MOSQUITO LARVICIDAL TRAP (MLT) AS SURVEILLANCE AND CONTROL TOOL FOR *AEDES* MOSQUITOES

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Abstract. The larvicidal potential and ovipositional effect of an insect growth regulator, 2% Novaluron (Mosquiron), in an oviposition trap or ovitrap on dengue mosquito vectors were determined under field condition. The set-up is hereafter referred to as Mosquito Larvicidal Trap (MLT). This MLT is a modification of an ovitrap utilized by De Las Llagas *et al* (2002, 2007, 2012) in all dengue mosquito surveillance and control studies in the Philippines. Variation entails mixing 40 parts per million (ppm) of an insect growth regulator (2% Novaluron), proven to have an attractant effect in an ovitrap clean water. The study was conducted from July 2012 to June 2013 in Barangay Batong Malake and Barangay San Antonio in Los Baños, Laguna, both places known as endemic sites for dengue transmission. The MLTs were installed and serviced every three weeks inside each household. Two hundred twenty households per study site were installed with MLT. Efficacy of MLT as a surveillance tool was measured against the conventional inspection of containers with water and the classical ovitrap. The Breteau Index (BI), Ovitrap Index (OI), and MLT positive for immatures of mosquito were determined. Breteau and ovitrap indices are known entomological indicators depicting the level of household mosquito infestation. The MLT as an autocidal/larvicidal trap was gauged in terms of its relative attractiveness to ovipositing mosquitoes compared to other containers and the classical ovitrap. Results showed that MLT was more attractive to *Aedes* for egg deposition than ovitrap or other containers inside the house. The mean MLT found positive with mosquito was 70.5% in Batong Malake while the OI was 53.5%. In San Antonio, the MLT was 56.6% compared to 50.4% OI. The added Novaluron increased the attractiveness to female mosquitoes to lay their eggs and has killing effect on the larvae. The findings indicate that MLT is more attractive than containers or ovitraps as oviposition medium. It is also lethal to the deposited eggs. Moreover, MLT prevented pupal development, indicating its autocidal activity to larvae. Thus, MLT prevents adult emergence by preventing pupal development. In conclusion, the use of MLT as an integral component of dengue vector control program will enhance reduction in density of mosquitoes at

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immature stages thus lessening dependency on adulticiding efforts. With the threat of vertical and transstadial dengue transmission in nature, managing the vector at its youngest developmental stage must be done.

Keywords: *Aedes* spp, dengue, ovitrap, mosquito larvicidal trap, surveillance and attractant tool, Philippines

INTRODUCTION

Aedes aegypti and *Aedes albopictus* mosquitoes are known vectors of dengue, chikungunya, and yellow fever (Gaedner and Ryman, 2010; Guzman *et al*, 2010). Presently, dengue is the most important among the arboviruses transmitted by mosquitoes throughout the world. It has become a public health problem in all of the continents, except Europe (Nogueira *et al,* 2001; Fontoura *et al*, 2012).

Researchers from the University of Oxford and the Wellcome Trust reported that around 390 million infections occur annually, of which 96 million are severe cases and approximately 300 million are mild or asymptomatic episodes (Hirschler, 2013). The data compares with the World Health Organization's (WHO) most recent estimate for overall infections of 50-100 million a year (WHO, 2007) and approximately 2.5 billion people live in areas where dengue can be transmitted (Gubler, 1998). Dengue has spread rapidly along with urbanization and globalization because it thrives in tropical mega-cities. Half of the world's population is now exposed to the disease and it occurs mostly in the developing countries (Hirschler, 2013).

