Research Article

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2019.19.003298

Development of A Novel Ultrasonic Sound-Generated Device: The Physical Tool for Controlling Immature Stages of Mosquitoes Transmitting Dengue Haemorrhagic Fever (*Aedes aegypti*) and Filariasis (*Culex quinquefasciatus*)

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ARTICLE INFO

Received: July 01, 2019

Published: July 05, 2019

Citation: Apiwat T, Usavadee T, Padet S , Sopon P. Development of A Novel Ultrasonic Sound-Generated Device: The Physical Tool for Controlling Immature Stages of Mosquitoes Transmitting Dengue Haemorrhagic Fever (*Aedes aegypti*) and Filariasis (*Culex quinquefasciatus*). Biomed J Sci & Tech Res 19(3)-2019. BJSTR. MS.ID.003298.

Keywords: Ultrasonic; Physical Control; Mosquitoes; *Ae. aegypti*; *Cx. quinquefasciatus*; Dengue Haemorrhagic Fever; Zika, Chikungunya; Filariasis

Abstract

Perseus is an ultrasonic sound-generated device developed as a physical tool for controlling the immature stages of mosquitoes by generating an ultrasonic wave at an appropriate frequency, which is specific to mosquito larvae and pupae. Its killing effect was evaluated against the larvae and pupae of Aedes aegypti and Culex quinquefasciatus, which are vectors of dengue haemorrhagic fever and filariasis respectively, under laboratory conditions. In addition, Perseus was investigated for the control of *Ae. aegypti* in the field. Perseus showed extreme efficacy in killing the larvae and pupae of *Ae. aegypti* and *Cx. quinquefasciatus* with 100% mortality within minutes after exposure. In the field, House Index (HI) and Container Index (CI) in a Perseus treated group were decreased dramatically from 73.3% to 6.7% and 66.1% to 1.6%, respectively. When compared with a non-treated control group, the HI fluctuated between 53.3% and 70% and CI remained between 41.2% and 59.6% during the same period. Negative impacts of the ultrasonic wave generated by Perseus also were studied against some aquatic non-target organisms usually found in mosquito breeding sites. No adverse effects were found in Guppy fish (*P. reticulata*), Swordtail fish (*X. hellerii*), small water-boatmen (*M. grisea*) or freshwater snails (*I. exustus*) after exposure to the ultrasonic wave. In conclusion, Perseus showed excellent killing effect against the immature stages of *Ae. aegypti* and *Cx. quinquefasciatus*. It is an innovative physical tool that could be used as supplementary equipment for controlling mosquito vectors, without using pesticides or having negative impact on beneficial aquatic organisms.

Introduction

Sound waves are physical energy caused by vibrating material. The energy of the vibrator is transferred to particles of the medium that are exposed to sound. These particles transfer the energy of vibrations together with the particles of the next medium, resulting in sound propagation for sound waves in air or water. Ultrasounds are high frequency waves (higher than 20kHz) that are used extensively in many industries and for medical purposes. They could be used in industry for detecting cracks and flaws in metal blocks or cleaning parts that are difficult to reach. Regarding medical purposes, ultrasound has been used in various instruments, such as echocardiograms and ultrasound scanners, and it is used to

 DOI: 10.26717/BJSTR.2019.19.003298

break up small kidney stones into fine grains. Moreover, ultrasonic waves have been used for marine purposes in measuring the speed, direction and distance of underwater objects. In addition, attempts have been made to exploit ultrasonic sound for insect and pest control. Many ultrasound devices were developed and tested for repellent efficacy against various kinds of insects and pests, and it was found that they were not effective against mosquitoes [1,2], bed bugs [3], fleas, ticks or cockroaches [4]. There have been very few studies on the larvicidal efficacy of ultrasound devices against mosquito larvae until recently. However, a 2015 study in the USA showed that two devices; the Larvasonic™ Field Arm Mobile Wetlands Unit and SD-Mini, were effective against Culex mosquitoes [5]. In Thailand, mosquito-borne diseases, such as dengue haemorrhagic fever (DHF), chikungunya, zika, filariasis and malaria are very important communicable diseases, which cause many deaths each year. Among these, DHF is the most dangerous causing a high incidence of over 100,000 patients in Thailand every 2 to 3 years.

