larval development at all larval instars and other stages. However, there are significant differences in diflubenzuron inhibiting activity among the instars, and the 3rd instar larvae were found to be the most resistant to the diflubenzuron inhibiting effect (Martins and da Silva, 2004) and diflubenzuron does not cause any reduction in the reproduction potential of *Ae. aegypti* (Fournet *et al*, 1993). The larvae on ingestion of diflubenzuron were unable to complete their molt and subsequently died. This is a completely different mode of action than the other synthetic chemical larvicides and thus provides a novel strategy for resistance management where resistance to conventional larvicides occurs. As temephos has been used for the control of *Ae. aegypti* larvae in Thailand for over 3 decades (Bang *et al*, 1972), it is now suspected that temephos resistance may already have developed or could soon occur in some areas subjected to temephos treatments for long periods. Development of some degrees of resistance to temephos in *Ae. aegypti* in the field has been reported in various places, such as the Caribbean (Georghiou *et al*, 1987), Santa Domingo (Mekuria *et al*, 1991), British Virgin Islands (Wirth and Georghiou, 1999), Brazil (Campos and Andrade, 2001), Thailand (Paeporn *et al*, 2004), and Malaysia (Chen *et al*, 2005).

The present study documents excellent larvicidal efficacy of two formulations of diflubenzuron at various dosages against 3rd instar larvae of *Ae. aegypti* in water-storage jars (200-l capacity) under field conditions. We used the 3rd instar larvae of *Ae. aegypti* for evaluation in order to determine the larvicidal efficacy against the most resistant stage of the tested species. Regarding the water-storage containers used in this study, we employed glazed-clay jars (200-l capacity) as they are the most commonly used containers by dwellers through out the country, which constitutes a major habitat for *Ae. aegypti* in Thailand (Chansang *et al*, 1993; Kittiyapong and Strickman, 1993; Thavara *et al*, 2001). To obtain actual larvicidal efficacy, we also simulated water-use and exchange practices by removal and refilling the water weekly. However, the longevity of diflubenzuron in water-storage jars against *Ae. aegypti* larvae was somewhat decreased by water exchange practices. In the jars without water removal and refill, the two higher dosages (0.5-1 mg/l a.i.) of both formulations provided almost complete efficacy (100 %EI) for 23-24 weeks post-treatment, whereas a similar residual efficacy of both dosages in the jars with water removal and refill lasted for 20-21 weeks (tablets) and 22-24 weeks (granules) post-treatment. The data in our study, support a high level of residual efficacy for both formulations of diflubenzuron against *Ae. aegypti* larvae in water-storage containers under field conditions. Recently, the World Health Organization (WHO) recommended the use of two formulations of diflubenzuron (2% DT and 2% GR) for the control of container-breeding mosquitoes, such as *Ae. aegypti*, at a dosage of 0.02-0.25 mg/l a.i., with an expected residual efficacy of 2 to 4 months (WHO, 2006). In addition, higher rates of application were also recommended for containers with exposure to sunlight or with high organic content. On the other hand, Ansari *et al* (2005) suggested using another two formulations of diflubenzuron (25WP and 22SL) at a dosage of 8 mg/m^2 to control *Ae. aegypti* larvae in unused coolers with complete inhibition (100%EI) for 7 weeks.

In Thailand, the most commonly used larvicide for the control of *Ae. aegypti* larvae is temephos which has been used since the early 1970s (Bang *et al*, 1972). Various formulations of temephos have shown excellent residual effectiveness against *Ae. aegypti* larvae in water-storage containers for several months (Mulla *et al*, 2004; Thavara *et al*, 2004). How-

ever, some formulations of temephos sand granules are objected to by dwellers for use in their water-storage containers because of unpleasant smell and water turbidity after application (Phanthumachinda *et al*, 1985; Thavara *et al*, 2001). Hence, it is likely that at least two formulations of diflubenzuron tested in this study could provide a substitute or an alternative larvicide for the control of *Ae. aegypti* larvae in Thailand as these formulations possess no unpleasant characteristics.

In addition to good effectiveness against *Ae. aegypti* larvae, diflubenzuron also has a wide spectrum of larvicidal activity against various mosquito species, such as *Anopheles quadrimaculatus* Say and *Culex tarsalis* Coquillett (Estrada and Mulla, 1986), *An. culicifacies* Giles, *An. stephensi* Liston and *Cx. quinquefasciatus* Say (Ansari *et al*, 2005). WHO (2006) has also recommended the use of diflubenzuron (2% GR and 25% WP formulations) for mosquito control in open bodies of water at the dosage of 25-100 g/ha a.i. However, higher dosages are required in polluted and vegetated habitats whereas lower dosages are probably adequate for the control of flood-water mosquitoes (WHO, 2006).

Regarding safety, WHO classifies diflubenzuron as unlikely to present an acute hazard in normal use since it has low-acute and chronic toxicity to mammals, with no indication of carcinogenicity, mutagenicity or teratogenicity (WHO, 2006). Diflubenzuron is also environmentally friendly with a low toxicity to birds, fish and aquatic plants. Although diflubenzuron is highly toxic to non-target biota, such as some crustaceans and macro-invertebrates, the resurgence of the affected target and non-target organisms takes place fairly quickly (WHO, 2006).

