FIELD EVALUATION OF NOVALURON, A CHITIN SYNTHESIS INHIBITOR LARVICIDE, AGAINST MOSQUITO LARVAE IN POLLUTED WATER IN URBAN AREAS OF BANGKOK, THAILAND

Apiwat Tawatsin[1], Usavadee Thavara[1], Payu Bhakdeenuan[1], Jakkrawarn Chompoosri[1], Padet Siriyasatien[2], Preecha Asavadachanukorn[3] and Mir S Mulla[4]

[1]National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi; [2]Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Bangkok; [3]Department of Statistics, Faculty of Commerce and Accountancy, Chulalongkorn University, Bangkok, Thailand; [4]Department of Entomology, University of California, Riverside, California, USA

Abstract. Novaluron, an insect growth regulator, a benzoylphenyl urea insecticide, was evaluated in the field against the larvae of polluted-water mosquitoes. The study was carried out in highly polluted sites infested with populations of mosquito larvae, mostly *Culex quinquefasciatus* Say, in low-income communities in urban areas of Bangkok, Thailand. An EC10 formulation was premixed in water and applied by pressurized spray tank to plots ranging from 180 to 1,000 m^2 at the rate of 0.1 ml EC10/m^2 (equal to 10 mg a.i./m^2) of the breeding sites. Assessments were made by sampling mosquito larvae and pupae to determine the trends of immature populations before treatment and weekly after treatment. Reduction of the populations in percents were then computed by comparing counts of immature mosquitoes (larvae and pupae) to the pretreatment counts at each particular site. It was found that the immature populations of mosquitoes in the treated areas were dramatically suppressed and remained at extremely low levels for 3-7 weeks after the treatment depending on the prevailing conditions of each experimental site. No negative impact on fishes or aquatic plants in the treated areas were detected during the study period and three months after the experiment was discontinued. Novaluron is an effective agent to control immature populations of polluted-water mosquitoes, especially *Cx. quinquefasciatus* in habitats in urban areas. This IGR larvicide may play an important role in vector control programs in terms of effectiveness, environmental friendliness and strategies for insecticide-resistance management in vector mosquitoes.

INTRODUCTION

The control of polluted-water mosquitoes, especially *Culex quinquefasciatus* Say in urban and suburban areas in Thailand is usually carried out by the municipality, district health office or local administrative unit of each area. These agencies always use thermal fogging with various chemical insecticides, mainly pyrethroids as the main strategy to control mosquitoes. This operation, however, mainly affects adult mosquitoes for a short period, Evidence of resistance to various groups of chemical insecticides by *Cx. quinquefasciatus* has been reported in several provinces of Thailand (Wattanachai *et al*, 1996; Somboon *et al*, 2003; Sathantriphop *et al*, 2006) as well as in various parts of the world, such as France (Yebakima *et al*, 1995), Colombia (Bisset *et al*, 1998), West Africa (Chandre *et al*, 1998),

Correspondence: Dr Apiwat Tawatsin, National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi 11000, Thailand.
Tel: 66 (0) 2951-0000 ext 99245; Fax: 66 (0) 2591-5449
E-mail: apiwat@dmsc.moph.go.th

USA (Liu *et al*, 2005) and Malaysia (Nazni *et al*, 2005). The resistance to chemical insecticides by *Cx. quinquefasciatus* mosquitoes may have been induced by repeated applications of the same kind or group of insecticides by mosquito control programs. The common use of household products containing mainly pyrethroids, carbamates and organophosphates to control insect pests in dwellings may cause insecticide resistance in mosquitoes (Charoenviriyaphap *et al*, 1999). Attempts have been made to control mosquitoes by using the microbial agent *Bacillus sphaericus* Neide. Many formulations of *B. sphaericus* larvicide have demonstrated high degrees of efficacy and long residual control of the larvae of *Cx. quinquefasciatus* in polluted water in artificial pools (Pantuwatana *et al*, 1989) and in urban and suburban communities in Thailand (Mulla *et al*, 1997, 1999, 2001). However, the emergence of resistance in field populations of *Cx. quinquefasciatus* to *B. sphaericus* has been described in Thailand (Mulla *et al*, 2003) and India in the past decade (Rao *et al*, 1995). This is an urgent need to develop strategies to control polluted-water mosquitoes, especially *Cx. quinquefasciatus* that have already developed resistance to chemical insecticides as well as the microbial larvicide *B. sphaericus*. Insect growth regulators (IGRs) are then considered as a novel group of insecticides for controlling these insecticide-resistant mosquitoes.