In the Philippines alone, the reported dengue cases were 42,207 with 193 deaths in 2013. Although there was almost 50% reduction of cases to 23,867 in January to May 31, 2014 (Herriman, 2014), the disease is still a major medical and public health problem. In the absence of vaccines or anti-drugs, prevention of dengue fever and its more severe forms is of primary importance. Rapid responses to dengue outbreaks are needed in order to control the spread of the virus and to manage the high number of cases (Pilger *et al*, 2010). Currently, vector control remains the key strategy in dengue prevention and control (Ng, 2011), as it reduces or interrupts the dengue virus transmission. It also plays a critical role in the prevention and containment of epidemics. One of the strategies in vector control is mosquito surveillance (NEA, 2006). Regis *et al* (2008) indicated that the implementation of preventive measures targeting critical locations at specific times requires efficient vector surveillance tools and methods sensitive enough to predict or detect in real time a sudden mosquito population growth.

Several tools and methods have been designed for vector surveillance to establish the minimum threshold of vectorial density (*eg*, vector indices). The use of oviposition traps or ovitraps (Fay and Eliason, 1966) is a possibility to indirectly estimate the female mosquito population. An ovitrap is a device which consists of a black cylinder with a piece of hardboard (Cheng *et al,* 1982; Wan Norafikah *et al,* 2011), cloth (Lenhart *et al,* 2005) or wood pallet (Focks, 2003; Regis *et al,* 2008). It is a technique recognized by the WHO, which can attract *Aedes* females to oviposit (WHO-WPRO, 1995).

The ovitrap was first used for the

control of *Aedes aegypti* in 1969 at the Singapore International Airport (Chan, 1973). Chan (1973) designed an autocidal screened ovitrap that attracted more *Ae. aegypti* than the other domestic container habitats in field tests. Rawlins *et al* (1998) reported that *Ae. aegypti* surveillance using ovitraps is more sensitive than visual inspection for larvae. A study showed that the ovitrap technique is a sensitive method in detecting *Aedes* population even at low infestation levels (Lee, 1992). De Las Llagas and Medina (2002) validated that the ovitrap technique can still detect *Aedes* density even during low density months or hot-dry months of the year. Lenhart *et al* (2005) recommend the modified ovitrap as a simple, inexpensive and sensitive tool for both monitoring oviposition and collecting large quantities of *Ae. aegypti* eggs. Ovitraps are now commonly used as a surveillance or monitoring tool in the field (Wan Norafikah *et al,* 2011). The simplicity, specificity and effectivity of the ovitraps are the proven advantages of this technique as surveillance tool.

Information generated from ovitrap has been successfully used to monitor the impact of various types of control measures involving infestation source reduction and insecticide applications (Focks, 2003). The ovitrap technique has been used to monitor the *Ae. aegypti* pre- and post- treatment density counts after cycles of thermal spraying (De Las Llagas and Cruz, 1994; De Las Llagas *et al*, 2007). It has been used to identify areas with high concentrations of mosquitoes based on the egg density index (Regis *et al*, 2008) and detection of mosquitoes from unexposed breeding sites and surrounding areas (Ho *et al,* 2005). Ovitrap data collected weekly can be used to identify mosquito breeding hotspots and risk areas when there is a danger of high *Ae. aegypti* infestation. Reports showed that ovitraps attracted more ovipositions of *Ae. albopictus* females than in natural and other artificial containers (Ang *et al,* 1995; Yap *et al,* 1995).

According to Tang *et al* (2007), ovitrap can be used for the control of dengue vectors. An ovitrap can be lethal to the eggs once a larvicide is incorporated on the oviposition substrate or in the water. The lethal ovitrap allows egg deposition but it prevents adult emergence, thus adversely affecting mosquito daily emergence rate. The modified ovitrap of De Las Llagas *et al* (1994, 2002, 2007, 2012) uses an evaporated milk-size can, containing 250-370 ml of water and a *lawanit* (a low cost alternate to ordinary plywood) paddle. The modified ovitrap is incorporated with either synthetic chemical or organic material which kills mosquitoes in different immature stages (ovicidal trap). Studies have shown that ovicidal traps have been effective in reducing the mosquito *Ae. aegypti* densities in Singapore (Teng, 2001; Tang *et al* 2007), Brazil (Perich *et al,* 2003), and Philippines (De Las Llagas and Cruz, 1994).