Since the disease was first reported in 1958, DHF has spread across Thailand and become an important mosquito-borne disease of the country [6]. Until now, an effective vaccine for DHF has not been available. Therefore, control of DHF vectors, such as Aedes aegypti and Aedes albopictus, is the best strategy for decreasing the incidence of this disease [6]. This study aimed to develop an ultrasonic-generated device and evaluate its efficacy for controlling the immature stages of 2 species of mosquito vectors. It also studied its impact on some aquatic animals that are not target organisms.

Materials and Methods

The Development of An Ultrasonic Sound-Generated Device

Perseus is an ultrasonic sound-generated device developed as a physical tool for controlling the immature stages of mosquitoes in water. Its control unit consists of a microprocessor, an amplifier, a rechargeable 24-volt battery, power switch and control button, with dimensions of 12x14x16 cm (WxLxH) and weight of about 4.8kg. An ultrasonic transducer probe (6-cm in diameter and 18-cm long) was connected to the control unit by a 2-meter cable. A piezoelectric resonator translated electrical energy into mechanical vibrations. The clamping mechanism of the transducer probe provided bonding tool mounting, which is a typical ceramic capillary in ball bonding. The oscillator output was given to a power amplifier that drove the piezoelectric crystal to generate ultrasound waves. Power amplification was achieved by replacing the transistor in a typical liquid crystal (LC) tuned Colpitt–oscillator by four power transistors placed in a bridge configuration (Figure 1). When turning on the power switch, the liquid crystal display (LCD) was activated from the 24-volt battery. After pressing the control button, the frequency was released to the transducer probe via an ultrasonic plug at a higher signal strength, ranging between 18-36 KHz [1hertz (Hz) - 1 cycle per second]. As the waves passed through the liquid, microscopic bubbles were formed and collapsed, thus destroying mosquito larvae or pupae.

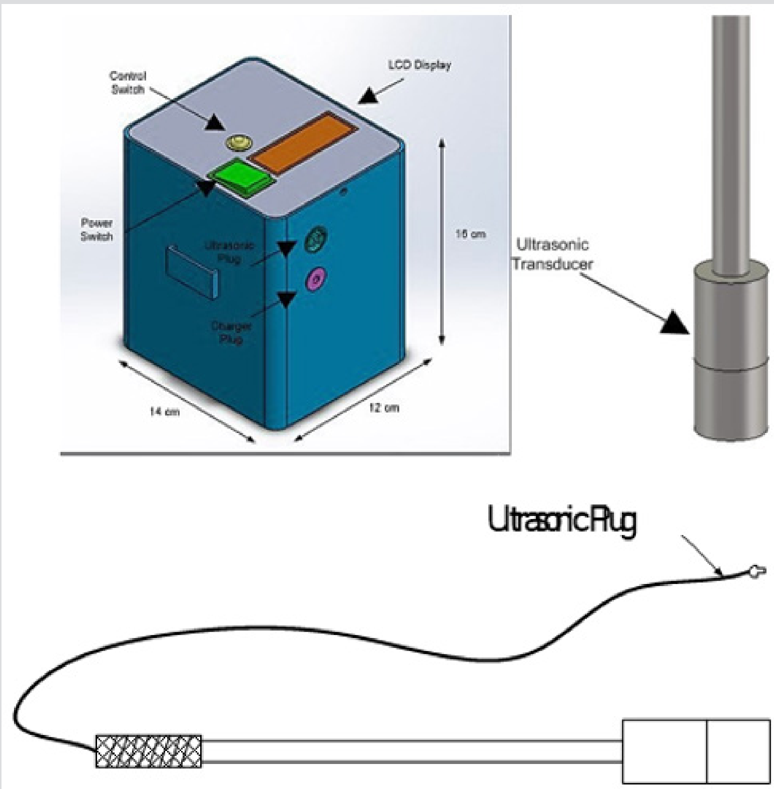

Figure 1: Component of the ultrasonic sound-generated device called "Perseus".