IGRs, in general, exhibit a good margin of safety to most non-target biota, thus offering some advantages in mosquito control programs (Mulla, 1995). Many IGR compounds and products have been evaluated for larvicidal activity against various mosquito species, such as *Culex peus* Speiser and *Cx. quinquefasciatus* (Mulla and Darwazeh, 1988), *Aedes aegypti* (L.) (Mulla *et al*, 2003), *Culex* mosquitoes (Su *et al*, 2003), *Anopheles* and *Culex* mosquitoes (Batra *et al*, 2005). Most IGRs provide good larvicidal efficacy for the control of targeted mosquitoes, depending on the active ingredients, formulations, dosages, and the habitats treated.

Novaluron is an IGR of the benzoyl urea group, which inhibits chitin synthesis, affecting the moulting process of insects. It has low acute, sub-acute and chronic toxicity to humans, birds, earthworms, fish and aquatic plants, but is highly toxic to some crustaceans (WHO, 2005). Recently, a few reports documented the larvicidal efficacy of novaluron under laboratory and field conditions against the larvae of *Ae. aegypti* (Mulla *et al*, 2003) and *Culex* mosquitoes (Su *et al*, 2003). The present study was carried out to evaluate the field efficacy of a formulation of novaluron against immature mosquitoes in highly polluted developmental sites infested with heavy populations of mosquito larvae and pupae in low-income communities in urban areas of Bangkok, Thailand.

MATERIALS AND METHODS

Experimental material

An EC10 (emulsifiable concentrate containing 10% active ingredient) formulation of novaluron was provided by SCK (269) Co, Ltd, Thailand. This material was produced by Makhteshim Chemical Works Ltd, Israel. This formulation is a new product that has not been previously evaluated against polluted-water mosquitoes in the field in Thailand.

Study sites

The four polluted-water mosquito breeding sites used in this study (three sites were waste-water accumulations but another one was a stagnant canal) are located in communities in the suburbs of Bangkok, Thailand. These communities have clusters of low-income housing without adequate waste-water disposal systems. All houses are elevated on posts; the domestic waste-water accumulates under and between the houses. The study sites are discribed below.

Krunai site 1. This site has waste-water accumulation, having the largest area of about 1,000 m^2, with a water depth ranging from 10 to 30 cm. Most of the area beneath the houses is covered with stagnant polluted water. The water level at this site is influenced by domestic water usage and rains; there is no gate for flood control.

Krunai site 2. This site has waste-water-accumulation, having an area of about 180 m^2, with a water depth ranging between 10 and 30 cm. The water level fluctuates with domestic water usage and rains. It is located near Krunai site 1 (~ 400 m apart).

Janpradittharam site 1. This site is located about 30 km from Krunai site 1 and Krunai site 2. It also has waste-water accumulation, covering an area of about 220 m^2, with a water depth ranging from 15 to 40 cm. The water level is influenced by domestic water usage and rains. The water is more polluted than the above 2 sites, there was some open space in the site exposed to the sun.

Janpradittharam site 2. This site is located near Janpradittharam site 1 (~ 600 m apart). It is a stagnant canal, having an area of about 500 m^2, with a water depth ranging from 50 to 100 cm. The water was highly polluted and the water level was influenced by opening and closing the flood-control gate, domestic water usage and rains. The flow rate of the water in the canal was relatively low, except some days when the gate was opened for water draining.