In this study, incorporation in the modified ovitrap by De Las Llagas *et al* (2002, 2007, 2012) of an insect growth regulator (IGR), that is, Novaluron, a benzoyliphenylurea (Mosquiron, Leads Philippines), as larvicide was done to determine its effect on the oviposition of *Aedes* mosquitoes. The ovicidal trap technique was assessed in terms of reduction in mosquito densities in the study sites, specifically the inhibition of pupal development. Results from this study will provide the operational basis for using ovicidal traps as a community-based dengue mosquito control technique and as an important component of an integrated vector control system.

MATERIALS AND METHODS

Study sites

The study was conducted in two sites (Batong Malake and San Antonio) in Los Banos, Laguna, Philippines from July 2012 to June 2013 following an experimental study design. The climate or temperature in Los Banos was generally cool throughout the year especially during the months of November to February. The rainy season started in May and lasted up to December while the rest of the year was dry. The temperature ranged from 27.1°C to 33.7°C with an average of 30.4°C in Batong Malake. In San Antonio, the lowest and highest temperatures recorded were 27.5°C and 33.7°C, respectively, with an average of 31.0°C.

The chosen study sites were communities in Los Banos, Site A, Barangay Batong Malake [Lat (DMS) 14º10´40N; Long (DMS) 121º14´32E] and Site B, Barangay San Antonio [Lat (DMS) 14º10´0N; Long (DMS) 121º15´0E]. These sites were chosen because they have high incidences of dengue cases, 8 and 10 reported cases of dengue as of April 2012 for Site A and Site B, respectively (Rural Health Unit 1, 2012).

Site A was considered as a high density residential area according to the Zoning Ordinance of Los Banos, Municipal Planning and Development Office in 2007. It has a population of 9,271 and 2,430 households living in 2,142 houses. Residents of Site A were composed of natives of Los Banos, Laguna and some informal settlers. Site B has a total land area of 301.52 hectares comprising of 6 *puroks* or districts. It is considered as a medium density residential area according to the Zoning Ordinance of Los Baños. It has a population of 13,411 and the number of houses in the barangay is 3,091.

These sites were chosen because they represent two different ecological environments of the two dengue vectors, *Aedes aegypti* and *Aedes albopictus*. *Aedes aegypti* breeds in artificial containers, such as old and unused tires, drums, vases and any container that will hold water for at least five days. *Aedes albopictus* can breed in both artificial and natural containers such as bamboo stumps and leaf axils. This species also prefers to breed in areas with vegetation.

Ovitrap set-up and monitoring

An empty evaporated milk can, painted black both inside and outside, and can hold approximately 370 ml of water was used as ovitrap (Fig 1). *Lawanit* board, measuring 6 x 1 in, was used as paddle or substrate for oviposition of *Aedes* mosquitoes. The paddle was soaked wet with water and positioned at the side of the ovitrap can containing 250-270 ml of water with or without larvicide (Fig 1A).

Mosquito Larvicidal Trap (hereafter referred to as MLT; Fig 1B) was prepared using an ovitrap containing 40 ppm of 2% Novaluron (Mosquiron), chemically known as 1-(3-chloro-4-(1, 1, 2-trifluro-2-trifluoromethoxy-ethoxy) phenyl)-3-(2, 6-difluorobenzoyl) urea, dissolved in water. Novaluron is a chitin synthesis inhibitor (CSI) type of insect growth regulator (IGR). The recommended application rate of Novaluron was 0.5-2.0 ml per cubic meter of water. Two MLTs were prepared for each household and these were installed inside (indoor) of each fixed sample house. The MLTs were installed and serviced every three weeks inside households. Two hundred twenty households per study site were installed with MLT trap. The efficacy of MLT as a surveillance tool was measured against the conventional inspection of containers