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The Immature Stages of Mosquitoes for Experiments

Ae. aegypti: To obtain the immature stages of *Ae. aegypti*, mosquito eggs (already prepared and kept in the insectary) were attached to filter paper, immersed in 5 liters of tap water in a plastic tray (300x400x50 mm) and left for about 1 hour. After hatching, the larvae were fed on dry powdered mouse food at a rate of 100 mg per tray twice daily until they developed completely. Four larval instars lasted for about 6-9 days, followed by the pupal stage. The third-instar larvae and pupae of *Ae. aegypti* were used in the experiments.

Cx. quinquefasciatus: To obtain the immature stages of *Cx. quinquefasciatus*, 6 to 7 egg rafts of the mosquitoes (previously prepared in the insectary) were placed in a plastic tray (300x400x50 mm) containing 5 liters of tap water. The larvae hatched within 2 days and were fed on dry powdered mouse food at a rate of 100 mg per tray twice daily. Fatty film on the water surface was removed every morning by wiping it with filter paper, with fresh water added to replace the water lost. Similar to *Ae. aegypti*, 4 larval instars lasted a total of 6-9 days, followed by the pupal stage. The third-instar larvae and pupae of *Cx. quinquefasciatus* were used in the experiments.

Non-Target Aquatic Organisms for The Experiments

Some aquatic organisms are found usually in the breeding places and natural habitats of *Ae. aegypti* and *Cx. quinquefasciatus*. Among these, some are predators of the immature stages of mosquitoes. These beneficial organisms should therefore be kept in natural habitats for the biological control of mosquitoes. The aquatic organisms used in this study included Guppy fish (*Poecilia reticulata*), Swordtail fish (*Xiphophous hellerii),* small water-boatmen (*Micronecta grisea*), and freshwater snails (*Indoplanorbis exustus*). The Guppy fish and the Swordtail fish were purchased from an aquatic pet shop in Nonthaburi province, while the small water-boatmen (*M. grisea*), and freshwater snails (*I. exustus*) were collected from natural breeding sites in ponds in the same province. The adults of these aquatic organisms were tested for any negative impact from ultrasonic exposure.

Evaluation of The Killing Efficacy Against the Target and Non-Target Organisms

The Immature Stages of Mosquitoes: Twenty of the 3rd instar larvae and 20 pupae of *Ae. aegypti* were transferred into a round-shaped plastic bucket containing 20 liters of water. Perseus was immersed into the water before the ultrasonic transducer probe swung around the larvae and pupae within a 5-10 cm radius. The number of larvae and pupae deaths from the ultrasonic wave was recorded at 5 minutes, 1 hour and 24 hours post exposure. The tests were carried out with 5 replications and then the average mortality rate (%) was calculated. A control group, with no exposure to the ultrasonic wave, also was tested with 5 replications and compared to the treated group in each experiment. If the average mortality rate of the larvae and pupae in the control group was between 5% and 20% in each experiment, the mortality rate of those in the treated group would be corrected and adjusted with Abbott's formula [7]. This experiment was carried out also in round-shaped plastic buckets containing 100 liters of water; however, the device operated for 2-3 minutes, as there was more space than that in 20-liter buckets. In addition, the same experiments were carried out against the larvae and pupae of *Cx. quinquefasciatus* in round-shaped plastic buckets containing 20 and 100 liters of water. The killing efficacy of ultrasonic exposure against the immature stages of both mosquito species was assessed by their mortality rates. The data obtained were normalized by transforming to natural log (x+1) prior to statistical comparison, and then analyzing by one-way analysis of variance (SPSS for Windows version 18, SPSS Inc., Chicago, IL). If statistical significance was observed, the means of mortality among the tested mosquitoes were then compared by Duncan's Multiple Range Test. The accepted level of significance for all comparisons was $P \leq 0.05$.