Application

The required amount of the EC10 formulation of novaluron for the given site and dosage was placed in a compression spray tank (8 liters), then the required amount of tap water was added and stirred with a wooden paddle. The tank was sealed, shaken well, and pumped to pressurize the mixture for spraying. The mixture was sprayed through a cone nozzle, which produced a stream that reached up to 5 meters away. In this study novaluron was applied at a concentration of 0.1 ml of EC10 per m^2 (equal to 10 mg a.i./m^2) to the breeding sites.

Sampling

Sampling for immature mosquitoes (larvae and pupae) was conducted by dipping with a standard 400 ml dipper at each study site before and after treatment. Samples were taken from locations where heavy aggregations of larvae and pupae occurred. The contents of each dipping were transferred into white plastic trays (30 x 15 x 4 cm deep) for counting. The study sites having large areas, such as Krunai site 1 and Janpradittharam site 2, were sampled by taking 30 dips for each assessment, whereas 15 dips were taken from the smaller sites (Krunai site 2 and Janpradittharam site 1). Some larvae and pupae from each study site were taken and brought to laboratory for species identification.

Efficacy assessment

Assessments were made by sampling mosquito larvae and pupae before treatment (two weeks) and weekly after treatment. Mean and standard error (SE) values for the immature populations (larvae and pupae) for each sampling date and treatment were determined. Reduction rates (%) for the immature mosquitoes post-treatment (weeks 3-12) were computed by comparing counts of immature mosquitoes to the average number obtained from the pre-treatment counts (weeks 1 and 2) for each particular site. These values (mean number, SE and % reduction) were inserted into the figures.

RESULTS

Krunai site 1, a treated area in this study, received a dose of EC10 of novaluron at 10 mg a.i./m^2. The population sizes of immature mosquitoes during preliminary surveys (weeks 1 and 2) were relatively high (average 310-434 larvae and pupae per dip) with an average number of 372 per dip. The treatment provided excellent control (92-100% reduction) in immature mosquitoes for seven weeks post-treatment (Fig 1). After this period, a high degree of control (81-82% reduction) was achieved for two more weeks, with a moderate resurgence of immature mosquitoes. Ten weeks after treatment, the efficacy had dramatically declined to about 49% compared to the average number of the immature mosquitoes before treatment. There were some light rains at the Krunai site 1 during the course of this study.

Fig 2 demonstrates the natural fluctuation in field populations of immature mosquitoes at Krunai site 2, the control area of this study. No treatments were made at this site in order to observe concurrent immature populations in comparison with those of the treated sites. The population of immature mosquitoes remained moderate to high throughout the experimental period (12 weeks). The average number of immature mosquitoes fluctuated between 216 and 355 per dip, whereas the average number obtained during weeks 1 and 2 (before treatment) was 279 per dip (Fig 2). The immature mosquito population in the control area did not change much compared to the average number for weeks 1 and 2 (279 per dip), but did during week 6 (21%), week 7 (-27%) and week 11 (23%). Some light rains were recorded in this area.

Janpradittharam site 1, another treated area, also received a single treatment with novaluron at the same dosage on the same day as the Krunai site 1. The population sizes of immature mosquitoes during preliminary surveys were heavy (average 226-272 larvae and pupae per dip). The average number of immature mosquitoes before treatment (weeks 1 and 2) was 249 per dip. Treatment with novaluron yielded excellent control (92-99% reduction) in all immature mosquitoes for three weeks post-treatment (Fig 3). After that, a moderate degree of control (69-71% reduc-

