Efficiency of an Ultrasonic Device in Eliminating *Aedes aegypti* Larvae and Pupae under Laboratory Conditions

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ABSTRACT *Aedes aegypti* mosquitoes, which are responsible for mosquito-borne diseases, cause a major public health challenge in Thailand. The Office of Disease Prevention and Control Region 9, Nakhon Ratchasima, in collaboration with Suranaree University of Technology, Thailand developed an ultrasonic wave generator named "SONiC BOOM" for mosquito management. This study aimed to evaluate the effectiveness of this device in controlling mosquito larvae and pupae under laboratory-controlled conditions. Mosquito larvae (n = 50) and pupae (n = 50) were tested in 20-liter containers in an entomological laboratory. The study included control and test groups exposed to 40 KHz ultrasonic waves for 3 seconds at five different points in the water. Percent mortality was recorded at 5 min and 24 hr post-exposure, and statistical analysis evaluated for significant differences. The results indicated that larval mortality was not different between 5 min and 24 hr post-exposure. The pupal stage showed a substantial difference in mortality between these time points. There was a significant difference in mortality ($p < 0.05$) between the group exposed to ultrasonic waves and the control group. Initial mortalities after 5 min exposure were 96.0% for larvae and 64.0% for pupae. After 24 hr, mortalities increased to 97.6% for larvae and 100% for pupae. Ultrasonic waves caused rupture of mosquito larvae and pupae's tissues or internal organs, leading to their death. Thus, "SONiC BOOM" is an efficient alternative tool for controlling mosquito larvae and pupae without chemicals, having minimal environmental impact. The device can be further developed and used in areas affected by future disease outbreaks.

Keywords: *Aedes* mosquito, Larva and pupa elimination, Ultrasonic

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Introduction

Mosquito-borne diseases, especially those transmitted by *Aedes aegypti* mosquitoes such as dengue hemorrhagic fever (DHF), Zika, and chikungunya, pose a severe threat to global health. These diseases infect hundreds of millions of populations annually, placing immense pressure on healthcare systems, particularly in tropical and subtropical regions. Factors like rapid urbanization and climate change are expanding the habitat of *Ae. aegypti*, facilitating the spread of these viruses. The absence of effective vaccines for these diseases intensifies their impacts, emphasizing the urgent need for improved vector control measures and strengthened public health strategies.[1] In Thailand, *Ae. aegypti* mosquitoes are the primary vectors responsible for dengue, Zika, and chikungunya diseases. The high prevalence of these diseases poses a significant public health challenge, particularly during the rainy season when mosquito populations surge. Controlling mosquito breeding sites is crucial, but insecticide resistance complicates eradication efforts.[2] Although chemical pesticides are frequently employed to control mosquito populations, they pose risks to human health, non-target organisms, and the environment. Widespread use of chemicals can also lead to contamination of water sources and soil, affecting local ecosystems or the environment and potentially harming beneficial species.[3] Additionally, these uses have accelerated the development of pesticide resistance, reducing the long-term effectiveness of these chemicals. This resistance makes outbreaks harder to manage. As a result, there is a growing need to explore alternative methods, such as biological control and community-based strategies, to achieve sustainable mosquito management.[4] To address these challenges, incorporating other safer technologies into the management of mosquito larvae and adults presents alternatives that reduce environmental and health risks. Moreover, the technologies not only minimize ecological impact but also engage communities in proactive mosquito control efforts, fostering a collaborative approach to public health.[5] Until now, few studies have investigated the larvicidal effectiveness of ultrasonic devices versus larvae of mosquitoes. Nowadays, the Larvasonic™ Field Arm Mobile Wetlands Unit and SD-Mini are efficient against mosquitoes in the genus Culex, according to research conducted in the USA.[6] Additionally, the Larvasonic SD-Mini Acoustic Larvicide device proved successful in controlling immature *Ae. aegypti* in various volumes typical of peri-domestic water containers.[7] Mosquito-borne diseases remain significant public health concerns in Thailand, augmenting a large number of deaths annually. DHF is particularly severe, impacting approximately 100,000 individuals in Thailand every 2 to 3 years.[8] The seasonal and geographical patterns of mosquito-borne illnesses underscore the need for sustained vector control efforts and enhanced public health infrastructure. Furthermore, climate change influences mosquito behavior and disease transmission dynamics, necessitating adaptive strategies to mitigate future risks effectively. The utilization of the Perseus ultrasonic device in Thailand demonstrates a promising approach to controlling mosquito larvae and pupae, specifically targeting *Ae. aegypti* and *Culex quinquefasciatus* (a vector for filariasis).[8] With the rapid spread of mosquito-borne diseases and rising resistance to traditional chemical pesticides, there is an urgent need for innovative alternatives.[9,10] Effective vector control can drastically reduce transmission rates, lowering disease burden in affected regions. Hence, the objective of this research is to evaluate the effectiveness of the ultrasonic wave generator "SONiC BOOM" in controlling mosquito larvae and pupae under laboratory-controlled conditions.