The Aquatic Non-Target Organisms: Ten adult Guppy fish (*P. reticulata*) were transferred into a round-shaped plastic bucket containing 20 liters of water. Perseus was immersed into the bucket and the ultrasonic transducer probe operated under the water for 1 minute. The probe swung around under the water within a 5-10 cm radius from the fish. The number of dead fish from the ultrasonic wave was recorded at 1 hour, 24 hour and 7 days post exposure. The tests were carried out with 5 replications and then the average mortality rate (%) was calculated. A control group, with no exposure to the ultrasonic wave, also was tested with 5 replications and compared to the treated group in each experiment. If the average mortality rate of the fish in the control group was between 5% and 20% in each experiment, the mortality rate of the fish in the treated group would be corrected and adjusted with Abbott's formula. This experiment was carried out also in round-shaped plastic buckets containing 100 liters of water; however, the device operated for 2-3 minutes as there was more space than that in the 20-liter buckets. In addition, the same experiments were carried out against the Swordtail fish (*X. hellerii*), small water-boatmen (*M. grisea*) and freshwater snails (*I. exustus*) in round-shaped plastic buckets containing 20 and 100 liters of water. The killing efficacy of ultrasonic exposure against the adult stage of the 4 species of organisms were assessed by their mortality rates. The data obtained were normalized by transforming to natural log (x+1) prior to statistical comparison, and then analyzing by one-way analysis of variance (SPSS for Windows version 18, SPSS Inc., Chicago, IL). If statistical significance was observed, the means of mortality among the tested organisms were compared by Duncan's Multiple Range Test. The accepted level of significance for all comparisons was $P \leq 0.05$.

Field Evaluation Against: *Ae. aegypti*: Field evaluation for the efficacy of Perseus against the immature stages of *Ae. aegypti* was carried out in 2 villages in Muang district, Nonthaburi province, Thailand. The 2 villages were selected randomly as study sites for this study, located about 5 km from each other. A total of 30 houses in each village was selected for the treatment and control (without treatment) group. All of the houses were single-storey with many water-storage containers of various types located inside and around the buildings. These water holders included glazed clay jars (50-200 L), plastic barrels (50-100 L), concrete tanks in the bathroom (100-1,000 L) and miscellaneous containers (20-100 L). Visual larval surveys were carried out prior to the start of this study by inspecting every water-storage container in each house in order to assess and record the prevalence of the immature stages (larvae and pupae) of *Ae. aegypti*. Then, Perseus treatment was administered for a few minutes to the treated group in some containers, whereas no treatment was given in the untreated group (control). In addition, each container was numbered and marked on its side with a permanent-color marker for subsequent surveys. The markings ensured that the same containers would be inspected for the presence or absence of larvae or pupae in the next surveys. In actual fact, assessments were carried out by inspection of the immature stages at the beginning of, and once a week after, treatment in both villages. The data obtained from the visual larval survey in the villages were then calculated as House Index (HI, the percentage of houses positive for larvae/pupae as compared with total houses) and Container Index (CI, the percentage of containers positive for larvae/pupae as compared with total containers). The experiments were carried out for 4 weeks after treatment. No larvicide or other mosquito agent was applied in the marked containers of either group during the 4 weeks of this study period.

Results and Discussion

Perseus, an ultrasonic sound-generated device, showed extreme efficacy in killing the immature stages of *Ae. aegypti* and *Cx. quinquefasciatus* in this study. It could kill the larvae and pupae of both species completely with an average mortality rate of 100% in 20- and 100-liter containers (Table 1). In actual fact, the ultrasonic sound emitted from the device could eliminate the larvae and pupae within a few seconds from a radius of about 5-10 cm away from the ultrasonic transducer probe. In principal, the immature stages of mosquitoes (larvae and pupae) have internal organs that contain a small air bladder that has acoustic resonance, especially underwater bubbles. The tissues of immature mosquitoes are fragile usually, and naturally match the acoustic resonance of the air bladder. Exposure to appropriate ultrasonic sound induces embolism and damages the surrounding tissues, resulting in the death of mosquito larvae and pupae. The Perseus developed in this study is an innovative ultrasonic tool that can kill both mosquito larvae and pupae. In a previous study, acoustic larvicide technology was developed to kill the larval stage of mosquitoes; however, it also was found that some *Ae. aegypti* larvae could survive and develop to adult stage even after long exposure in small volumes of water [8].