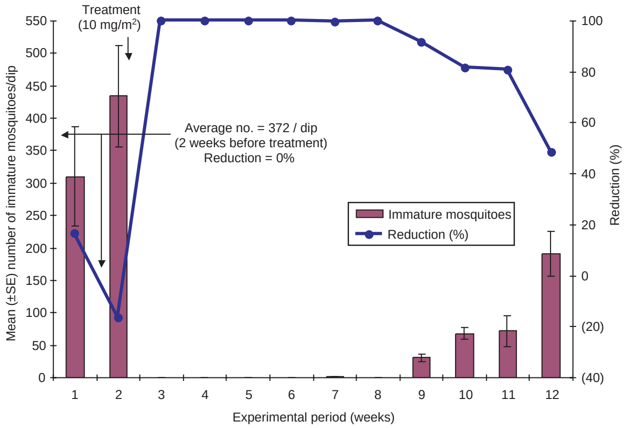

Fig 1–Field efficacy of novaluron treated at the dosage of 10 mg/m^2 at Krunai site 1.

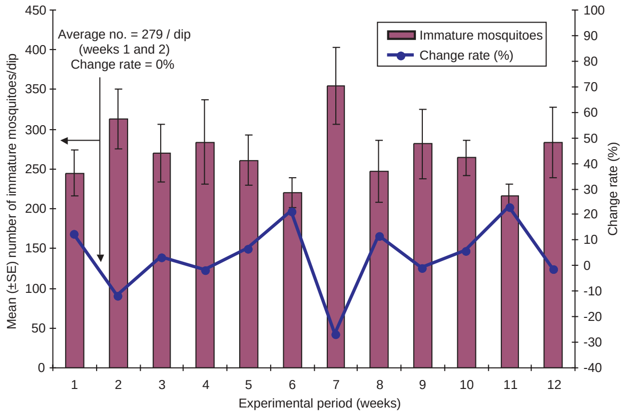

Fig 2–Field populations of immature mosquitoes at Krunai site 2 (check area).

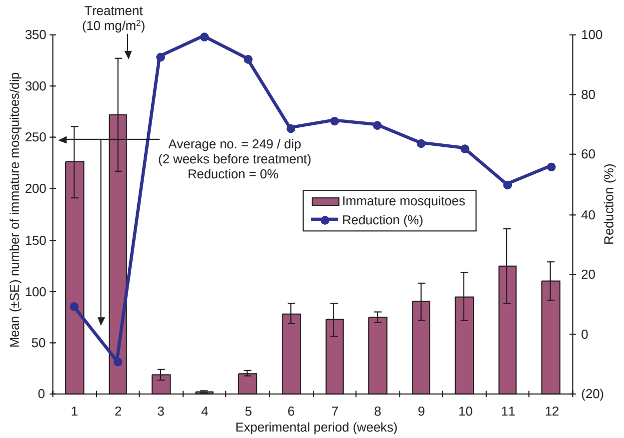

Fig 3–Field efficacy of novaluron treated at the dosage of 10 mg/m^2 at Janpradittharam site 1.

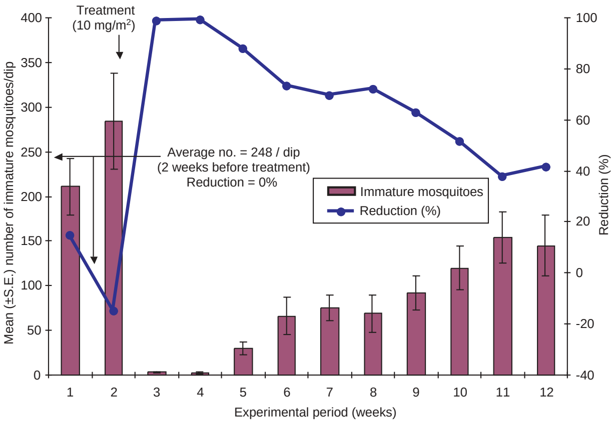

Fig 4–Field efficacy of novaluron treated at the dosage of 10 mg/m^2 at Janpradittharam site 2.

tion) was achieved for three more weeks. The residual efficacy then dropped over the time and remained at about 56% by the end of the experiment (10 weeks post-treatment). Light to heavy rains occurred in the Janpradittharam site 1 during the study period.