Materials and Methods

Mosquito rearing

Ae. aegypti immature stages were produced by hatching mosquito eggs at the Entomological Laboratory (ISO/IEC 17025:2017), the Center for Vector-Borne Disease Control 9.2 in Buri Ram, Office of Disease Prevention and Control Region 9 Nakhon Ratchasima, Department of Disease Control, Thailand. Eggs of *Ae. aegypti*, sourced from Department of Medical Sciences,Thailand, were placed in 1 liter of chlorine-free water in a plastic tray (18 cm length × 13 cm width × 4 cm depth) and incubated for approximately 24 hr. The larvae of mosquitoes were reared at a temperature of 27±2°C with over 40% relative humidity and a 12:12 light-dark cycle. After hatching, the larvae were fed daily with finely powdered pig feed until they developed into fully grown larvae (larval stages 3-4), typically within 7-9 days, at which point they entered the pupal stage. Both larvae and pupae used in this study were of a susceptible strain to chemical insecticides that were reared in the laboratory for over five generations.

Guppy fish

Adult guppy fish (*Poecilia reticulata*) which is one of the non-target aquatic organisms were collected and selected from ponds near the Entomological Laboratory. The adult guppies were also evaluated for any adverse effects resulting from exposure to ultrasonic waves.

Ultrasonic wave generator

The SONiC BOOM device was developed by Center of Excellence in Electromagnetic Wave, Suranaree University of Technology, Thailand, in collaboration with Office of Disease Prevention and Control Region 9 Nakhon Ratchasima, Department of Disease Control, as an ultrasonic tool to eliminate immature mosquito stages in water under laboratory-controlled conditions. An ultrasonic transducer device called SONiC BOOM operates at a frequency of 40 KHz and outputs 100 W using piezoceramic transducer elements. The specifications of this device include input DC12.6V, 3A, power 100W, frequency 40KHz, battery Li-ion 12V, 15Ah, and power switch, with a size of 15.4 cm length × 10.3 cm width × 20.3 cm height and weighing approximately 3 kg. A 7-cm-diameter, 6.3-cm-long ultrasonic transducer probe is connected via a 2-meter cable to the control unit, which includes a control button (Figure 1). Press the button to activate the transmission of a 40 KHz frequency signal to the transducer probe, which generates fine bubbles in the water.

Effectiveness of ultrasonic treatment under controlled laboratory conditions

Experiments were conducted in earthen jars at the Entomological Laboratory. Each jar

Figure 1 The ultrasonic wave generator named "SONiC BOOM"

of 30 cm diameter, containing 20 liters of water at a height of 22.5 cm, was added 50 larvae and 50 pupae of *Ae. aegypti*. The SONiC BOOM transducer probe was submerged under water and operated within a 10–15 cm range around the larvae and pupae. The probe was activated for 3 seconds per area and repeated 5 times to ensure water surface coverage. The mortality of larvae and pupae was observed at the 5 min and 24 hr time points post-exposure. Control groups, consisting of larvae (n = 50) and pupae (n = 50) not exposed to ultrasonic waves, were included for comparison in each experiment. Each experiment was independently replicated three times using new batches of larvae and pupae, and each repeat was conducted at the same time of the day. Dead larvae and pupae were examined under a stereo microscope (C-LEDS, Nikon, China) to confirm rupture of the exoskeleton. The experiment was conducted for approximately two months. Additionally, 50 guppy fish (*P. reticulata*), were tested under similar conditions of exposure and timing as *Ae. aegypti* groups.