Table 1: Mortality rates of the immature stages of *Ae. aegypti* and *Cx. quinquefasciatus* at 24 hours after exposure to Perseus used in 2 sizes of water containers (20 and 100 liters).

The Immature Stages of Each Species In 2 Sizes of Water Containers	Mortality (Mean + S.E.) of the Immature Stages of Each Species	
	Treatment with Perseus	Control (without treatment)
Ae. aegypti larvae in 20-L barrels	100^{a} + 0.0	0^{b} + 0.0
Ae. aegypti pupae in 100-L barrels	100^{a} + 0.0	0^{b} + 0.0
Cx. quinquefasciatus larvae in 20-L barrels	100^{a} + 0.0	0^{b} + 0.0
Cx. quinquefasciatus larvae in 100-L barrels	100^{a} + 0.0	0^{b} + 0.0

Remark: The mean mortality in the same column followed by the same letter is not significantly different.

(P>0.05, Duncan's multiple range test) No mortality was observed in the control group.

This could be due to inappropriate frequency used, inadequate strength of the ultrasonic wave emitted from the device and insufficient duration of exposure time. However, there are many advantages of Perseus use in controlling the immature stages of *Ae. aegypti* and *Cx. quinquefasciatus*. Firstly, Perseus can kill mosquito larvae and pupae within seconds when used properly within a radius of 5-10 cm, which means that the ultrasonic transducer probe must be moved as carefully as possible to the targeted larvae and pupae inside the water container. Although this maneuver is not difficult, it does need concentration and good eyesight to work it correctly. In general, Perseus may take a few minutes to kill all of the larvae and pupae in 200-L water-storage containers. Secondly, it exploits the physical property of ultrasonic sound to kill the targeted larvae and pupae without using pesticides, which results in Perseus being used in all containers in the house, including potable water-storage containers. Thirdly, Perseus can kill immature stages of mosquitoes that are resistant to insecticides, and this could reduce insecticide resistance in mosquito vectors in Thailand. In fact, resistance to various kinds of insecticides, especially pyrethroid group has developed in *Ae. aegypti* and *Ae. albopictus* in various provinces of Thailand [9, 10]. Fourthly, Perseus requires a small power supply from battery operated equipment, without the use of diesel or gasoline. This means that Perseus uses green energy for its operation, and it can therefore reduce environmental pollution caused by fossil fuel. Finally, using Perseus dramatically reduces the use of larvicides or thermal fogging with adulticides, and decreases water and air pollution.

The possible negative effects of ultrasonic sound or Perseus on some aquatic non-target organisms were studied, and the results are demonstrated in Table 2. No adverse effects were apparent on the Guppy fish (*P. reticulata*), Swordtail fish (*X. hellerii*), small water-boatmen (*M. grisea*) or freshwater snails (*I. exustus*) after exposure to ultrasonic waves in 20-L and 100-L water containers. No mortality of the 4 species was found 7 days after the test in the treated or control group. In general, Guppy fish (*P. reticulata*), Swordtail fish (*X. hellerii*) and small water-boatmen (*M. grisea*) are beneficial organisms used as biological control agents for controlling *Ae. aegypti* and other mosquito species [11,12]. In Thailand, some people in rural areas put larvivorous fish, such as Guppy (*P. reticulata*), Siamese fighter (*Betta splendens*) or Emerald Betta (*Betta smaragdina*) in big water-storage containers, for example, 200-L jars or concrete tanks in their restroom, in order to control mosquito larvae. However, it has been found frequently that these larvivorous fish could not consume all of the larvae inside the containers if the larval density was high, and some of the larvae developed into adult mosquitoes. In this case, Perseus is used as a supplementary tool with the fish to eliminate the larvae and pupae in the containers.

Table 2: Mortality rates of the 4 species of adult aquatic non-target organisms at 7 days after exposure to Perseus used in two sizes of water containers (20 and 100 liters).

