An innovative year-round larvicide for mosquito control

Dr Nayem Hassan and Dr Abu Imroz Ali*

Aedes aegypti. Photo: WHO, CDC/Pablo Carera, Muhammad Mahdi Karim; Mosquiton (novaluron 0.12P) larvicide tablet. Photo: Russell Bio Solutions

Mosquitoes are considered one of the most serious vectors of some of the world's deadly diseases afflicting the tropical and subtropical countries. One of the devastating mosquito transmitted diseases, malaria is known to be endemic in 117 countries with 3.2 billion people living at risk all over the world and about 350 to 500 million clinical cases of malaria worldwide with over 1 million deaths annually (*Nwabor et al, 2017)*.

Most of the deaths occur in children under the age of 5 years. According to the WHO, unmanaged insecticide resistance may lead to an increase in malaria-related mortality. Mosquitoes are the most common nuisance to urban life in developing countries. It is also responsible for transmitting other diseases of public health significance including dengue fever, chikungunya, filariasis and yellow fever.

Among the other vector borne diseases, dengue is the most prevalent viral infection transmitted by Aedes mosquitoes. More than 3.9 billion people in over 129 countries are at risk of contracting dengue, with an estimated 96 million symptomatic cases and an estimated 40,000 deaths every year.

Chemical control methods are the most popular choice, due to their fast action and ease of handling (WHO 1981). Chemical insecticides are undoubtedly powerful in their role as the prime choice in the control of mosquito borne diseases. Their role of pest control also cannot be over emphasized, nor can it be overlooked. However, irrespective of these roles, their detrimental effect on the environment and the eco-system has long been a cause of concern.

Resistance of malaria vector *Anopheles vagus* to dichlorodiphenyltrichloroethane (DDT) in Bangladesh has been reported since the 1980s. Other Southeast Asian countries have reported to have insecticide resistance in this species to several pyrethroids including deltamethrin, permethrin, alpha-cypermethrin, and lambda-cyhalothrin (Mittal *et all,* 2004). Recently, Alam et all., (2002) reported, *Anopheles vagus*, the primary vector of malaria, was found to be resistant to permethrin and deltamethrin, with only

* Russell Bio Solutions, Flint, CH6 5XA, UK

29% and 55% mortality at 30 minutes respectively.

In polluted water, Culex mosquitoes in urban and suburban areas are usually controlled by using thermal fogging with various chemical insecticides, mainly pyrethroids as the main strategy. This operation, however, mainly affects adult mosquitoes for a short period. Evidence of resistance to various groups of chemical insecticides by *Culex quinquefasciatus* has been reported in several countries including Thailand, France (Yebakima et al, 1995), Colombia (Bisset etal, 1998), West Africa (Chandre et al, 1998), USA (Liu *et al*, 2005) and Malaysia (Nazni *et al*, 2005). Continued use of the same or different types of chemical insecticides like permethrin and malathion, results in mosquitoes developing multi-insecticide resistance (Forstinus et al. 2017).

So, an alternative approach for mosquito control is now being increasingly explored (Vivekanandhan, Karthi, et al. 2018). However, the emergence of resistance in field populations of *Culex quinquefasciatus* to a bacteria *Bacillus sphaericus* has been described in Thailand (Mulla *et al*, 2003) and India in the past decade (Rao *et al*, 1995). There is an urgent need to develop strategies to control mosquitoes, especially *Culex quinquefasciatus* that have already developed resistance to chemical insecticides as well as the microbial larvicide *Bacillus 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 programmes (Mulla, 1995). Many IGR compounds and products have been evaluated for larvicidal activity against various mosquito species, such as *Culex quinquefasciatus* (Mulla and Darwazeh, 1988), *Aedes aegypti* (Mulla *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 being 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 *Aedes 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 sites infested with heavy populations of mosquito larvae in urban areas of Bangladesh.

Russell Bio Solutions Ltd. has developed a unique, novel and innovative product MOSQUITON 0.12P to control mosquito larvae. The formulation of MOSQUITON has been developed to achieve the controlled release of the active ingredient, novaluron (0.12%) over a long period of time. The Mosquiton tablet contains inert material that helps to disperse the active novaluron in water as an emulsion form and eventually it helps to float the active ingredients uniformly on the water surface. The larvicide is released at a slower and steadier rate in areas of standing water (small or large) that can control around 95-98% of Culex and other mosquito larvae in all the aquatic stages of mosquito development to inhibit adult development. The tablet lasts in the field for a period of 90-150 days depending on the conditions of the water surface.

