

SCG Mosquito Trap: A New Lethal Ovitrap for Controlling *Aedes aegypti* (L.) and *Ae. albopictus* (Skuse); the Vectors of Dengue Haemorrhagic Fever, and Chikungunya and Zika Fever

Apiwat Tawatsin

Department of Medical Sciences, Ministry of Public Health
Nonthaburi 11000, Thailand

Usavadee Thavara

Department of Medical Sciences, Ministry of Public Health
Nonthaburi 11000, Thailand

Navy Srivarom

Department of Medical Sciences, Ministry of Public Health,
Nonthaburi 11000, Thailand

ABSTRACT

Aedes aegypti (L.) and *Ae. albopictus* (Skuse) are known to be the vectors of dengue haemorrhagic fever (DHF), and chikungunya and zika fever. Of these diseases, DHF is the most dangerous because it has high incidence and mortality in many countries worldwide, especially in Asia. As there is currently no effective vaccine available to prevent these diseases, vector control is the best strategy for controlling them. Therefore, the SCG Mosquito Trap: a new lethal ovitrap combined with attractant and biological larvicide, was developed in this study for controlling *Ae. aegypti* and *Ae. albopictus* mosquitoes. The trap without attractant showed significantly higher oviposition efficacy than that of a conventional one in luring gravid *Ae. aegypti* and *Ae. Albopictus* to lay their eggs under laboratory conditions by approximately 3.1 and 2.6 times, respectively. The attractant derived from carpet shell extract increased the oviposition efficacy of the SCG Mosquito Trap by about 1.7 and 4.1 times against *Ae. aegypti* and *Ae. albopictus*, respectively, under laboratory conditions. *Bacillus thuringiensis israelensis* (Bti) was the biological larvicide used in the SCG Mosquito Trap. It provided a complete killing effect (100% mortality) against the larvae of *Ae. aegypti* and *Ae. albopictus* for 4 weeks post single application, as no larvae hatched from sponge sheets applied with Bti, which were obtained from the SCG Mosquito Trap. In the field study against *Ae. aegypti*, the SCG Mosquito Trap could reduce the number of houses infested from 80% (with 75% from the positive trap and an average of 242 eggs/trap) to 30% (with 22.5% from the positive trap and an average of 64 eggs/trap) by the fourth week after initiation. In conclusion, the SCG Mosquito Trap developed from this study could be used additionally as an effective device for controlling *Ae. aegypti* and *Ae. albopictus* mosquitoes, in order to prevent the diseases that are transmitted by these vectors.

Key words: Lethal ovitrap, Bti, Control, *Aedes aegypti*, *Aedes albopictus*

INTRODUCTION

Many diseases, for example, dengue haemorrhagic and yellow fever, Japanese encephalitis, malaria, etc. are caused by pathogens, such as viruses or protozoan that are transmitted to humans through mosquito bites [1]. *Aedes aegypti* (L.) and *Ae. albopictus* (Skuse) are accepted as vectors of dengue, chikungunya and Zika viruses [2,3]. Researchers have attempted to develop effective vaccines against these diseases, but there are none currently available. Therefore, control of vector mosquitoes is the most effective strategy for controlling the diseases. At present, many strategies are used to prevent and control *Aedes* mosquitoes, such as surveying and eliminating mosquito breeding sites, using biological agents [4,5], applying larvicides and adulticides [6-9], and using lethal ovitraps [10-20]. In practice, ovitraps have been used for monitoring and surveillance of *Ae. aegypti* and *Ae. albopictus* in high-risk areas, where *Aedes* mosquito populations seem to be lower than usual. However, the ovitrap could be modified and developed as a lethal ovitrap for controlling *Aedes* mosquitoes when combined with larvicides [16-18,20], adulticides [10,12,14,17,19] or a sticky agent [11,13,15]. The objective of this study was to develop a new lethal ovitrap combined with biological larvicide for controlling *Ae. aegypti* and *Ae. albopictus* mosquitoes. It could be used as a tool for integrating vector control programs and controlling *Aedes*-borne diseases.

MATERIALS AND METHODS

Development of the SCG Mosquito Trap

The SCG Mosquito Trap was designed and developed as a new lethal ovitrap to control *Ae. aegypti* and *Ae. albopictus* mosquitoes. It is cylindrical in shape, 19 cm high and 14 cm in diameter at its base, and made from black-colored polypropylene plastic. There are 2 drainage holes (1x4 cm) located 12 cm from the bottom of the trap. The trap is covered with a lid, 19.5 cm in diameter and 6 cm thick to protect from sunlight and water. In addition, 12 windows (2.5x3.7 cm) are placed around the lid that is attached to the body of the trap. These windows are designed as routes for gravid mosquitoes to fly into the trap and lay their eggs (Figure 1). On the other hand, the conventional ovitrap is used for oviposition of *Ae. aegypti* or *Ae. albopictus* mosquitoes in the laboratory or surveillance of *Aedes* mosquitoes in the field. It usually is made of a black polypropylene plastic cup (8.5 cm high, and 7 cm and 9.5 cm in diameter at its base and top, respectively). The oviposition efficacy of the SCG Mosquito Trap was then compared with the conventional ovitrap for oviposition of gravid *Ae. aegypti* and *Ae. albopictus*, with 10 replications for each species under laboratory conditions.