Janpradittharam site 2, the stagnant canal, was treated with novaluron at a dosage of 10 mg a.i./m^2 on the same day, as the other two treated sites (Krunai site 1 and Janpradittharam site 1). The populations of immature mosquitoes before treatment were high (211-284 larvae and pupae per dip) with an average number of 247 per dip. Similar to the Janpradittharam site 1, the treatment provided excellent control (88-99% reduction) of all immature mosquitoes for three weeks post-treatment (Fig 4). Afterward, a moderate degree of control (70-73% reduction) was obtained for three more weeks. The field efficacy of novaluron then declined over the experimental period. The reduction rate remained about 41% by the time the study was discontinued (10 weeks post-treatment). Janpradittharam site 2 also received some light to heavy rains during the course of this study; as a result, some water was drained out several times.

In this study, *Cx. quinquefasciatus* was the dominant species (>98%) found in all experimental sites, whereas the other species, *Cx. gelidus* Theobald and *Armigeres subalbatus* Coquillett were present in small proportions (< 1%). According to our direct observations, no negative impact on mosquito fish (*Gambusia affinis* Baird & Girard) or aquatic plants, such as water morning glory (*Ipomoea aquatica* Foisk) and water hyacinth [*Eichhornia crassipes* (Mart.) Solms] in the treated areas was detected during the study and for three months after the experiment was discontinued.

DISCUSSION

In our experiment, we used the dosage (10 mg a.i./m^2) for novaluron as a single treatment and we attempted to obtain residual effects against target mosquitoes in the treated sites, containing highly polluted waters. It is interesting to note that a single treatment of an EC10 formulation of novaluron at a dosage of 10 mg a.i./m^2 provided excellent control (90-100% reduction) of the immature populations of polluted-water mosquitoes in the treated areas for three to seven weeks. The differences in residual efficacy depended on the prevailing conditions for each site, such as the degree of water pollution, sun exposure and extent of rains and water drainage. These conditions were encountered in two treated sites (Janpradittharam site 1 and Janpradittharam site 2), which yielded excellent residual control for only three weeks. In contrast, Krunai site 1, another treated site where less rain and drainage was noted provided longer residual control for seven weeks. Similarly, a study conducted in India also showed that novaluron 10% EC when applied at the same dosage (10 mg a.i./m^2) exhibited high degrees of residual effects against *Cx. quinquefasciatus* larvae for 13 days in cesspits, 17 days in drains and 69 days in unused wells (Jambulingam *et al*, unpublished report to the WHO Pesticide Evaluation Scheme, 2004). In smaller breeding sites, such as buckets (18 liter capacity), novaluron 10% EC provided excellent control (>90% inhibition of the emergence) of wild populations of *Cx. quinquefasciatus* for as long as 10 weeks (Arredondo-Jimenez and Valdez-Delgado, unpublished report to the WHO Pesticide Evaluation Scheme, 2004).

Su *et al* (2003) revealed the duration of a high level of efficacy (>90% inhibition of emergence) of novaluron against the larvae of *Cx*. *quinquefasciatus* tested in mesocosms (27 m^2) were 7 and 13 days at dosages of 1-5 and 10 mg/m^2, respectively; whereas a duration of 14 days was achieved at a dosage of 1.25-5 mg/m^2 tested in a microcosm (240 liters) where the water was enriched with rabbit pellets. Field evaluation of novaluron at dosages of 1-10 mg/m^2 against *Cx*. *quinquefasciatus* conducted in India also demonstrated that the effective duration (>80% inhibition of emergence) obtained in cesspits (1-15 m^2), street drains (4-6 m^2) and wells (1.3-4 m^2) were 11-13, 8-17 and 33-69 days, respectively (Jambulingam *et al*, unpublished report to the WHO Pesticide Evaluation Scheme, 2004). As can be seen, the extent residual activity of novaluron against *Cx*. *quinquefasciatus* depends markedly on two major factors: dosages used and types of larval habitats. The activity of novaluron is also degraded by environmental factors, such as ultraviolet light and organic pollution (Su *et al*, 2003).