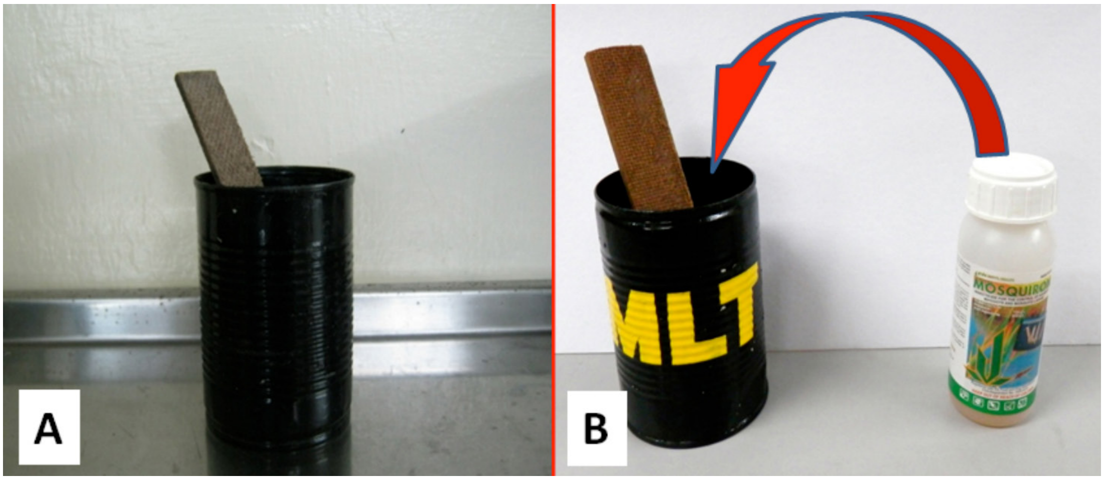

Fig 1–(A) An ovitrap and (B) Mosquito Larvicidal Trap (MLT) incorporated with 2% Novaluron with paddle made of *lawanit* as oviposition substrate for *Aedes* mosquito (Los Banos, Laguna, 2013 June).

Fig 2–Examples of water-holding containers monitored in each fixed household to determine the presence of the immature stages of mosquito (Los Banos, Laguna, June 2013). (A) Defrost tray of a refrigerator; (B) Drums, pails, and basins; (C) Coconut shell used as receptacle for rain water; (D) Dish rack tray.

with water and the classical ovitrap. Two ovitraps with water only were installed per household to serve as control (hereafter referred to as Ovitrap). Installation of traps, inspection and monitoring were done weekly and carried out by a team of 10 persons per study site. The MLTs were installed and collected every 21 days, that is, on the 4th, 7th, 10th, 13th, 16th, 19th, 22nd, 25th, 28th, 31st, 34th, 37th, 40th, and 43rd week of the study.

Monitoring of indoor ovitraps was conducted every three and five days to observe for the presence of eggs deposited on the sides of each trap and larvae and/or pupae in the water, respectively. The presence of eggs, larvae and pupae in the water as well as the conditions of the MLT and Ovitrap (absence of water due to accidental spillage, broken paddle, and so forth) was observed and recorded every monitoring period. Surveillance of water-holding containers, such as drum, pail, basin, defrost tray, dish rack and others, in each household was also conducted weekly to determine whether or not they were infested with mosquitoes (Fig 2).