Statistical analysis

Mortality data were analyzed using descriptive statistics, including mean and standard deviation, with GraphPad Prism software version 8. Mortality percentages were examined through one-way analysis of variance (ANOVA). To compare the mean mortalities among various mosquito groups, a post-hoc analysis using Duncan's multiple range test was conducted. Additionally, t-tests were utilized to compare mean mortalities across different treatment periods. A *p*-value of less than 0.05 was considered statistically significant for all comparisons.

Results

Results of the SONiC BOOM treatment are shown in Table 1, presenting the percent mortality of dead *Ae. aegypti* mosquitoes with the ultrasonic wave set to 40 KHz and positioned 10–15 cm from the probe. Within five minutes of exposure, the ultrasonic wave achieved 96.0% mortality in larvae and 64.0% in pupae.

Table 1 Percent mortality of *Ae. aegypti* larvae and pupae and guppy fish after exposure to SONiC BOOM for 5 min and 24 hr.

No. of Replication	Percent mortality of *Ae. aegypti* and guppy fish after exposure to SONiC BOOM																	
	5 minutes									24 hours								
	larvae			pupae			Guppy fish			larvae			pupae			Guppy fish		
1	92	94	100	100	86	90	0	0	0	84	100	100	100	100	100	0	0	0
2	100	96	96	30	68	56	0	0	0	98	98	98	100	100	100	2	0	4
3	96	90	100	54	36	56	0	0	0	100	100	100	100	100	100	6	4	4

By 24 hr post-exposure, mortality increased to 97.6% in larvae and reached 100% in pupae. No significant difference in larval mortality was observed between the 5 min and 24 hr time points, whereas pupal mortality showed a significant increase ($p < 0.01$). Percent mortality was also assessed in guppy fish (*P. reticulata*), with no significant difference between the 5 min and 24 hr post-exposure periods (Figure 2).

At 5 min post-exposure, there was a significant difference in larval percent mortality between the control group (non-exposure) and the treated group ($p < 0.0001$). Additionally, the percent mortality of pupae showed a significant difference between the control group and the treated group ($p < 0.01$) (Figure 3).

At 24 hr post-exposure, there was a significant difference in larval percent mortality between the control group and the treated group ($p < 0.001$). Additionally, the percent mortality of pupae showed a significant difference between the control group and the treated group ($p < 0.0001$) (Figure 4).

No mortality was observed in the control group of mosquito larvae, as they were not treated. The immature *Ae. aegypti* mosquitoes killed with SONiC BOOM treatment were shown in Figure 5. The mortality of fourth instar larvae caused by the SONiC BOOM device was likely attributed to high-frequency sound energy transmitted through the water. This ultrasonic wave caused ruptures in the tissues or internal organs of the mosquito larvae, resulting in significant damage and death, as observed under a stereo microscope (C-LEDS, Nikon, China). However, tissue ruptures were not observed in the pupae. The pupae were unable to move and develop into adult mosquitoes.