Figure 1. The conventional ovitrap (left) and SCG Mosquito Trap (right).

Oviposition experiments in the laboratory

Ae. aegypti and *Ae. albopictus* were used for the oviposition experiments in this study, with the *Aedes* mosquitoes cultured in the insectary of the National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Thailand. They were reared according to standard operation procedure of the institute, as described by Tawatsin et al. [20]. Sponge sheets (11×43 cm and 7×23 cm) were used in each experiment as substrates for *Aedes* mosquitoes to lay their eggs separately in the SCG Mosquito Trap and conventional ovitrap. Both traps were filled with tap water until the level reached about halfway up the sponge sheet, which was wound around the inside of each trap. A total of 100 gravid females of *Ae. aegypti* or *Ae. albopictus* (aged 4-6 days, and 2-3 days after blood feeding) were released into a large mosquito cage made from polyester net (180×200×180 cm), in which the SCG Mosquito Trap and conventional ovitrap were placed about 90 cm away from each other. During the test period, 10% sugar syrup was poured onto cotton wool, put in a small bottle and placed in the cage as food for the mosquitoes. The mosquito cage was located in a test room (4×4×2.6 m) for 4 days, and the temperature, relative humidity (RH) and photo period were controlled at 26±2 °C, 60-80 %RH and 12 hours light:12 hours dark (12L:12D), respectively. Finally, the sponge sheets of both traps were removed and dried, and the number of *Aedes* eggs was counted. The experiments were carried out against the gravid *Ae. aegypti* and *Ae. albopictus* under laboratory conditions, as described previously, with 10 replications for each species.

Enhancement of SCG Mosquito Trap efficacy with oviposition attractant

Carpet shell (*Paphia undulata*) extract, formulated and described by Tawatsin et. al. 2019 [20], was used as an oviposition attractant to lure gravid *Ae. aegypti* and *Ae. albopictus* to lay their eggs in the SCG Mosquito Trap [21]. The lower side of the SCG Mosquito Trap lid was coated with the attractant. For comparison, sponge sheets were wound around the inside of two SCG Mosquito Traps (one with and one without attractant on the lid), which were filled with 2 liters of water. They were placed about 90 cm apart from each other in a large polyester net cage (180×200×180 cm) containing 100 gravid *Ae. aegypti* or *Ae. albopictus* mosquitoes. Both traps were left in the cage for 4 days in the test room (4×4×2.6 m), and the temperature, RH and photo period were controlled as described previously. Then, both traps were removed from the cage

and the number of mosquito eggs laid on the sponge sheets was counted. The experiments were carried out against the gravid *Ae. aegypti* and *Ae. albopictus* under laboratory conditions, as described previously, with 10 replications.

Development of the SCG Mosquito Trap as a lethal ovitrap for controlling *Aedes* mosquitoes

The SCG Mosquito Trap was designed and developed as a lethal ovitrap with attractant and larvicide. Carpet shell extract was used as attractant (Tawatsin et. al. 2019) [20], while *Bacillus thuringiensis israelensis* (Bti) was utilized as the larvicide in this study. The Bti used in this study was a commercial product (Vectobac WDG, Valent Bioscience, USA). The experiments were carried out to compare the number of mosquito eggs in the SCG Mosquito Traps, with and without Bti, and study the lethal effect of the larvicide. Approximately 1 g of Bti was used in each treated trap. In each experiment, sponge sheets (11×43 cm) were wound around the inside of two SCG Mosquito Traps filled with 2 liters of water (one with and one without Bti), which were placed about 90 cm apart from each other in a large polyester net cage (180×200×180 cm) containing 100 gravid *Ae. aegypti* or *Ae. albopictus* mosquitoes. Both traps were left in the cage for 4 days in the test room (4×4×2.6 m), and the temperature, RH and photo period were controlled as described before. The number of eggs laid on the sponge sheets in both traps were counted and compared. The experiments were carried out against *Ae. aegypti* and *Ae. albopictus* under laboratory conditions, with 10 replications for each species.

In the lethal effect experiment in this study, the sponge sheets containing *Ae. aegypti* and *Ae. albopictus* mosquito eggs obtained from the SCG Mosquito Traps (with and without Bti) were dried at room temperature for 2 days for embryonic development and egg conditioning. Each substrate was then submerged in water from the trap that had been placed inside in a plastic tray (26×36×6 cm) for hatching observation. The number of larvae that hatched from each substrate was counted and recorded 5 days after hatching. A total of 10 replications were carried out against each species.

Longevity of the lethal efficacy of Bti in the SCG Mosquito Trap

An experiment was carried out in the laboratory to study the longevity of lethal efficacy in Bti added in the SCG Mosquito Trap. Sponge sheets (11×43 cm) were wound round the inside of 20 SCG Mosquito Traps, each filled with 2 liters of tap water for *Aedes* egg laying. About 1 gram of Bti was added to the water in each trap and stirred with a wooden stick until dissolved completely. To assess lethal efficacy in the trap, 20 *Ae. aegypti* larvae (third instar) were put into each trap and larval mortality was observed 3 days later. The same 20 traps were evaluated continuously for lethal efficacy by adding larvae every week for 6 weeks after the start of single Bti treatment.

