

Field Evaluation of Sumilarv® 2MR Efficacy Against *Aedes aegypti* Larvae in Water-Storage Containers in Thailand

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ABSTRACT

Vector-borne disease transmission by *Aedes* mosquitoes, including Dengue, Chikungunya, and Zika fever, is estimated to increase massively worldwide. An alternative strategy for disease control is to limit the vector populations by using a larvicide. This study was initiated to evaluate the residual efficacy of a newly developed larvicide, Sumilarv® 2 MR, against larvae of *Aedes aegypti* that thrive in water-storage containers under simulated field conditions in Thailand. The Sumilarv® 2 MR, a 5.5 cm diameter resin disc containing 2% (w/w) pyriproxyfen, was used in this study against *Aedes aegypti* larvae in the simulated field trial method. The discs were immersed in 40L, 80L, and 160L of tap water in water-storage containers (1 piece/container); resulting in 3 different dosages of Sumilarv® 2 MR treatment. The controls were immersed in similar containers filled with tap water but without the disc. Five containers were used for each treatment and the controls. Pupal skins in each container were counted weekly, and the Emergence Inhibition Rate (%IE) was calculated. For the field simulation, half the volume of water in each container was removed weekly and replaced with new tap water for 114 weeks. During the 114 weeks of evaluation, average IE rates of $98.58\% \pm 1.73$, $98.25\% \pm 2.20$, and $97.94\% \pm 2.55$ were observed in the 40L, 80L and 160L containers, respectively. There was no significant difference ($p > 0.05$) in residual efficacy among the three dosages used in this study. Furthermore, the average %IE of all dosages was not less than 96.0%, even at the lowest dosage (160-liter containers) until the last weeks of evaluation. All dosages of Sumilarv® 2 MR in this study provided a high level of larvicidal efficacy (almost 100 %IE) in a water exchange regimen for up to 2 years post-treatment. This could be an effective environmentally friendly device for controlling *Aedes aegypti* larvae in water-storage containers.

Keywords: Sumilarv® 2 MR, Pyriproxyfen, Larvicide, *Aedes aegypti*, Field evaluation

INTRODUCTION

Diseases caused by arboviruses are one of the most important infections for human beings [1]. Over decades, the health burden of dengue fever, in terms of health and economic impact has been enormously increasing worldwide. In the absence of an effective vaccine, controlling mosquito vectors using various agents such as repellents, insecticides and larvicides were used as the strategy to eradicate and prevent the disease outbreak. *Aedes aegypti* mosquito is known as an important vector of Dengue and Chikungunya fever worldwide [2-3]. In Thailand, a large number of *Ae. aegypti* breeding sites are still found due to the use of outdoor containers around the houses to collect and store water. As the water is kept for domestic use, a larvicide used in the container is an important alternative strategy for limiting vector populations and thus preventing diseases. Insect growth regulators (IGRs) appear to be an advantage in mosquito larval control, with a different mode of action such as mimicking the juvenile growth hormone and chitin synthesis inhibitor, which prevent mosquito larvae from developing into reproduction-capable adults [4]. Pyriproxyfen (PPF) is the juvenoid broad-spectrum IGR, which acts on the hormonal system of insects. It has been used worldwide as a pesticide and was approved by the World Health Organization (WHO) for controlling mosquito vectors [5], due to its excellent residual efficacy and much lower dose than other larvicides. Sumilarv® 2 MR is a novel mosquito larval control product, based on the PPF, which has been evaluated as a safe insecticide for application in drinking water [6-7]. It is contained in a small resin disc with slow-release technology for easy use in water storage [8]. The main objective of this study was to

evaluate the residual efficacy of this newly developed larvicide against *Aedes aegypti* larvae in water-storage containers under simulated field conditions in Thailand.

MATERIALS & METHODS

Study Area, Temperature Conditions, and Test Duration

This study was carried out at a field research station in Bang Bua Thong district, Nonthaburi province, Thailand, to evaluate larvicidal products and other experimental agents for vector control. Water jars were located under a roof. During this study, the ambient temperatures at this site were between 24°C and 37°C. The ranges of minimum and maximum temperatures in the jars were 22-29°C and 28-36°C, respectively. This study was carried out continuously for 114 weeks.

