A Comparative Study on the Efficiency of Heart-Leaved Moonseed *(Tinospora crispa* L.)Extract Concentrations in Eliminating Aedes Mosquito Larvae*(Aedes aegypti)*

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30th January 2026

Abstract:

This study aimed to evaluate the effectiveness of ethanolic extract from Heart-leaved moonseed(*T. crispa)* stems in eliminating dengue mosquito larvae and to investigate the relationship between extract concentration and larval mortality under controlled conditions. The experimental design consisted of four extract concentrations: 2.5, 5, 7.5, and 10 ppm. Each concentration was tested at three different volumes: 10, 20, and 30 milliliters, and compared with a control group using clean water. Each treatment was conducted in triplicate. Environmental factors, including temperature, light, and pH, were controlled to ensure suitable conditions for larval survival. Larval mortality and physical changes were recorded continuously for 7 days.

The results showed that the Heart-leaved moonseed*(T. crispa)* extract caused a statistically significant increase in larval mortality ($P < 0.05$). The mortality rate was directly proportional to the extract concentration; as the concentration increased from 2.5 ppm to 10 ppm, the mortality of mosquito larvae increased accordingly. In addition, noticeable physical changes were observed in larvae exposed to higher extract concentrations, such as reduced movement and immobility prior to death.

In conclusion, the extract of Heart-leaved moonseed*(T. crispa)* contains bioactive compounds with high potential to inhibit growth and effectively eliminate dengue mosquito larvae. Therefore, it can be used as an alternative method for controlling mosquito breeding sites to prevent dengue fever outbreaks, replacing synthetic chemical insecticides. This approach is safer for humans and environmentally friendly for community use.

Keywords: Heart-leaved moonseed*(T. crispa)*, dengue mosquito larvae, herbal extract, insect control, dengue fever

Research Question:

Does the extract of Heart-leaved moonseed*(T. crispa)* have different levels of effectiveness in controlling mosquito larvae at different concentrations?

Hypothesis

Different concentrations significantly affect the effectiveness of mosquito larval control.

Introduction and Review of Literature

Mosquito-borne diseases are major public health problems in many tropical countries, including Thailand. Mosquitoes act as vectors that transmit viruses, bacteria, and parasites from infected individuals to others, leading to widespread disease outbreaks. Common and severe mosquito-borne diseases include dengue fever, chikungunya, and Zika virus disease, all of which significantly affect public health, quality of life, and national healthcare systems.

In Thailand, these diseases are primarily caused by *Aedes* mosquitoes, which thrive in environments with stagnant water. This is especially evident during the rainy season, which provides favorable conditions for egg laying and rapid population growth. Therefore, controlling and reducing the population of *Aedes* mosquitoes is a crucial strategy for preventing disease transmission. One effective approach is the elimination of mosquito larvae at their aquatic breeding sites.

However, current mosquito larval control methods mainly rely on chemical insecticides. Although these chemicals provide rapid results, they present several limitations and adverse effects, such as toxicity to humans, domestic animals, and the environment. In addition, prolonged and repeated use of chemical insecticides may lead to insecticide resistance in mosquitoes, resulting in reduced effectiveness of control measures.

Heart-leaved moonseed*(T. crispa)*, commonly known as Boraphet, is a traditional Thai medicinal plant that has long been used in Thai traditional medicine. Previous studies have reported that Boraphet contains bioactive compounds capable of affecting the biological systems of certain insects. Therefore, interest has arisen in investigating the potential of Boraphet extract as a natural alternative for mosquito larval control.

Accordingly, this project entitled **"A Study of Heart-leaved moonseed*(T. crispa)* Extract for Mosquito Larval Control"** was conducted to evaluate the effectiveness of Boraphet extract in eliminating mosquito larvae. The results of this study are expected to provide fundamental information for developing safe, environmentally friendly, and sustainable methods for mosquito larval control, while also promoting the utilization of Thai herbal resources.