Field evaluation of SCG Mosquito Trap efficacy against *Ae. albopictus*

The SCG Mosquito Trap was evaluated for field efficacy in a mangosteen and durian orchard in Makham district, Chantaburi province, Thailand, where *Ae. albopictus* mosquitoes are abundant. Thirty pairs of SCG Mosquito Traps (with and without attractant) were set up to collect *Aedes* eggs in the field. All of them had sponge sheets placed inside and were filled with 2 liters of tap water and 1 gram of Bti. Each pair of traps was placed about 10 cm apart and around 10 m away from other pairs. The traps were left in the field for 15 days and the sponge sheet of each trap was removed for *Aedes* egg counting. Another 30 pairs of SCG Mosquito Traps

(with and without attractant) also were prepared, as described previously, and set up to collect the sponge sheets for counting *Aedes* eggs in the field after 30 days. Each trap also was inspected for mosquito larvae, insects and animals 15 and 30 days after they had been left in the field.

Field evaluation of the SCG Mosquito Trap efficacy against *Ae. aegypti*

The SCG Mosquito Trap was evaluated for field efficacy against *Ae. aegypti* in a village in Muang district, Nonthaburi province, Thailand. A piece of sponge sheet (11×43 cm) was wound around the inside of each trap as substrate for *Ae. aegypti* egg laying, and about 2 liters of tap water was poured into the trap until its level reached the middle of the sponge sheet. One gram of Bti (Vectobac WDG) was added to the water and stirred with a wooden stick until dissolved completely. Then, the trap was covered with a lid. Pairs of traps were set up for *Ae. aegypti* egg laying and placed at suitable locations inside individual houses, for example, the living room, toilet, under the stairs, clothes hanger, etc. A total of 80 traps were set up in 40 houses in this study. The sponge sheets were collected from the traps 4 days after placement, and replaced with new ones for a further 4 weeks of weekly evaluations. The number of eggs laid on the sponge sheet in each trap was counted and recorded. The percentage of houses positive for *Aedes* eggs was calculated against the total number of houses. The ovitrap index also was calculated from the number of positive *Aedes* eggs from the ovitrap against the total number of ovitraps retrieved from the field.

DATA ANALYSIS

The efficacy of the SCG Mosquito Trap was evaluated by comparing the mean number of *Ae. aegypti* or *Ae. albopictus* eggs with that from the conventional ovitrap. The enhancement of oviposition efficacy from the attractant also was determined under laboratory and field conditions. The t-test analysis was applied for comparisons of data obtained from all experiments. The statistical significance for all comparisons was accepted with $p < 0.05$. The SPSS program for windows (SPSS for Windows version 28, SPSS Inc., Chicago, IL) was used for all statistical analysis.

RESULTS

The SCG Mosquito Trap demonstrated significantly higher oviposition efficacy than the conventional ovitrap in luring gravid *Ae. aegypti* to lay their eggs (Figure 2). The mean number ($\pm$ S.E.) of *Ae. aegypti* eggs in the SCG Mosquito Trap (981.1 ± 77.3) was about a significant 3.1 times higher than that of the conventional trap (314.8 ± 103.9). Similar results against the gravid *Ae. albopictus* also were obtained from the SCG Mosquito Trap (Figure 2). The mean number of *Ae. albopictus* eggs ($\pm$ S.E.) in the SCG Mosquito Trap (691.6 ± 44.5) was significantly greater than that of the conventional ovitrap (267.5 ± 33.6) by about 2.6 times.