In conclusion, a high degree of larvicidal efficacy (96-100%EI) was achieved with 4 dosages (0.05, 0.1, 0.5 and 1 mg/l a.i.) of both formulations of diflubenzuron for a period of 23 weeks post-treatment, whereas the efficacy of the lowest dosage (0.02 mg/l a.i.) of the tablet and granular formulations lasted for 21 and 22 weeks post-treatment, respectively. Under conditions of water removal and refilling weekly, high degrees of larvicidal efficacy (96-100%EI) at the 3 higher dosages was obtained with the tablet formulation for 18 to 21 weeks post-treatment, whereas the efficacy of the granular formulation persisted 15 to 23 weeks post-treatment depending on the dosage used. This study clearly demonstrated a high level of residual activity for both formulations of diflubenzuron (tablets and granules) against the larvae of *Ae. aegypti* in water-storage containers. Considering environmental factors and water-use conditions, it is likely that dosages 0.05 to 0.1 mg/l a.i. will be effective dosages to provide long-lasting control for at least 3 to 4 months in the field.

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REFERENCES

Ansari MA, Razdan RK, Sreehari U. Laboratory and field evaluation of Hilmilin against mosquitoes. *J Am Mosq Control Assoc* 2005; 21: 432-6.

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.

Batra CP, Mittal PK, Adak T, *et al*. Efficacy of IGR compound Starycide 480 SC (triflumuron) against mosquito larvae in clear and polluted water. *J Vector Borne Dis* 2005; 42: 109-16.

Campos J, Andrade CF. Larval susceptibility to chemical insecticides of two *Aedes aegypti* populations. *Rw Saude Publica* 2001; 35: 232-6.

Chen CD, Nazni WA, Lee HL, *et al*. Susceptibility of *Aedes aegypti* and *Aedes albopictus* to temephos in four study sites in Kuala Lumpur City Center and Salangor State, Malaysia. *Trop Biomed* 2005; 22: 207-16.

Chansang C, Chansang U, Thavara U, *et al*. The distribution of *Aedes aegypti* in rural areas during 1989-1991. *Bull Dept Med Sci* 1993; 35: 91-106.

Estrada JG, Mulla MS. Evaluation of two new insect growth regulators against mosquitoes in the laboratory. *J Am Mosq Control Assoc* 1986; 2: 57-60.

Fournet F, Sannier C, Monteny N. Effects of the insect growth regulators OMS 2017 and diflubenzuron on the reproductive potential of *Aedes aegypti*. *J Am Mosq Control Assoc* 1993; 9: 426-30.

Georghiou GP, Wirth M, Tran H, *et al*. Potential for organophosphate resistance in *Aedes aegypti* (Diptera: Culicidae) in the Caribbean area and the neighboring countries. *J Med Entomol* 1987; 24: 290-4.

Kittiyapong P, Strickman D. Distribution of container-inhabitation *Aedes* larvae (Diptera: Culicidae) at a dengue focus in Thailand. *J Med Entomol* 1993; 30: 601-6.

Martins F, Da Silva IG. Evaluation of the inhibiting activity of the diflubenzuron on the ecdysis of larvae of *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae). *Rev Soc Bras Med Trop* 2004; 37: 135-8.

Mekuria Y, Gwinn TA, Williams DC, *et al*. Insecticide susceptibility of *Aedes aegypti* from Santo Domingo, Dominican Republic. *J Am Mosq Control Assoc* 1991; 7: 69-72.

Mulla MS. The future of insect growth regulators in vector control. *J Am Mosq Control Assoc* 1995; 11: 269-73.

Mulla MS, Darwazeh HA. Efficacy of new insect growth regulators against mosquito larvae in dairy wastewater lagoons. *J Am Mosq Control Assoc* 1988; 4: 322-5.

Mulla MS, Thavara U, Tawatsin A, Chompoosri J, Zaim M, Su TY. 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.

Paeporn P, Ya-umphan P, Suphapathom K, *et al*. Insecticide susceptibility and selection for resistance in population of *Aedes aegypti* from Ratchaburi Province, Thailand. *Trop Biomed* 2004; (suppl): 1-6.

Phanthumachinda B, Phanurai P, Samutrapongse W, *et al*. Studies on community participation in *Aedes aegypti* control at Panus Nikhom District, Chonburi Province, Thailand. *Mosq-Borne Dis Bull* 1985; 2: 1-8.

Su T, Mulla MS, Zaim M. Laboratory and field evaluations of novaluron, a new insect growth regulator (IGR), against *Culex* mosquitoes. *J Am Mosq Control Assoc* 2003; 19: 408-18.

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.

WHO. Report of the ninth WHOPES working group meeting, WHO/HQ, Geneva, 5-9 December 2005. *WHO/CDS/NTD/WHOPES/2006.2*. 2006.

Wirth MC, Georghiou GP. Selection and characterization of temephos resistance in a population of *Aedes aegypti* from Tortola, British Virgin Islands. *J Am Mosq Control Assoc* 1999; 15: 315-20.