Development background

Novaluron is an insecticide of the class diflubenzoylureas. The compound shows the killing effect against various larvae of genus Lepidoptera, Coleoptera, Hemiptera and Diptera. Novaluron has been registered as an insecticide for food crops in many countries, including South Africa, Argentina, and Australia. The United States authorities approved the compound's registration as a pest control product for floriculture in September 2001. The acute toxicity of novaluron tested on rats through oral, dermal and inhalation administrations show LD_{50} of novaluron is determined as >5000 mg/kg body weight (bw) for oral dose acute toxicity to male and female rats, >2000 mg/mg bw for dermal treatment acute toxicity to male and female rats, and >5150 mg/m^3 for inhalation acute toxicity to male and female rats.

Regarding environmental concerns, novaluron at a dosage of 10 mg ai/m^2 had no impact on 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 ai/l (Yapabandra, unpublished report to the WHO Pesticide Evaluation Scheme, 2004).

WHO has assessed novaluron for use as a mosquito larvicide in drinking-water in containers, particularly to control dengue fever. The recommended dosage of novaluron in potable water in containers should not exceed 0.05 mg/litre under the WHO Pesticides Evaluation Scheme. In view of the absence of a carcinogenic potential in rodents and the lack of genotoxic potential in vitro and in vivo, the JMPR (Joint Meeting on Pesticide Residues) concluded that novaluron is unlikely to pose a carcinogenic risk to humans. JMPR also concluded that novaluron is not a developmental toxicant (World Health Organization Guidelines for Drinking-Water Quality, 3rd edition including 1st and 2nd addenda, 2008).

The WHO (2005) recommends the use of novaluron as a larvicide applied in non-drinking water-storage containers, temporary mosquito habitats and polluted waters at the dosage of 10-50 g ai/l or 10-100 g ai/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).

How Mosquiton works?

MOSQUITON 0.12P Novaluron larvicide affects the development of immature stages by disrupting the moulting process. It is an effective means for preventing the emergence of adult mosquitoes from water bodies. Consequently, beneficial species are not affected. The use of Mosquiton eliminates the risk of people getting infected by mosquito borne diseases.

How Mosquiton is used?

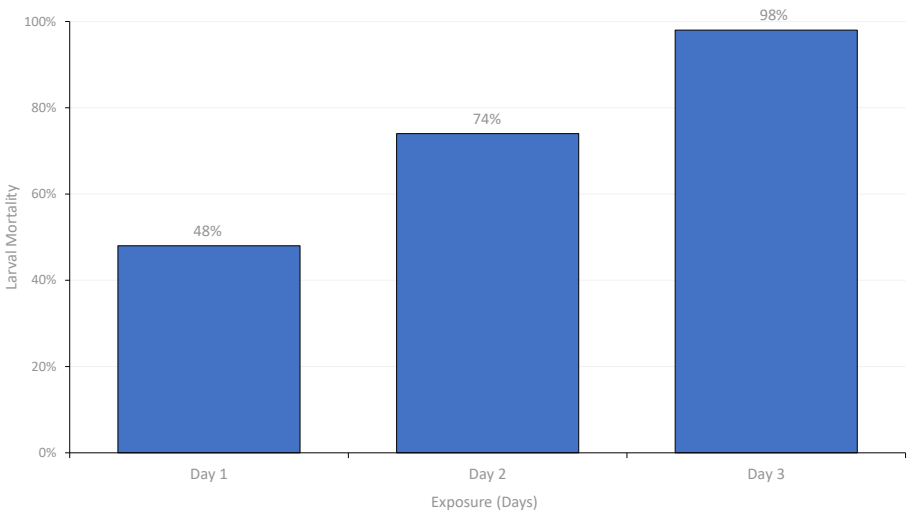
Apply 1 Tablet (1 gm) per 10 litres of water, the dose will be double if content of organic matter is too high in the water body. In case of a large water body, apply a 10 gm tablet for every metre distance. It can control all kinds of mosquito transmitted diseases including dengue, chikungunya, malaria and Zika.