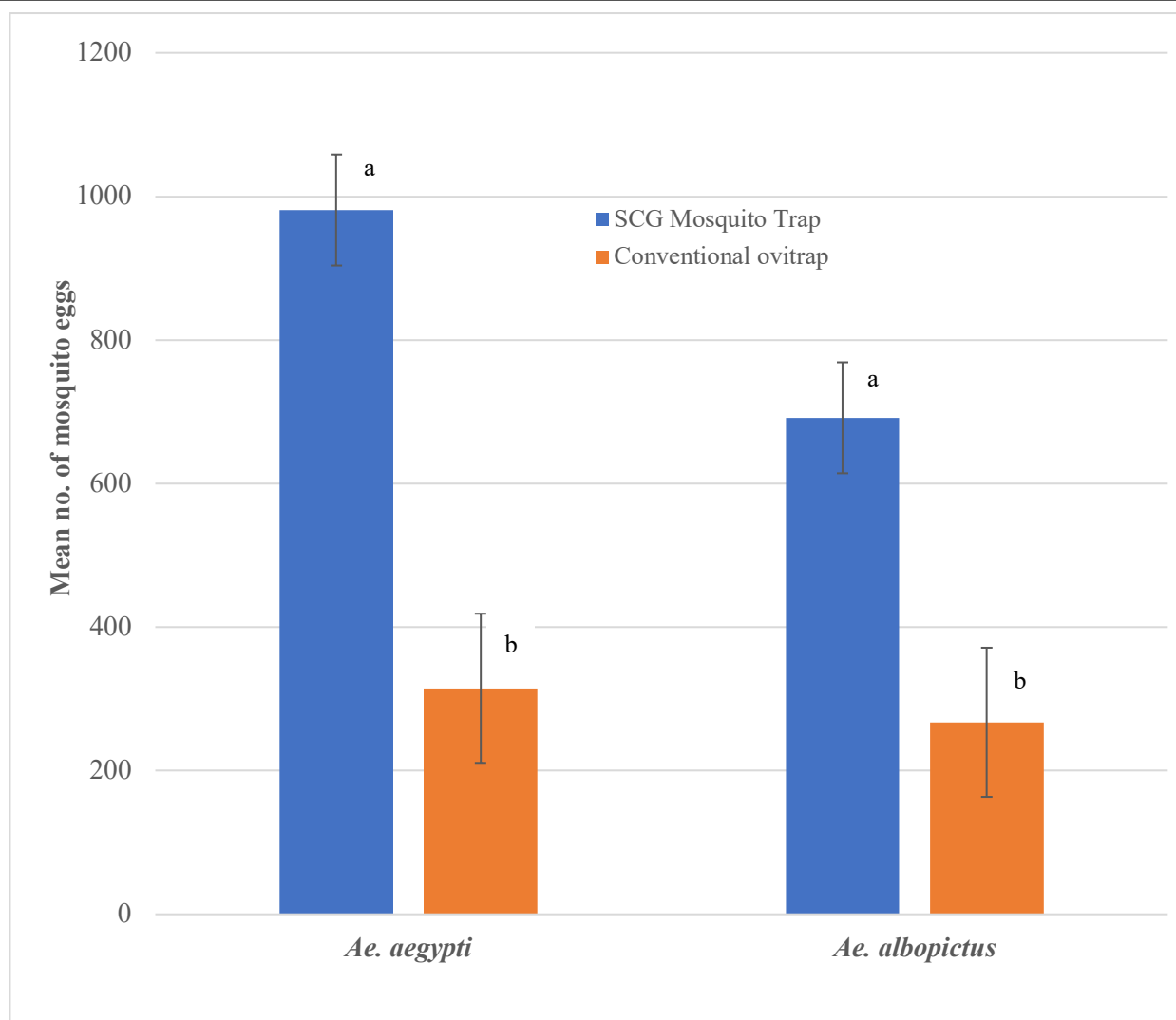


Figure 2. Comparison of efficacy of the SCG Mosquito Trap and the conventional ovitrap for oviposition of *Ae. aegypti* and *Ae. albopictus* under laboratory conditions. The mean number ($\pm$ S.E.) of eggs of the same species followed by different letters are significantly different ($p < 0.05$).

The attractant derived from carpet shell extract played an important role in increasing the oviposition efficacy of the SCG Mosquito Trap when luring gravid *Ae. aegypti* and *Ae. albopictus* (Figure 3). When testing against *Ae. aegypti*, the mean number ($\pm$ S.E.) of eggs collected by the SCG Mosquito Trap coated with the attractant ($1,615.7 \pm 252.7$) was significantly higher than that of the trap without attractant (931.3 ± 213.5). Similar results were obtained from the experiments against *Ae. albopictus*. The SCG Mosquito Trap coated with the attractant significantly urged gravid *Ae. albopictus* to lay their eggs with a mean number ($\pm$ S.E.) of about 850 ± 52.9 , while the trap without the attractant provided approximately 208.3 ± 23.2 . It was clear that the attractant increased the oviposition efficacy of the SCG Mosquito Trap against *Ae. aegypti* and *Ae. albopictus* by about 1.7 and 4.1 times, respectively.