The Adult Stages of Each Species In 2 Sizes of Water Containers	Mortality (Mean + S.E.) of the Adult Stage of Each Species Within 7 Days Post Exposure	
	Treatment with Perseus	Control (Without Treatment)
Guppy fish (*P. reticulata*) in 20-L barrels	$0^a + 0.0$	$0^a + 0.0$
Guppy fish (*P. reticulata*) in 100-L barrels	$0^a + 0.0$	$0^a + 0.0$
Swordtail fish (*X. hellerii*) in 20-L barrels	$0^a + 0.0$	$0^a + 0.0$
Swordtail fish (*X. hellerii*) in 100-L barrels	$0^a + 0.0$	$0^a + 0.0$
Small water-boatmen (*M. grisea*) in 20-L barrels	$0^a + 0.0$	$0^a + 0.0$
Small water-boatmen (*M. grisea*) in 100-L barrels	$0^a + 0.0$	$0^a + 0.0$
Freshwater snail (*I. exustus*) in 20-L barrels	$0^a + 0.0$	$0^a + 0.0$
Freshwater snail (*I. exustus*) in 100-L barrels	$0^a + 0.0$	$0^a + 0.0$

Remark: The mean mortality in the same column followed by the same letter is not significantly different.

(P>0.05, Duncan's multiple range test) No mortality was observed in the control group.

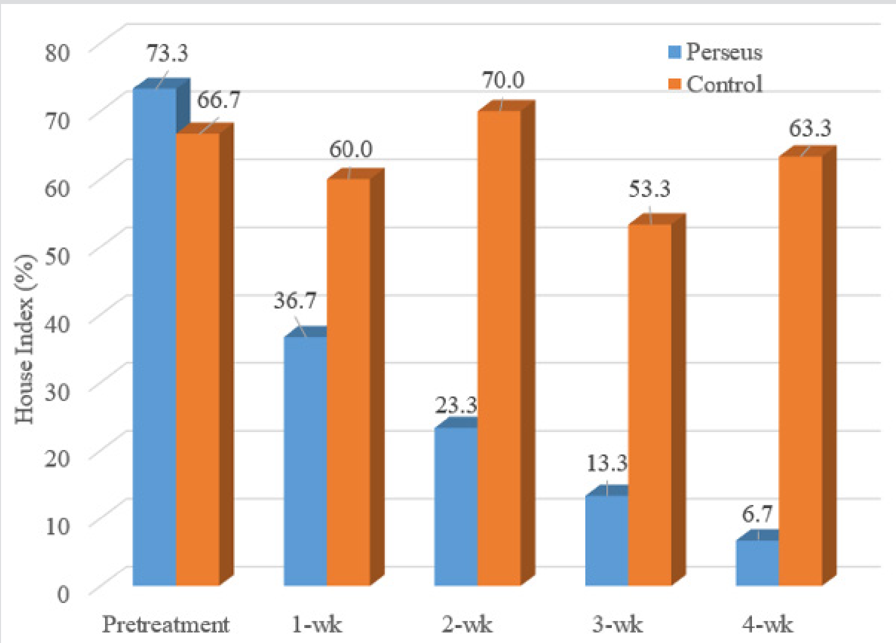

Figure 2: Assessment of the House Index (HI) in the group of houses treated with Peseus during 4 weeks after treatment and those without treatment (control).

Field evaluation for the efficacy of Perseus against the immature stages of *Ae. aegypti* was carried out in various kinds of water containers in 2 villages for a period of 4 weeks and the results are shown in Figures 2 and 3. Thirty houses in the treated group had a total of 124 water-storage containers, while the 30 houses in the control group had 136. The HI in the treated group was dramatically decreased from 73.3% at the start of the study to 6.7% at 4 weeks post treatment (Figure 2). In contrast, the HI in the control group was about 66.7% at the start of the study and fluctuated between 53.3% and 70% during the 4-week period until the end of the study (Figure 2). Similar to the HI, the CI in the treated group also was reduced substantially from 66.1% to 1.6% in the last week of this study, whereas the CI in the control group also fluctuated between 41.2% and 59.6% (Figure 3). The results obtained from this study revealed that the proper use of Perseus substantially reduced the population of immature *Ae. aegypti* mosquitoes in infested areas, especially those in large containers containing at least 10 liters of water. In Thailand, the main breeding places for *Ae. aegypti* are mainly man-made containers, such as 200-L jars, 50-100 L plastic barrels, 50-200 L concrete tanks in restrooms, ant traps, flower pots and vases [6]. Operating Perseus treatment needs extra concentration and care in moving the ultrasonic transducer probe to the targeted larvae and pupae inside the water container. However, it is too difficult for Perseus to eliminate the immature stages of *Ae. aegypti* in small containers that have a capacity of less than 10 liters such as ant traps, flower pots, vases, etc. Therefore, larvicides or other agents should be used in small amounts of water. In this study, Perseus was not evaluated against *Cx. quinquefasciatus* in the field because limitation of its design. Actually, the current device has been designed for using in the water storage containers containing 10-200 liters of water. The larger device would be developed for controlling Culex larvae in their breeding places, such as polluted canals, ponds or drains.