The formulation is effective for up to 3 months and in a year-round for sub-tropical and tropical locations mosquito control can be achieved with 2-3 applications. MOSQUITON 0.12P Novaluron Insecticide can be used in governmental mosquito control programmes by professional pest control operators or homeowners.

Russell Bio Solutions has been conducted trial in Bangladesh, Sri Lanka, Pakistan and Thailand to evaluate the efficacy of Mosquiton tablets. In all trial locations, a single treatment of Mosquiton at a dose of 1g (1.2 mg ai novaluron) / m^2 provided excellent control of 95-98% of immature larval mortality. The Bangladesh lab trial conducted by Institute of Epidemiology Disease Control and Research (IEDCR) against female mosquito larvae of *Culex quinquefasciatus* at 28 degrees Celsius and 76% humidity. The average mortality of was recorded, 74% on 3 days and 98% 5 days after exposer to Mosquiton treated water (see graph)

Tawatsin et al., 2007 used the dosage (10 mg ai/m^2) of novaluron as a single treatment and attempted to obtain residual effects against target mosquitoes in the treated sites, containing highly polluted waters. The study revealed that a single treatment of an EC10 formulation of novaluron at a dosage of 10 mg ai/ 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. Tawatsin et al., 2007 described the differences in residual efficacy depending on the prevailing conditions for each site, such as the degree of water pollution, sun exposure and extent of rains and water drainage.

A similar trend of mortality has found in the simulated field trial up to one month. The trial was conducted by National Institute of Health of Thailand. A single treatment of Mosquiton at a dose of 1g / 10 litres of water provided excellent control of 100% larval mortality within 7 days.



Trial of Mosquiton Larvicide (0.12 P Novaluron) against *Culex quinquefasciatus*. Trial has been conducted by Institute of Epidemiology Disease Control and Research (IEDCR) Bangladesh against female mosquito larvae of *Culex quinquefasciatus* at 28°C and 76% humidity.

TABLE 1

TRIAL CONDUCTED BY NATIONAL INSTITUTE OF HEALTH OF THAILAND. THE TRIAL WAS CONDUCTED AGAINST AEDES MOSQUITO LARVAE DURING THE PERIOD OF NOVEMBER 2020.

INSECTICIDE	EXPOSURE (DAYS)	PERCENT LARVAL MORTALITY
Mosquiton Larvicide (0.12 P Novaluron) Against *Aedes aegypti* larvae in simulated field by 1 month	Day 1 (Treatment)	45
	Control (water)	0
	Day 2(Treatment)	77
	Control (water)	0
	Day 7 (Treatment)	100
	Control (water)	2

Similarly, a study conducted in India also showed that novaluron 10% EC when applied at the same dosage (10 mg ai/m^2) exhibited high degrees of residual effects against *Culex 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 litres capacity), novaluron 10% EC provided excellent control (>90% inhibition of the emergence) of wild populations of *Culex 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 *Culex 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 litres) where the water was enriched with rabbit pellets. Field evaluation of novaluron at dosages of 1- 10 mg/m^2 against *Culex 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).

It has been reported that the extent of residual activity of novaluron against *Culex quinquefasciatus* depends evidently 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 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 ai/l) for 63 days and in gem pits (at dosages of 0.01- 0.1 mg ai/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 ai/l) against the larvae of *An. albimanus* Wiedemann and *An. pseudopunctipennis* in sentinel cages placed in artificial pools was also achieved for 16 weeks (Arredondo-Jimenez and Valdez-Delgado, unpublished report to the WHO Pesticide Evalu- ation Scheme, 2004).

Novaluron provided excellent control of *Aedes aegypti* larvae in 200 litres of water storage jars at dosages of 10-20 µg ai/ l for at least two months (Mulla *et al*, 2003); applications at dosages of 0.055 -0.165 mg ai/l yielded high mortality (>85%) for *Aedes aegypti* and *Aedes albopictus* (Skuse) larvae in sentinel cages placed in 18 litres buckets for 14 weeks (Arredondo-Jimenez and Valdez- Delgado, unpublished report to the WHO Pesticide Evaluation Scheme, 2004).

In conclusion, our findings show novaluron (0.12 P) can be used as an effective larvicide to control immature, clean and polluted water mosquitoes, such as *Culex quinquefasciatus* larvae, with residual effects for three to 12 weeks after a single treatment at a dosage of 1.2 mg ai/m^2. Retreatment 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 programmes 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 cities in Bangladesh will continue. ■

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