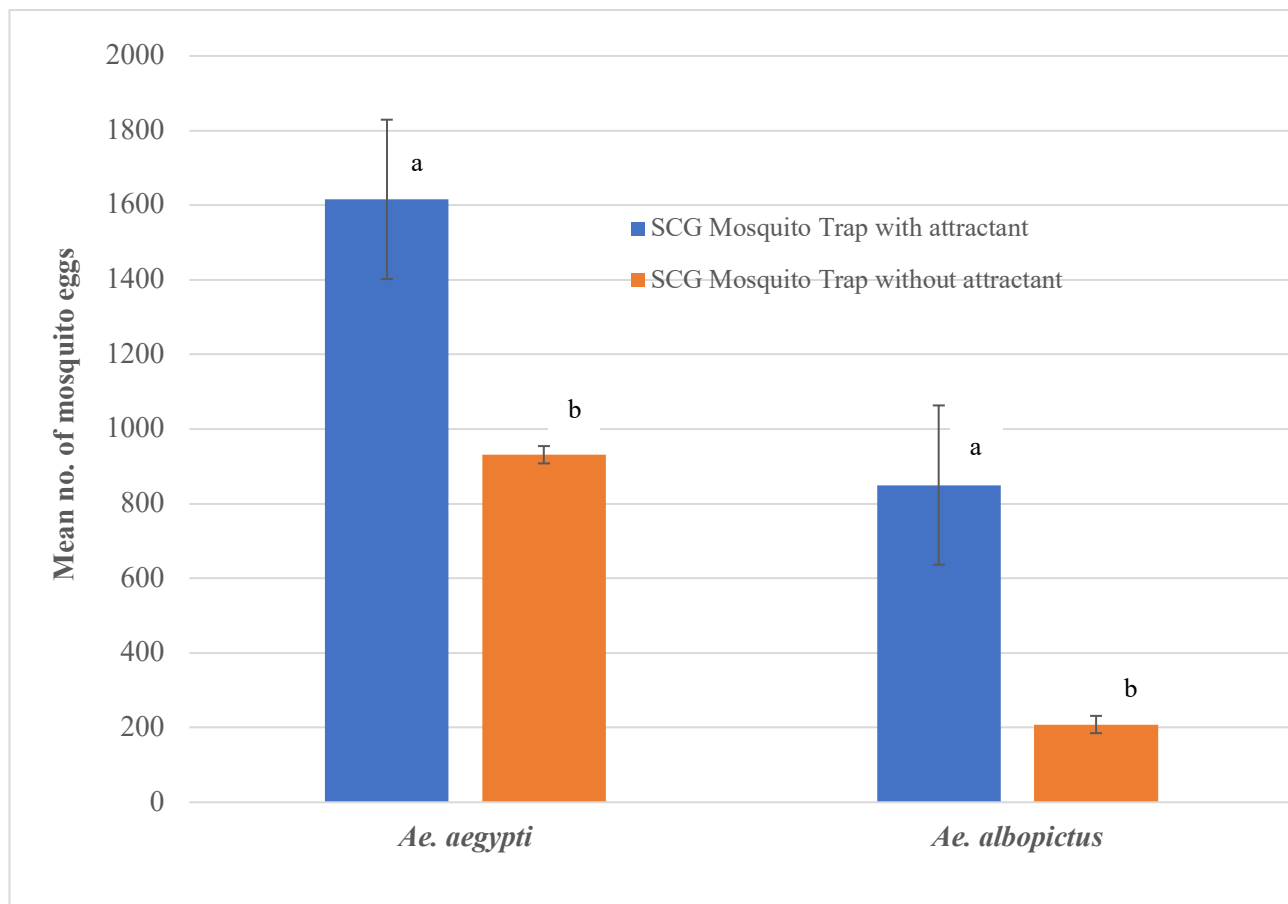


Figure 3. Comparison of efficacy of the attractant coated on SCG traps against *Ae. aegypti* and *Ae. albopictus* under laboratory conditions. The mean number ($\pm$ S.E.) of eggs of the same species followed by different letters are significantly different ($p < 0.05$).

Bti provided an excellent killing effect in this study, as there were no live larvae of *Ae. aegypti* or *Ae. albopictus* emerging from the sponge sheets obtained from the SCG Mosquito Trap with Bti. On the other hand, many larvae of *Ae. aegypti* and *Ae. albopictus* hatched from the sponge sheets of the SCG Mosquito Trap without Bti larvicide, with a mean number ($\pm$ S.E.) of about 765.6 ± 88.7 and 586.3 ± 46.4 , respectively (Table 1).

Table 1. Lethal efficacy of Bti added in the SCG Mosquito Trap

Mosquito species	No. of live mosquito larvae (Mean + S.E.)	
	SCG Mosquito Trap with Bti	SCG Mosquito Trap without Bti
<i>Ae. aegypti</i>	0.0 ^a $\pm$ 0.0	765.6 ^b $\pm$ 88.7
<i>Ae. albopictus</i>	0.0 ^a $\pm$ 0.0	586.3 ^b $\pm$ 46.4

Remark: The mean number of larvae in the same row followed by a different letter is significantly different ($p < 0.001$).

The longevity of lethal efficacy of Bti added in the SCG Mosquito Trap against *Ae. aegypti* larvae during the study period of 6 weeks is demonstrated in Figure 4. It was found that the Bti added in the traps provided complete larvicidal efficacy (100%) for 4 weeks before dropping dramatically to 64.8% and 43.0% at week-5 and week-6, respectively.