The number of larvae and/or pupae per trap in each study site and the number of traps with immature stages of mosquito were recorded using Microsoft Excel 1997-2003 in Windows Vista (2007, Microsoft, Singapore). Data obtained were used for the computation and analysis of the Breteau Index (BI), Ovitrap Index (OI), and MLT positive for the immature stages of mosquito using the following formula:

Surveillance tool

Breteau Index (BI). BI refers to the percentage of positive containers per 100 households inspected in the study sites.

$$BI = \frac{\text{Number of positive containers (w/larvae/pupae)}}{\text{Number of households inspected}} \times 100$$

Ovitrap Index (OI). OI refers to the percentage of ovitraps positive for *Aedes* immatures.

$$OI = \frac{\text{Number of ovitraps with larvae/pupae in water and/or paddles with eggs}}{\text{Number of ovitraps collected}} \times 100$$

Mosquito Larvicidal Trap (MLT). MLT refers to the percentage of MLT positive for *Aedes* immatures.

$$MLT = \frac{\text{Number of positive paddles from MLT}}{\text{Number of traps installed and collected in the households (one trap per household)}} \times 100$$

Vector control tool

Breteau Index Pupa (BIP)

$$BIP = \frac{\text{Number of positive containers (with pupae)}}{\text{Number of households inspected}} \times 100$$

Ovitrap Index Pupa (OIP)

$$OIP = \frac{\text{Number of ovitraps with pupae}}{\text{Number of ovitraps collected}} \times 100$$

Mosquito Larvicidal Trap Pupa (MLTP)

$$MLTP = \frac{\text{Number of MLT with pupae}}{\text{Number of traps installed and collected in the households (one trap per household)}} \times 100$$

Mosquito Larvicidal Trap (MLT) was compared with the conventional ovitrap, with regards to its field performance both as surveillance and a control tool. MLT was also compared with Breteau Index in terms of surveillance of mosquito vector infestation.

Ethical consideration

Permission to conduct the study in the two *barangays* (villages) in Los Banos, Laguna was obtained from their respective barangay captains. Informed consent from the head of each household was also obtained prior to the conduct of the survey. An orientation-training on the scope of the study, use of the entomological forms, surveillance of containers, monitoring of ovitraps and community engagement for the field workers was conducted before the actual field collection. In the orientation, emphasis was given on the

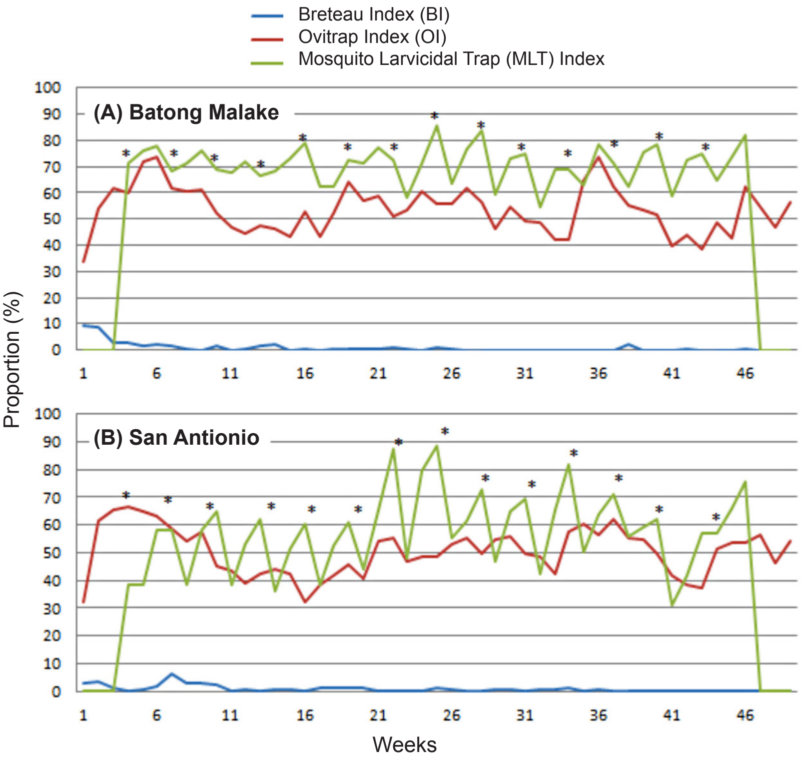

Fig 3–Weekly Breteau (BI), Ovitrap (OI) and Mosquito Larvicidal Trap (MLT) indices in (A) Batong Malake and (B) San Antonio, Los Banos, Laguna; June 2013. Asterisks indicate the day of MLT installation.