Discussion

In this study, we evaluated the effectiveness of the device of SONiC BOOM in eliminating mosquito larvae and pupae under entomological laboratory-controlled conditions. The mosquitoes used in this study were a susceptible strain of *Ae. aegypti*, specifically reared in the laboratory for over five generations to ensure consistent susceptibility to chemical insecticides as the baseline mosquitoes. Rearing conditions included regulated temperature, humidity, and a consistent light-dark cycle, which supported standardized development stages and minimized external variability across experimental repeats. In our study, the SONiC BOOM effectively killed immature stages of *Ae. aegypti* mosquitoes of larvae and

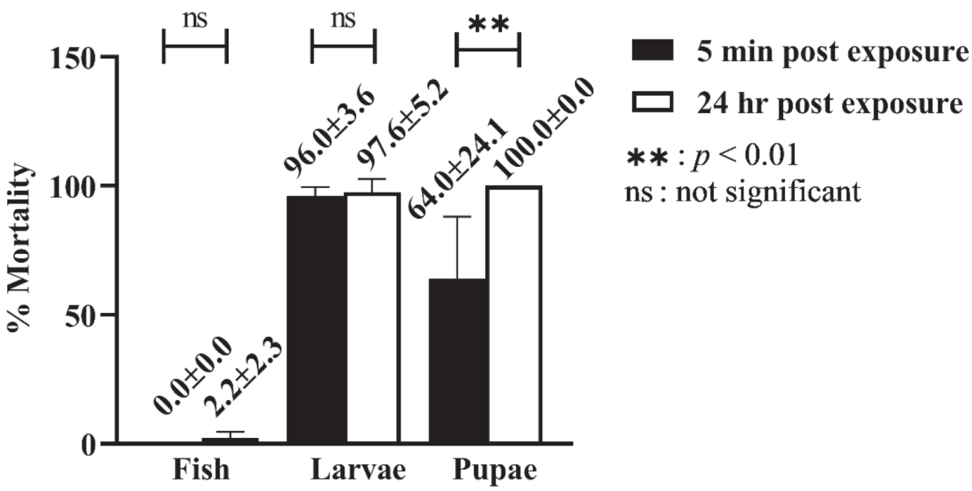

Figure 2 Average percent mortality (±SD) of *Ae. aegypti* larvae and pupae and guppy fish between 5 min and 24 hr post-exposure to SONiC BOOM

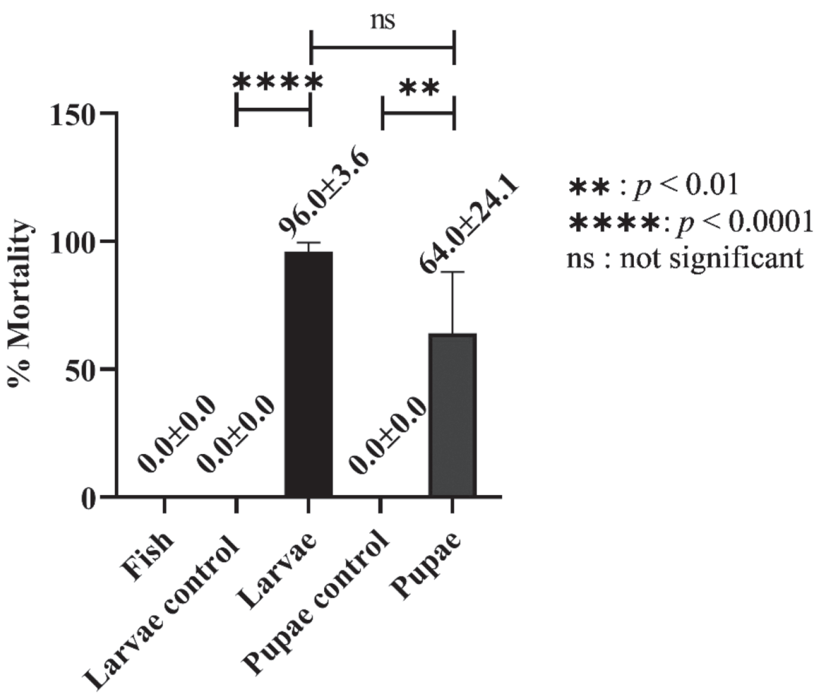

Figure 3 Average percent mortality (±SD) of *Ae. aegypti* larvae and pupae and guppy fish at 5 min post-exposure compared to their respective controls (non-exposure).