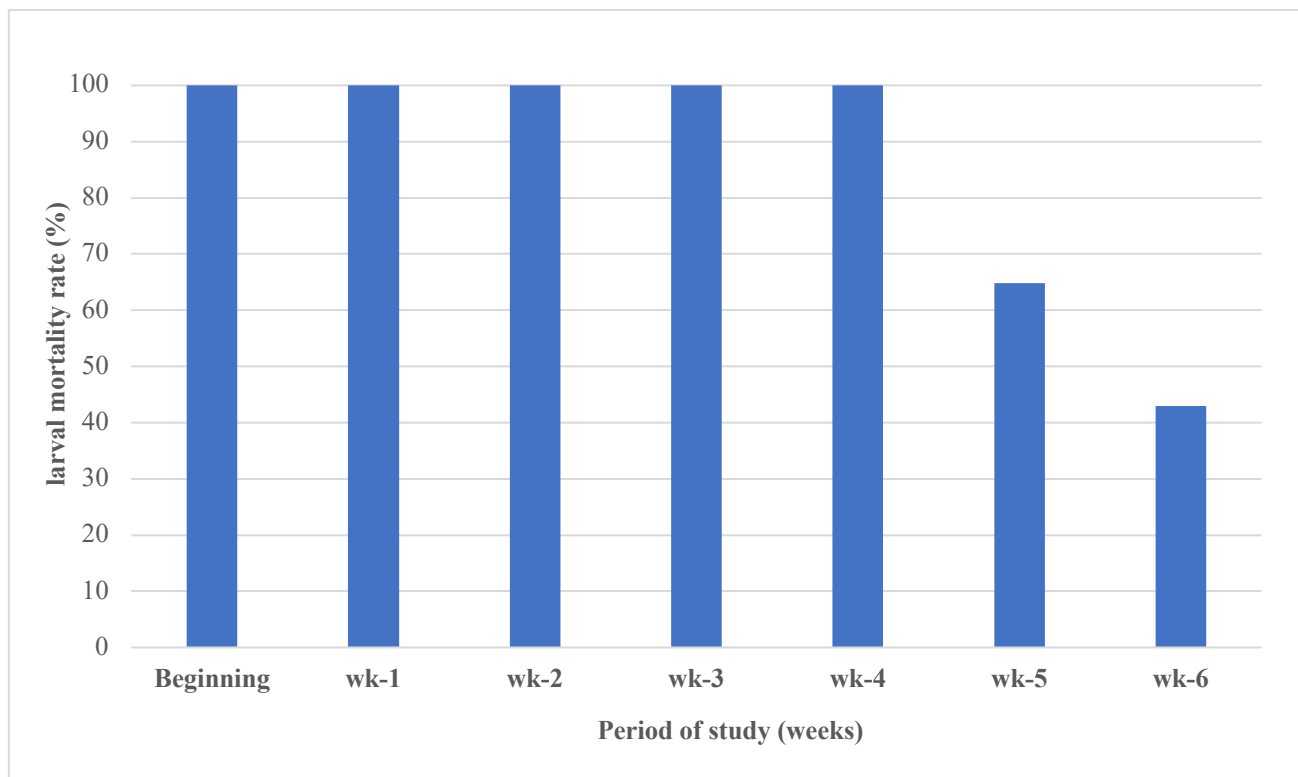


Figure 4. Longevity of lethal efficacy (larval mortality rate, %) of Bti added in the SCG Mosquito Trap against *Ae. aegypti* larvae during 6 weeks of evaluation.

The SCG Mosquito Trap coated with the attractant showed significantly higher oviposition efficacy than the trap without against *Ae. albopictus* in the field for a period of 15 days, with a mean number ($\pm$ S.E.) of mosquito eggs of about 398.4 ± 73.1 and 179.2 ± 41.6 , respectively (Figure 5). It was clear that the attractant increased the oviposition efficacy of the SCG Mosquito Trap by 2.2 times. In the longer experimental period of 30 days, the SCG Mosquito Trap with and without attractant could collect many mosquito eggs with a mean number ($\pm$ S.E.) of $1,382.3 \pm 84.1$ and 904.7 ± 55.2 , respectively. Therefore, the attractant significantly enhanced the oviposition efficacy of the SCG Mosquito trap by about 1.5 times when compared with the trap without the attractant.