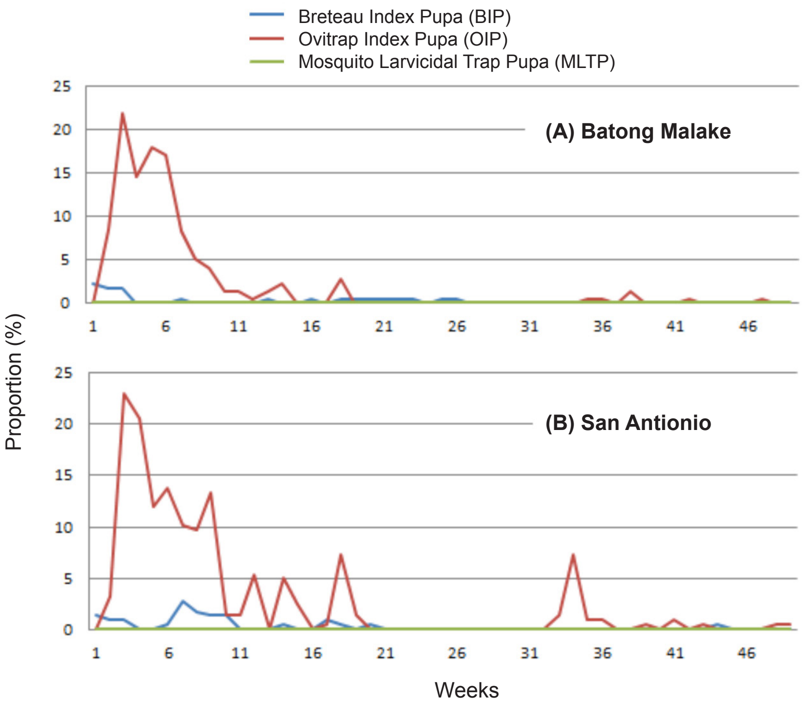

Fig 4–Proportion (%) of weekly pupal development in containers (BIP), ovitraps (OIP), and Mosquito Larvicidal Trap (MLT) in (A) Batong Malake and (B) San Antonio in Los Banos, Laguna; 2013 Jun3.

significance of engaging the household cooperation throughout the conduct of the study. There was no human subject used in the present study.

RESULTS

Mosquito Larvicidal Trap (MLT) has been observed to be more attractive to female mosquito for oviposition than ovitrap and containers in the households (Fig 3). The mean MLT index in Site 1, Batong Malake, was computed as 70.5% [Confidence interval (CI): 67.9-73.0], which was relatively higher than the mean Ovitrap Index (OI) at 53.5% (CI: 50.9-56.1) and mean Breteau Index (BI) at 0.9% (CI: 0.3-1.4). The trends in the fluctuation of the indices were the same in MLT and OI throughout the study. MLT indices in Batong Malake were significantly higher than the OI and BI throughout the study ($p = 0.0056$). Similarly, the mean MLT index in San Antonio at 56.6% (CI: 52.5-60.8) was higher compared to mean OI at 50.4% (CI: 48.0-52.9) and BI at 0.7% (CI: 0.4-1.1). However, mean OI was not significantly different from the mean MLT. The findings indicate that MLT for both sites were more sensitive in detecting dengue vector populations than ovitrap and containers, particularly in Batong Malake.

In the present study, the ovitrap density indices (ODI), that is, the average number of eggs laid by a single female in the ovitrap, in Batong Malake at 25.5 (CI: 19.5-31.4) and San Antonio at 23.1 (CI: 18.0-28.1) were not significantly different from each other ($p = 0.5384$).