Mosquito Larvae

Laboratory reared 3rd instar larvae of *Aedes aegypti* used in this study were provided by the National Institute of Health (NIH), Thailand. Larvae were reared at ambient temperature in a round plastic tub filled with filtered non-chlorinated water and fed with ground mouse food.

Sumilarv® 2MR Treatment

The Sumilarv® 2MR, developed by Sumitomo Chemicals Company, Japan, is a resin concave-shaped disc containing 2% (w/w) PPF. A Sumilarv® 2MR disc was immersed into a water jar filled with 40L, 80L, and 160L of tap water, respectively. Water jars in the control group were filled with the same volume of water without placing the disc. Five replicates were evaluated for each set. Then, each jar was covered with a lid to minimize water evaporation and contamination of debris. The water jars were allowed undisturbed for a week.

Simulated Field Trial

Every week, a new cohort of third-instar *Ae. aegypti* larvae were used in testing. A total number of 25 larvae were transferred into each jar, and one gram of ground mouse food was added as larval food. As to the warm temperature in Thailand, mosquito larvae tend to develop quickly within a week. Therefore, the adult emergence was observed after one week by counting the pupal skins. After adults emerge from pupae, their skins are floated at the water surface and can easily be aspirated with the transferable pipette. Then, the pupal skins were placed in white plastic tray filled with water and counted. It is notable that as an effect of IGRs, the mosquito mortality occurs at pupal stage. Therefore, pupal skins provide a good evaluation yield of emerging inhibition of IGRs. After aspirated all pupal skins in each water jar, all of the remained dead and alive immature mosquitoes were removed by using fishing net prior to replacing a new cohort of *Ae. aegypti* larvae. After stirring the water, 50% of its volume was removed and replaced with the same amount of tap water. The replacement of water and the new cohort of *Ae. aegypti* larvae was carried out every week. All of the jars were covered with lids to prevent the oviposition by wild mosquitoes and the invasion of predators.

Emergence Inhibition Rate & Data Analysis

The Emergence Inhibition Rate expressed as a percentage (%IE) is calculated based on the number of larvae that did not develop into emerging adults, as compared to the initial number of larvae added, as by the following formula [9-10]:

$$\% \text{ IE} = 100 - [(T \times 100) / C]$$

where

- T = adults emerged in the treated sets
- C = adults emerged in the control sets

Once the mortality rate of mosquito larvae in the control group was between 5-20%, the corrected using Abbott's formula. If the % IE in the control group was above 20%, the test was discarded and then retested.

The residual efficacy among the three dosages of Sumilarv® 2MR was compared by using ANOVA and LSD. The significant difference was accepted at a 95% confidential interval.

RESULTS

Sumilarv® 2MR was evaluated against *Aedes aegypti* larvae in simulated field conditions with a half volume of water replacement for 114 weeks. As of the 40L jar, % IE was found ranging from 90.2% to 100% with an average of $98.58\% \pm 1.73$. Levels of % IE was found above 92.8% from the 2nd week until the last week of the experiment (Fig.1). In the 80L jar, the minimum level of emergence inhibition rate (81.6% IE) was observed in the first week, then increased to 99.2% in the 2nd week, and maintained at 94.4% - 100% until the last week, with an overall average of $98.25\% \pm 2.20$. (Fig.2). Similarly, high levels of the inhibition rate were also found in the 160L-jar experiment, ranging from 88.0% to 100.0% throughout the experiment with an overall average of $97.94\% \pm 2.55$. (Fig.3). Interestingly, high levels of emergence inhibition rate (above 96.0%) were observed in all dosages of Sumilarv® 2MR from the beginning to the end of the study. No mortality of mosquito larvae was observed in the control group. In addition, no significant difference in the residual efficacy among the three dosages of Sumilarv® 2MR was found ($p>0.05$).