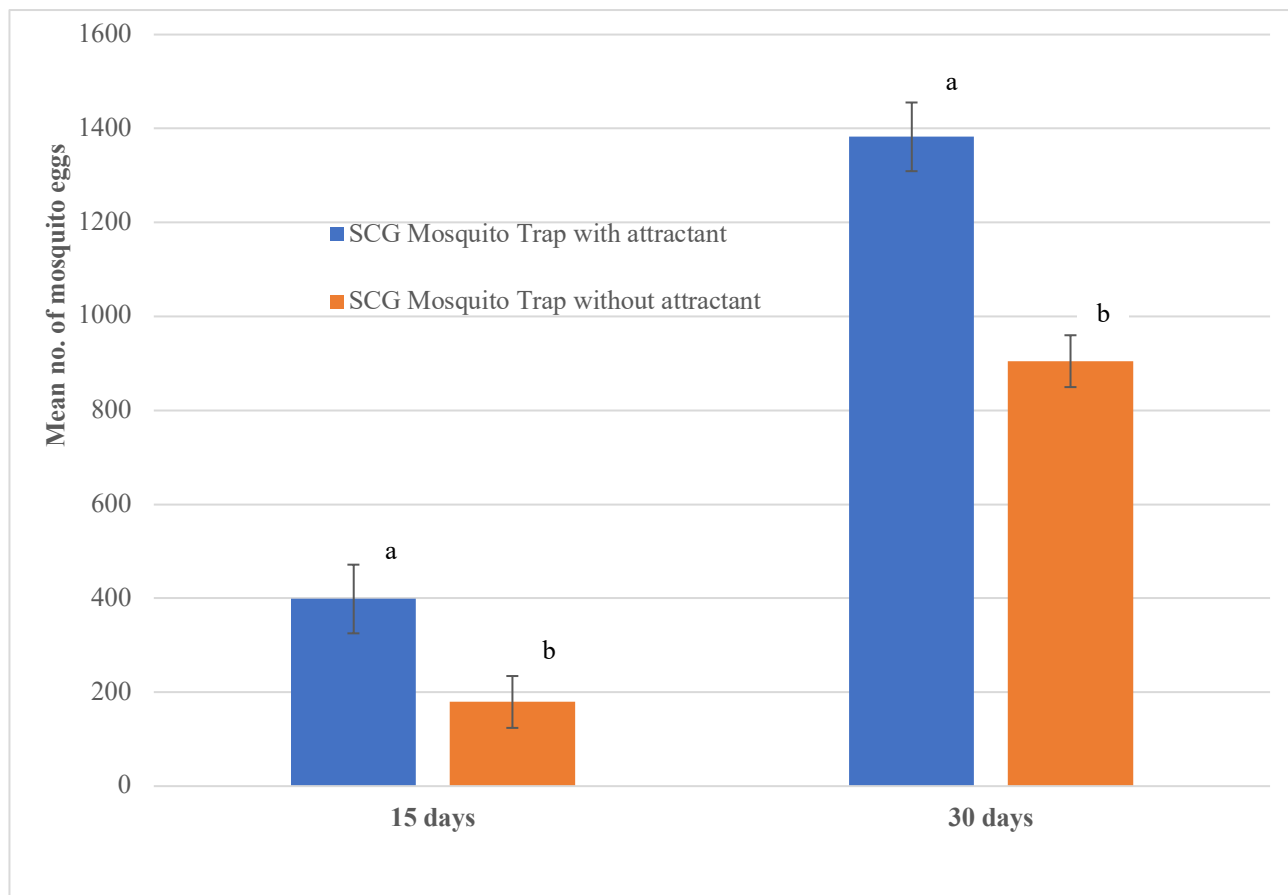
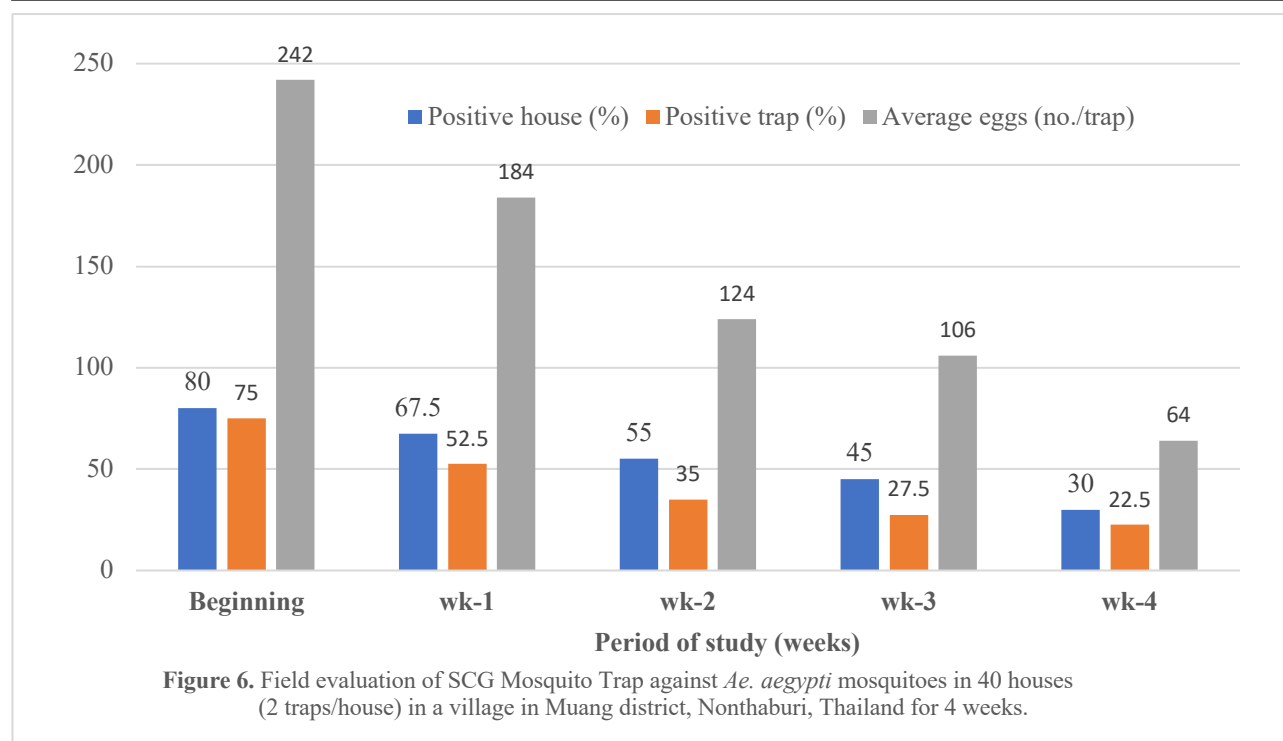


Figure 5. Comparison of mean numbers ($\pm$ S.E.) of *Ae. albopictus* eggs collected by the SCG Mosquito Trap with and without attractant under field conditions for 15 and 30 days. The mean numbers ($\pm$ S.E.) of eggs of the same period followed by different letters are significantly different ($p < 0.05$).