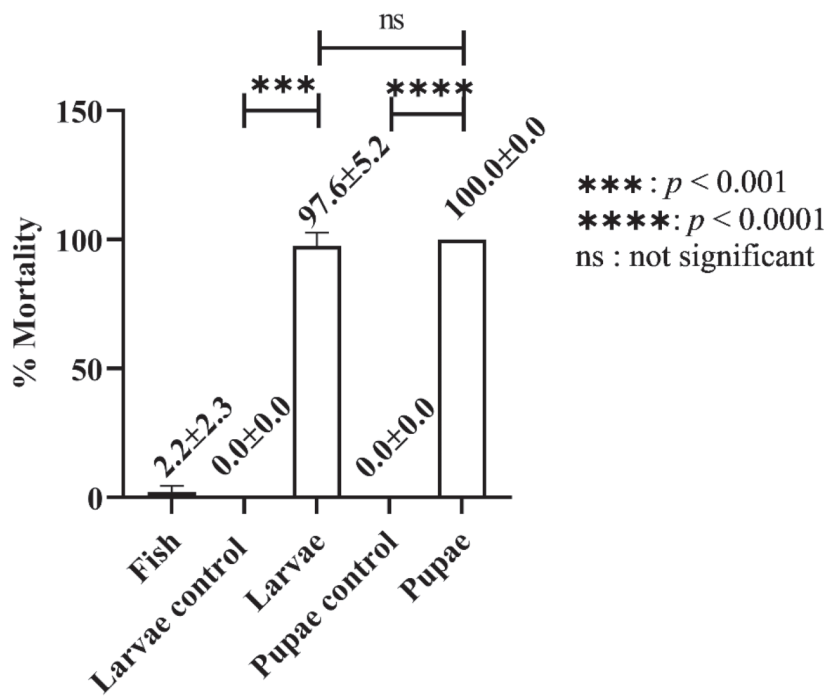

Figure 4 Average percent mortality (±SD) of *Ae. aegypti* larvae and pupae and guppy fish at 24 hr post-exposure compared to their respective controls (non-exposure).