In both study sites, the mean BIP was less than 1.0%, and OIP mean values were, 2.3% (CI: 0.8-3.7) and 2.9% (CI: 1.4-4.5), for Batong Malake (Fig 3A) and San Antonio (Fig 3B), respectively. These results indicate that at least 1 adult mosquito may be produced per container while 2 to 3 adult mosquitoes may be produced per ovitrap. In Batong Malake, BIP and OIP were not significantly different from each other ($p = 0.0065$) while in San Antonio, the OIP was significantly higher ($p = 0.0009$) compared to those in BIP and MLTP. All MLTP had a weekly pupal development of 0% (Fig 4) hence; there was no adult development. Results indicated that MLT was an autocidal trap or a lethal trap since it prevented pupal development (Fig 4) and consequently adult emergence.

DISCUSSION

The added Novaluron may serve as attractant to the female mosquitoes to lay their eggs as shown by the increase in MLT indices. This is the first report on the attractant potential of Novaluron to female mosquito. According to Ponnusamy *et al* (2008), oviposition attractants could be used to lure females to lethal ovitraps or stimulants could be used to increase their exposure to insecticide-impregnated substrates. The attraction mechanism of the MLT to female mosquito is unknown. The presence of the IGR might serve as stimulant to the female mosquito to oviposit in the MLT. In a study done by Sihuincha *et al* (2005), IGR like Pyriproxyfen, was used to exploit the conspecific attraction of the ovipositing females.

According to Farnesi *et al* (2012) and Fontoura *et al* (2012), the application of Novaluron inhibited adult emergence and induced mortality in a dose-dependent manner in larvae. It has been reported that 10-20 μg active ingredient of Novaluron applied in 200 liters of water in storage jars provided excellent control of *Ae. aegypti* larvae. Second instars were found slightly more susceptible than fourth instars (Mulla *et al*, 2003). Mortality of larvae occurred because Novaluron delays larval development by preventing ecdysis or molting (Farnesi *et al*, 2012), *ie*, prolonging the duration of *Ae. aegypti* immature stages. Studies revealed that larvae exposed to chitin synthesis inhibitors develop fragile cuticles unable to support the increased tension during the molting process. The exposed larvae have difficulty shedding their exuviae, dying due to starvation, suffocation or have weak and malformed cuticle (Reynolds, 1987; Graf, 1993). Farnesi *et al* (2012) reported that Novaluron effectively reduced the chitin content of *Ae. aegypti* larvae in a dose-dependent manner. Treatment with Novaluron also affected the epidermis and other internal larval organs. In the study conducted by Fontoura *et al* (2012), Novaluron inhibited 70% of adult emergence in *Ae. aegypti* and the residual effect of the chemical lasted for eight weeks and five-six weeks in indoor and outdoor conditions, respectively.

The induction of immature mortality (Mulla *et al*, 2003, Farnesi *et al*, 2012), delay in larval development, inhibition of adult emergence (Fontoura *et al*, 2012), alteration of adult sex ratio (Farnesi *et al*, 2012) and induction of morphogenetic anomalies in the life stages beyond the one treated (Mulla, 1991) are some of the reported effects of Novaluron on mosquitoes.

The results of the present study indicate that Novaluron is effective against field populations of mosquitoes. The findings suggest that Novaluron may play a significant role in operational vector control programs in terms of effectiveness, safety to the environment and as a strategy for managing insecticide-resistant vector mosquitoes.

Mosquiron Larvicidal Trap (MLT) is sensitive and effective as a surveillance tool as well as autocidal/lethal trap, because there is larval mortality and consequently no pupal development. The use of MLT as an integral component of dengue vector control program will enhance reduction in the density of the immature stages thus lessening dependency on adulticiding efforts. The larvicide used in the study may play a significant role in operational vector control programs in terms of effectiveness, environmental friendliness and as a strategy for managing insecticide-resistant vector mosquitoes. With the threat of dengue vertical and transstadial transmission in nature, managing the vector at its youngest stage must be done. It is recommended that Mosquiron must be repackaged for easier application through use in MLT.

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