The data from a field experiment evaluated efficacy of the SCG Mosquito Trap against *Ae. aegypti* in a village in Muang district, Nonthaburi province, Thailand, as demonstrated in Figure 6. It was found at the start of this study that 60 (75%) out of 80 SCG Mosquito Traps were positive for *Ae. aegypti* eggs in 32 houses (80%), with an average 242 eggs/trap. The percentages of positive houses for *Ae. aegypti* eggs gradually decreased to 67.5%, 55%, 45% and 30% at week-1, week-2, week-3 and week-4, respectively. During the 4 weeks after initiation of the study, the ovitrap index (rates of positive traps) declined dramatically to 52.5%, 35%, 27.5% and 22.5%, respectively, while the average of *Ae. aegypti* eggs per trap dropped from 184 eggs at week-1 to 64 at week-4.



DISCUSSION

The results obtained from this study revealed that the SCG Mosquito Trap without attractant showed significantly higher oviposition efficacy in luring gravid *Ae. aegypti* and *Ae. albopictus* to lay their eggs under laboratory conditions than the conventional trap by approximately 3.1 and 2.6 times, respectively. These results were similar to those obtained by the LeO-Trap® when gravid female *Ae. aegypti* and *Ae. albopictus* preferred to lay their eggs in the LeO-Trap® rather than the conventional ovitrap by about 1.9 and 2.5 times, respectively [20]. This was the effect of using the same black color and physical design for both traps. However, the SCG Mosquito Trap may have the advantage of size over the LeO-Trap® because it could hold about 2 liters of water that lasted for a month, whereas the LeO-Trap® held about 250 ml. of water, which lasted for 2 weeks [20]. It is important that all lethal ovitraps always have enough water, as empty containers cannot lure any mosquitoes to lay their eggs. When lethal ovitraps are implemented in a village, it is essential to remind the users to refill them with water when they are near empty and put in the appropriate amounts of larvicides.

The attractant derived from the carpet shell extract used in this study was developed originally by Thavara et al. 2004 [21] and modified and formulated by Tawatsin et al. 2019 [20]. It could increase the oviposition efficacy of the SCG Mosquito Trap against *Ae. aegypti* and *Ae. albopictus* by about 1.7 and 4.1 times under laboratory conditions, respectively. These results of oviposition efficacy were similar to those of the LeO-Trap®, which were increased with attractant against both *Ae. aegypti* and *Ae. albopictus* by about 1.5 and 2.7 times, respectively [20]. There was similar attraction against *Ae. aegypti* that derived from both the LeO-Trap® and the SCG Mosquito Trap. However, the difference of attraction against *Ae. albopictus* between the LeO-Trap® and SCG Mosquito Trap in this study may be due to different sources of the carpet shells used, which may provide different amounts of chemical constituents of the extract.

In this study, Bti was used as a biological larvicide in the SCG Mosquito Trap in order to avoid using chemical insecticides. As expected, Bti demonstrated excellent killing effect with complete mortality (100%) against the larvae of *Ae. aegypti* and *Ae. albopictus* for 4 weeks post single application. This showed good advantage of using the lethal ovitrap with biological larvicide that is environment friendly. However, Bti has the limitation of short residual efficacy, with longevity of complete larvicidal effect of about 4 weeks after single application, whereas temephos used in the LeO-Trap® has larvicidal efficacy for 3 months [20]. Therefore, it is important to develop new formulations of environment friendly Bti that have longer residual larvicidal efficacy in the SCG Mosquito Trap. An advanced ovitrap of low-density polyethylene (LDPE), with larvicide pyriproxyfen, was developed and showed emergence inhibition of 100% for over 30 weeks [16]. Another interesting study developed the novel durable dual-action lethal ovitrap (DDALO), which combined with larvicide, adulticide and a slow-release polymer (isobutyl methacrylate) [17]. The DDALO provided high adult mosquito mortality and prevented *Ae. aegypti* eggs from hatching for 6 months [17]. Therefore, an innovation to enhance long lasting efficacy of the lethal ovitrap, in luring and killing *Aedes* larvae, must be developed.

In conclusion, the SCG Mosquito Trap demonstrated outstanding efficacy in luring *Ae. aegypti* and *Ae. albopictus* to lay their eggs in the trap, and all of the larvae that emerged from the eggs were killed by Bti. This trap could be used as an additional effective device for controlling *Ae. aegypti* and *Ae. albopictus* mosquitoes, and preventing diseases that are transmitted by these vectors. However, more field studies of the SCG Mosquito Trap should be carried out to confirm its impact on the reduction of mosquito populations as well as the incidence of diseases in the areas implemented.

ACKNOWLEDGEMENT

This study was supported by the Department of Medical Sciences, Ministry of Public Health, Thailand and SCG Chemicals Co., Ltd.

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