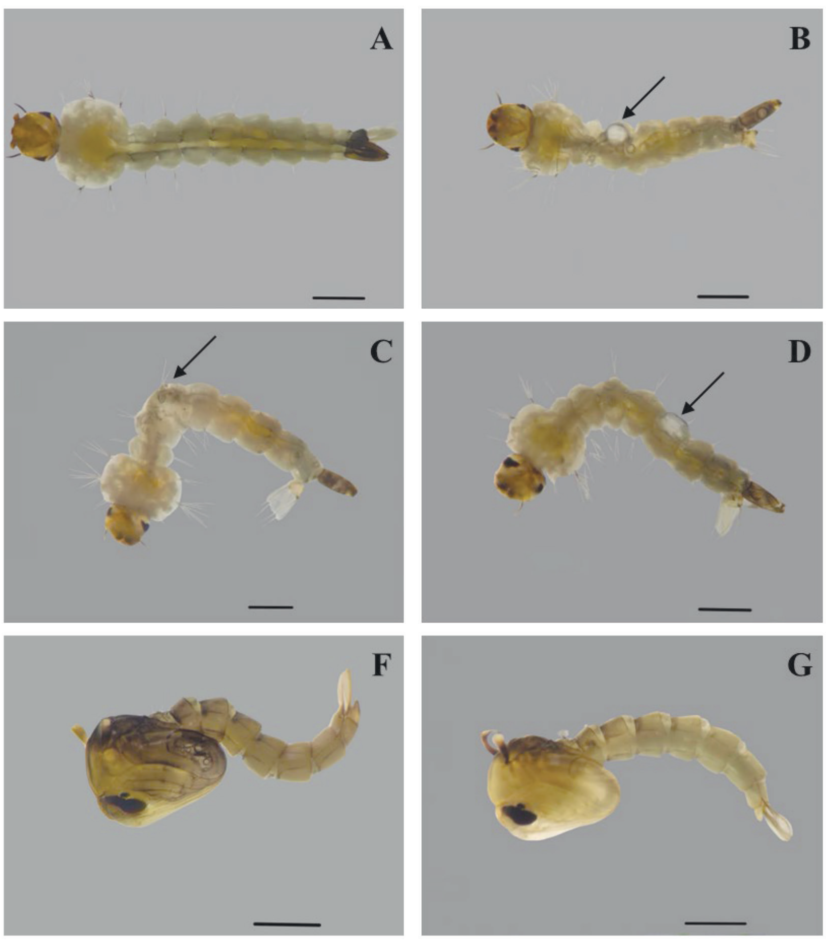

Figure 5 Immature *Ae. aegypti* mosquitoes without (A and F) and with (B, C, D, and G) SONiC BOOM treatment. (A–D: IV instar larvae; F–G: pupae; scale bars = 1 mm).

pupae. The observed mortality in *Ae. aegypti* larvae exposed to the device is likely due to the transmission of high-frequency sound energy into the water[11], with morphological analyses, it caused ruptures in the dorsal tracheal trunk of the larvae and damaged the internal organs of mosquito pupae leading to severe tissue damage and eventual death. The results strongly supported our hypothesis that the acoustic energy generated by the transducer underwater effectively caused fractures in the tracheal trunks of this mosquito species. This phenomenon results in the rupturing of the dorsal tracheal trunk in young mosquito instars, causing significant tissue damage and eventual death.[11] In principle, the internal organs of mosquito larvae and pupae including a small air bladder can emit sound resonance, especially when underwater bubbles are present. Therefore, studying to further understand the resonance characteristics could aid in developing novel pest control strategies that leverage acoustic waves. Exposure to specific ultrasonic frequencies induces embolism, resulting in the destruction of surrounding tissues and effectively eliminating mosquito larvae and pupae.[8]

In this study, the device was demonstrated to kill both larvae and pupae within a brief 5 min timeframe when utilized at a proximity of 10-15 cm. Tawatsin and colleagues[8] reported that the optimal distance and exposure time for the ultrasonic transducer probe (operating between 18-36 KHz) were 5-10 cm and 60 seconds. It also showed 100% mortality of mosquito larvae and pupae, with no adverse effects observed in guppy fish (*P. reticulata*), which is an organism utilized in biological control targeting *Ae. aegypti* and other mosquito species.

Effective operation of the ultrasonic transducer requires precise positioning towards targeted larvae and pupae within water containers, therefore, it needs to focus on adjusting for optimal performance. However, the device exhibits certain limitations, such as the unidirectional release of waves from the transducer probe, following a straight line without radial dispersion. Another constraint is the limited exposure duration of the control button, lasting only 3-5 seconds. This led to a significant limitation as prolonged exposure times indicated increased cumulative effects, influencing control efficiency.[11] Remarkably, this device offers a pesticide-free finding ideal for use in household water containers or portable water storage. Moreover, since it is a chemical-free method, it may exhibit efficacy against mosquito larvae and pupae resistant to traditional insecticides. This is particularly important because populations of both *Ae. aegypti* and *Ae. albopictus* in many parts of Thailand have developed widespread resistance to several insecticides, especially those in the pyrethroid category.[12,13] This kind of device may be superior to larvicides or thermal fogging with adulticides which decreases both water and air pollution. The results indicate that ultrasonic wave devices could be a practical alternative technology for mosquito management.

Conclusively, the findings highlight the potential of ultrasonic treatment as a non-chemical method for mosquito control, offering environmentally friendly. Further studies are needed to optimize parameters such as frequency and intensity to enhance efficacy across diverse mosquito populations and environmental conditions.