As mentioned earlier, *Cx. quinquefasciatus* has already developed resistance to some chemical insecticides as well as the microbial larvicide *B. sphaericus* in Thailand and other places around the world; therefore, IGRs including novaluron may be effective larvicides for the control of *Cx. quinquefasciatus* mosquitoes. There have been no reports of resistance to novaluron in this mosquito species. However, monitoring of resistant status in target mosquitoes should be carried out when novaluron or other IGRs are applied in the field.

As tested against other mosquito species, novaluron demonstrated a high level of residual activity against *Anopheles* larvae (*An. culicifacies* Giles and *An. subpictus* Grassi) in riverine pools (at a dosage of 0.01 mg a.i./l) for 63 days and in gem pits (at dosages of 0.01-0.1 mg a.i./l) for 124 days (Yapabandra, unpublished report to the WHO Pesticide Evaluation Scheme, 2004). Novaluron provided complete coverage (at dosages of 0.0166-0.0498 mg a.i./l) against the larvae of *An. albimanus* Wiedemann and *An. pseudopunctipennis* Theobald confined in sentinel cages placed in artificial pools was also achieved for 16 weeks (Arredondo-Jimenez and Valdez-Delgado, unpublished report to the WHO Pesticide Evaluation Scheme, 2004). Novaluron provided excellent control of *Ae. aegypti* larvae in 200 liter water-storage jars at dosages of 10-20 µg a.i./l for at least two months (Mulla *et al*, 2003); applications at dosages of 0.055 -0.165 mg a.i./l yielded high mortality (>85%) for *Ae. aegypti* and *Ae. albopictus* (Skuse) larvae in sentinel cages placed in 18 liter buckets for 14 weeks (Arredondo-Jimenez and Valdez-Delgado, unpublished report to the WHO Pesticide Evaluation Scheme, 2004).

The WHO (2005) recommends the use of novaluron as larvicide applied in non-drinking water-storage containers, temporary mosquito habitats and polluted waters at the dosage of 10-50 µg a.i./l or 10-100 g a.i./ha; however, the higher dosages are required for polluted and vegetated habitats and for obtaining longer residual efficacy. It was stated by the WHO (2005) the level of control by novaluron at various dosages is comparable to pyriproxyfen and better than methoprene (Mulla *et al*, 1986, 1989).

Regarding environmental concerns, novaluron at a dosage of 10 mg a.i./m^2 had no impact on mosquito fishes and aquatic plants in treated areas during and after the experiment. A similar safety profile for novaluron with non-target fauna in riverine pools was also found for guppies (*Poecilia reticulata*), a native fish species of Sri Lanka (*Rosbora daniconis*) and aquatic beetles, when applied at concentrations of 0.01-2.5 mg a.i./l (Yapabandra, unpublished report to the WHO Pesticide Evaluation Scheme, 2004).

In conclusion, our findings show novaluron (10% EC) may be used as an effective larvicide to control immature polluted-water mosquitoes, such as *Cx. quinquefasciatus* larvae, with residual effects for three to seven weeks after a single treatment at a dosage of 10 mg a.i./m^2. Re-treatment with this IGR, may be justified, depending on the prevailing conditions of the treated sites. This IGR larvicide may play an important role in operational vector control programs in terms of effectiveness, environmental friendliness and as a strategy for managing insecticide-resistant vector mosquitoes. Further field studies of novaluron against a range of mosquito species in various habitats in Thailand are warranted.

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