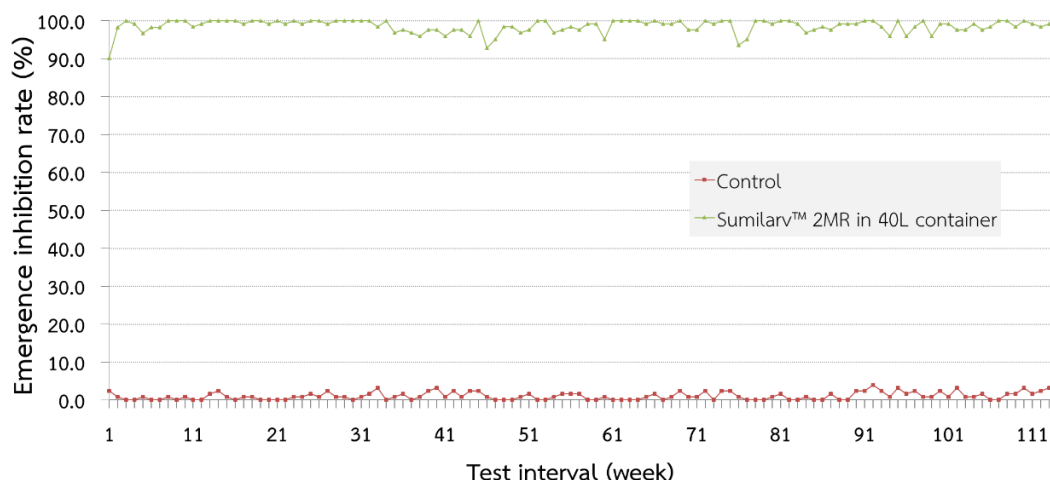


Figure 1: Larvicidal efficacy of Sumilarv® 2MR in 40L water jars during 114 weeks.

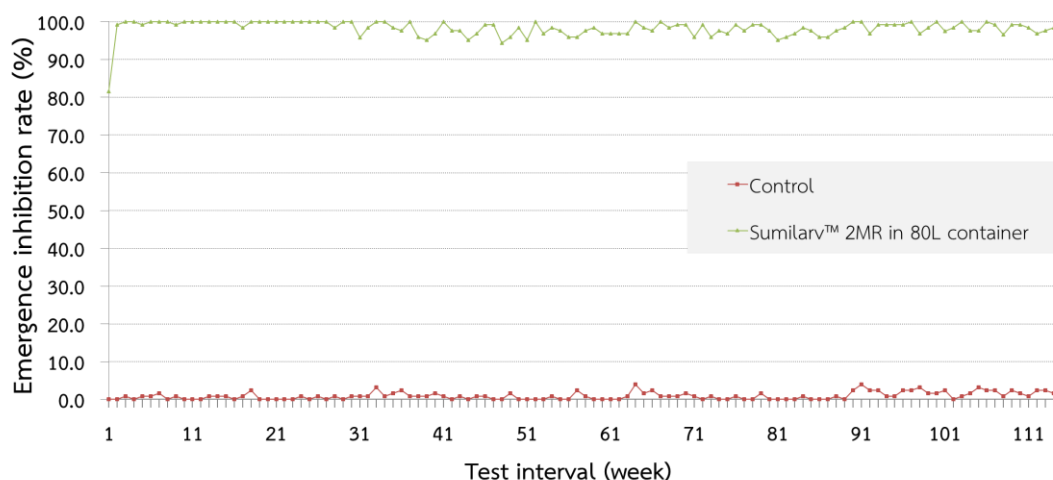


Figure 2: Larvicidal efficacy of Sumilarv® 2MR in 80L water jars during 114 weeks.