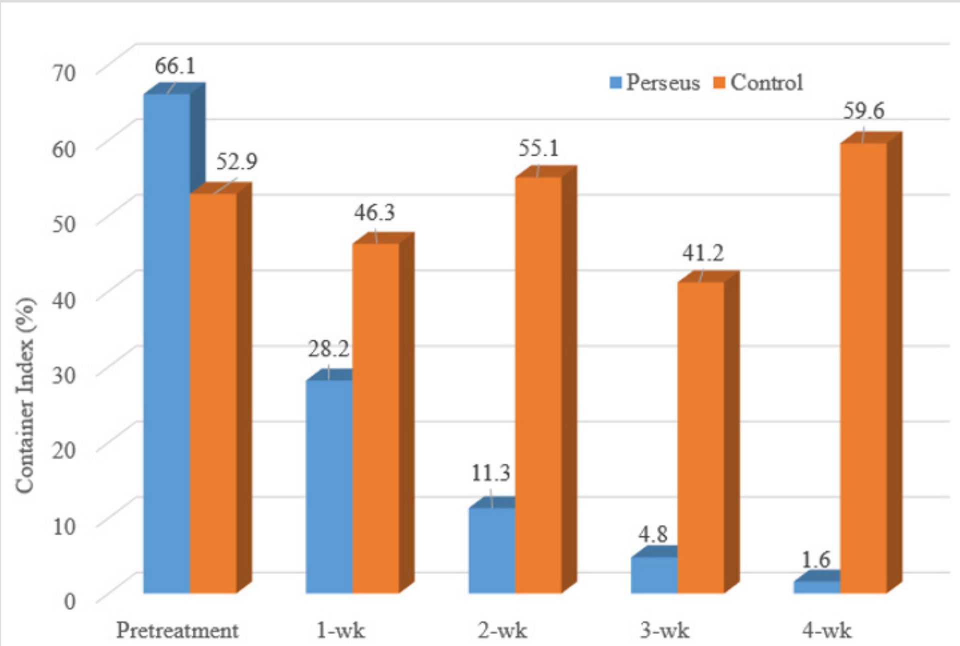

Figure 3: Assessment of the Container Index (CI) in the group of houses treated with Peseus during 4 weeks after treatment and those without treatment (control).

In conclusion, Perseus showed excellent killing effect against the immature stages of Aedes aegypti and *Cx. quinquefasciatus* under laboratory and field conditions. It is an innovative physical tool that can be used as supplementary equipment for controlling mosquito vectors in Thailand without the use of pesticides or impact on beneficial aquatic organisms. On the other hand, it also has some disadvantages in that no residual effect is apparent in treated containers and Perseus is a time and labor-intensive method. However, this physical tool can help to reduce the use of pesticides, save on costs for purchasing pesticides and minimize the negative impact that pesticides have on human health. In addition, Perseus can eliminate the problem of insecticide resistance in mosquitoes, as found in various regions of Thailand.

Acknowledgement

The authors appreciated kind assistance of Mr. Navy Srivarom and Mrs. Thanittha Chumphonrak in laboratory and field evaluations.

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ISSN: 2574-1241

DOI: 10.26717/BJSTR.2019.19.003298

Apiwat Tawatsin. Biomed J Sci & Tech Res