Conclusion

This research presented the primary findings obtained from utilizing an ultrasonic sound-based device against *Ae. aegypti* larvae and pupae. The SONiC BOOM ultrasonic device demonstrated high effectiveness in eliminating *Ae. aegypti* larvae and pupae under laboratory-controlled conditions. The findings showed that ultrasonic waves caused significant damage to the tissues or internal organs of mosquito larvae and pupae, leading to their death. Within five minutes of treatment with the SONiC BOOM device, the percent mortality of larvae and pupae reached 96.0% and 64.0%, respectively, increasing to 97.6% and 100% at 24 hr post-treatment. Based on these experiments, the SONiC BOOM device could be a potential practical tool for managing mosquito populations. Moreover, the device could be developed for use in public health interventions and epidemiological areas.

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ประสิทธิภาพของเครื่องอัลตราโซนิคในการกำจัดลูกน้ำและตัวโม่งยุงลายบ้าน (*Aedes aegypti*) ในห้องปฏิบัติการ

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บทคัดย่อ ยุงลายบ้าน (*Aedes aegypti*) เป็นพาหะนำโรคที่เป็นปัญหาสาธารณสุขที่สำคัญของประเทศไทย สำนักงานป้องกันควบคุมโรคที่ 9 จังหวัดนครราชสีมา ร่วมกับมหาวิทยาลัยเทคโนโลยีสุรนารี ได้พัฒนาเครื่องกำเนิดอัลตราโซนิค ชื่อ "SONiC BOOM" เพื่อจัดการลูกน้ำและตัวโม่งของยุง การศึกษานี้มีวัตถุประสงค์เพื่อประเมินประสิทธิภาพของอุปกรณ์ในการควบคุมลูกน้ำและตัวโม่งของยุงภายในห้องปฏิบัติการ โดยใช้ลูกน้ำยุง จำนวน 50 ตัว และตัวโม่ง จำนวน 50 ตัว ทดสอบในโอ่งดินขนาด 20 ลิตร ในห้องปฏิบัติการทางกีฏวิทยา แบ่งเป็น 2 กลุ่ม ได้แก่ กลุ่มควบคุมและกลุ่มทดลองที่ได้รับอัลตราโซนิคที่ความถี่ 40 กิโลเฮิร์ตซ์ ปล่อยคลื่นในบริเวณน้ำ จำนวน 5 จุด ๆ ละ 3 วินาที บันทึกเปอร์เซ็นต์การตายหลังจากปล่อยคลื่นไปแล้ว 5 นาที และ 24 ชั่วโมง วิเคราะห์ความแตกต่างทางสถิติ ผลการศึกษาพบว่าเปอร์เซ็นต์การตายของลูกน้ำในระยะเวลา 5 นาที และ 24 ชั่วโมง หลังการสัมผัสคลื่นไม่แตกต่างกัน โดยในตัวโม่งพบเปอร์เซ็นต์การตายมีความแตกต่างกันอย่างมีนัยสำคัญ นอกจากนี้เปอร์เซ็นต์การตายระหว่างกลุ่มที่ได้รับอัลตราโซนิคและกลุ่มควบคุมมีความแตกต่างอย่างมีนัยสำคัญ ($p < 0.05$) โดยเปอร์เซ็นต์การตายเริ่มต้นของลูกน้ำ เท่ากับ 96.0% และตัวโม่ง เท่ากับ 64.0% และหลังจากได้รับอัลตราโซนิคแล้ว 24 ชั่วโมง พบเปอร์เซ็นต์การตายของลูกน้ำเพิ่มขึ้นเป็น 97.6% และตัวโม่งเป็น 100% โดยอัลตราโซนิคมีผลทำลายเนื้อเยื่อและอวัยวะภายในของลูกน้ำยุง เครื่อง SONiC BOOM จึงเป็นอีกทางเลือกหนึ่งที่มีประสิทธิภาพในการควบคุมลูกน้ำและตัวโม่งของยุงโดยไม่ใช้สารเคมี ทำให้ส่งผลกระทบต่อสิ่งแวดล้อมน้อย สามารถนำไปพัฒนาและนำไปใช้ควบคุมโรคในพื้นที่ที่มีการระบาดได้

คำสำคัญ: ยุงลาย, การกำจัดลูกน้ำและตัวโม่ง, อัลตราโซนิค