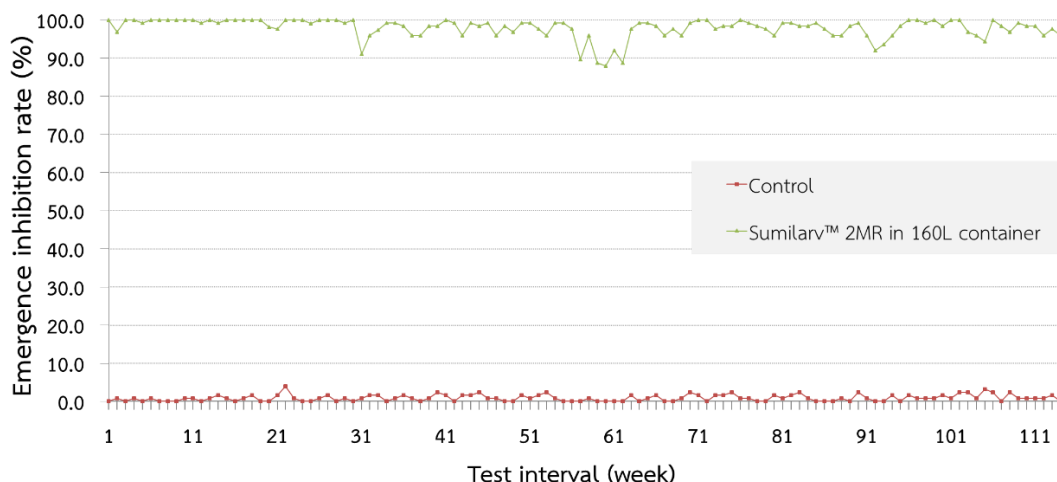


Figure 3: Larvicidal efficacy of Sumilarv® 2MR in 160L water jars during 114 weeks.

DISCUSSION

As of the conventional way in Thailand, water containers are still used, which can become the breeding sites for mosquito vectors, particularly for *Aedes aegypti* in the residential areas. Disease control by managing vector populations with larvicides is an alternative method, recommended by WHO. PPF, an insect growth regulator, plays a significant role in controlling diseases spread by mosquitoes. Due to its very low toxicity to humans and animal pets, WHO has recommended this IGR for use in household water. Various forms of PPF such as granules has been developed with high efficacy in mosquito control [11].

Sumilarv® 2MR disc contains PPF in resin matrix, with the property of gradually release of active ingredient, thus inhibiting the growth of mosquito larvae. This property helps to maintain concentration of PPF in the water container, even when the water is drawn or replaced. This product has the advantage of not leaving an unpleasant odor and high efficacy at lower concentrations than other larvicides [12]. High levels of residual efficacy (last to 6 months) of Sumilarv® 2MR have been found in semi-field tests conducted in Malaysia, Myanmar, and Pakistan, respectively [12-14]. In addition, a longer residual effect of up to 1 year

has also been reported in Brazil, using 100-liter water containers [15]. This study demonstrated greater efficacy of up to 2 years with 50% of weekly water replacement. Efficacy in 40L and 80L of water showed no difference in the first four weeks and in the last month. Although test results in 160L of water showed significant differences in larvicidal efficacy between the first and the last month, but its average level in the last month was still as high as 96.6%. Müller et al. (2024) reported that in the simulated field condition, smaller containers showed lower residual effects when compared to the larger ones [15]. This may be related to temperature or product concentration, which requires further investigation. This study found no significant difference in the larvicidal efficacy among the three concentrations of Sumilarv® 2MR, indicating that temperature and the size of the container do not affect the residual activity of this product.

Since the test in this study was semi-field, results might differ in the actual field conditions. There may be various factors affecting the product's residual activity, such as the frequency of household water usage, temperature, sunlight, and the replacement of water in the container due to rainfall. Field studies in Myanmar and Laos found that the product had residual effects lasting for up to 6 months and it was well accepted by the public [8,12]. In addition, the product significantly reduced the density of dengue vector mosquitoes in the treated areas, compared to untreated areas [13]. However, factors affecting control included loss of the product from water containers, due to its small size and light weight [15]. It should be noted that the reduction in mosquito populations when using the product took about one month, which may affect user's confidence [12-13,16]. This limitation should be addressed through campaigns to the public.

The longevity of Sumilarv® 2MR activity was found in all dosages, with up to 2 years in the water exchange regimen. However, longevity testing should be extended further to achieve a suitable residual efficacy, or PPF concentration should be adjusted downward to make it appropriate. PPF in the resin disc is easy to use and conducive for controlling *Aedes* mosquitoes. This disc can be reused many times when the water in the container is removed and replaced, or when the container is washed.

CONCLUSION

Evaluation of Sumilarv® 2 MR in this study provided a high level of larvicidal efficacy (almost 100% IE) for up to 2 years in the water exchange regimen. It could be an effective agent for controlling *Aedes aegypti* larvae in water-storage containers with environmentally friendly.

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