Materials and methods

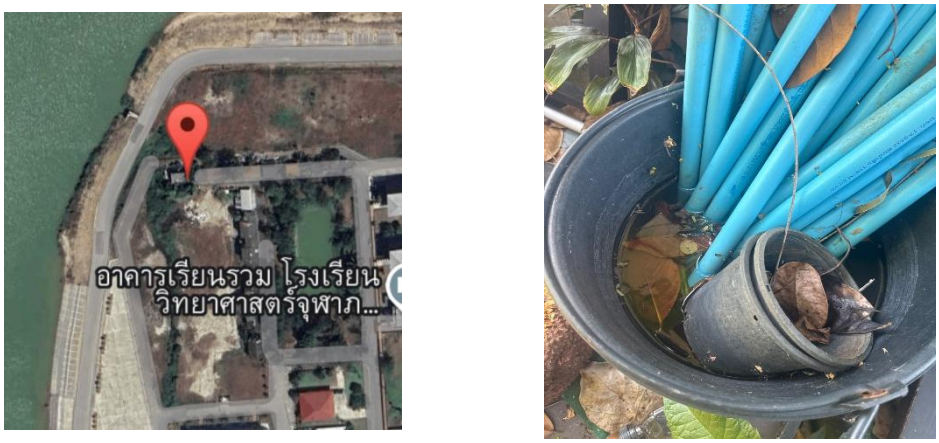
Materials

1.Mosquito
2.muslin cloth
3.Rubber band
4.Aquarium net
5. 250 ml beaker
6.Rotary Evaporater
7.Ethanol
8.Dropper
9.Wormwood extract
10.Fish tank
11.Waterbath

Methods

Study site

This study located 2 study sites, first is Teacher's residence of Princess Chulabhorn Science High School Trang. 196 Village 4, Trang-Si Kao Road, Bang Rak Subdistrict, Mueang Trang District, Trang Province on latitude 7.5552 Longitude 99.5566
Second is 112/2 Village No.5,Bang Kung Subdistrict,Huai Yot District,Trang Province on latitude 7.7512 Longitude 99.4650



Picture 1 : Teacher's residence of Princess Chulabhorn Science High School Trang.
196 Village 4, Trang-Si Kao Road, Bang Rak Subdistrict, Mueang Trang District, Trang Province

Picture 2 : **112/2 Village No.5,Bang Kung Subdistrict,Huai Yot District,Trang Province**

2. Research Method

2.1. Time duration and sampling point setting

For sampling, the researchers collected mosquito larvae at Princess Chulabhorn Science High School Trang **during December to January 2026. Dividing the study into 4 areas such as backyard area, front yard area, outdoor waste bin area, Outdoor tub in the backyard, as shown in Figure 2.**

(a) **(b)** **(c)** **(d)**

Figure 2 Study area (a) backyard area, (b) front yard area, (c) outdoor waste bin area, and (d) Outdoor tub in the backyard area.

4.Mosquito larvae collection and identification

1. Collection of Aedes mosquito larvae

Aedes mosquito larvae were collected from stagnant water sources.

2. Separation and identification of mosquito larvae

The collected mosquito larvae were separated, and only Aedes mosquito larvae were selected for the experiment. The characteristics of Aedes mosquito larvae include resting at an angle of approximately 45–60 degrees to the water surface and having a short respiratory siphon.

Aedes aegypti larvae have five pairs of setae, and their comb scales are broad in the middle with serrated edges. *Aedes albopictus* larvae have four pairs of setae, and their comb scales are slender and pointed.

The selected larvae were transferred into an aquarium and reared for experimental use.

Research Method

1) Preparation of Plant Samples

The preparation of plant samples is an important step to obtain clean, uniform, and suitable raw materials for the extraction of bioactive compounds.

1.1 Fresh Boraphet stems weighing 50 grams were washed thoroughly with clean water several times to remove dirt, dust, and other contaminants attached to the plant material. The samples were then allowed to drain until excess water was removed.

1.2 The cleaned Boraphet stems were cut into small pieces approximately 1–2 centimeters in length to increase the surface area of the plant material, thereby improving the efficiency of drying and compound extraction.

1.3 The chopped Boraphet samples were dried in a hot-air oven at 60°C until completely dry. The dried samples were then ground into a fine powder using a grinder or mortar and pestle to obtain Boraphet powder suitable for extraction.

2. Herbal Extraction Process

Herbal extraction is the process of separating bioactive compounds from plant materials using an appropriate solvent.

2.1 The finely ground Boraphet powder was placed into a 500-milliliter beaker. Subsequently, 400 milliliters of ethanol were added. The beaker was covered with aluminum foil or plastic wrap to prevent solvent evaporation and external contamination.

2.2 The mixture was left to stand at room temperature for one week to allow the bioactive compounds in Boraphet to dissolve completely into the ethanol.

2.3 After the extraction period, the solution was filtered through Whatman No. 1 filter paper to separate plant residues from the extract solution.

2.4 The clear filtrate, free of solid residues, was transferred into an evaporation flask and subjected to solvent evaporation to increase the concentration of the extract.

2.5 The extract was further concentrated using a water bath at 60°C for 6 hours until a viscous Boraphet extract was obtained. The extract was then ready for subsequent efficacy testing.

3. Preparation of Mosquito Larvae for Testing

This step involved the preparation of mosquito larvae for use in the experiment.

3.1 Mosquito larvae were collected from natural water sources and identified to species level. Only *Aedes aegypti* larvae were selected for the experiment. The larvae were reared

in breeding trays until suitable for testing and transferred to a mosquito rearing chamber for use in evaluating the effectiveness of Boraphet extract.

4. Mosquito Control Efficacy Testing Procedure

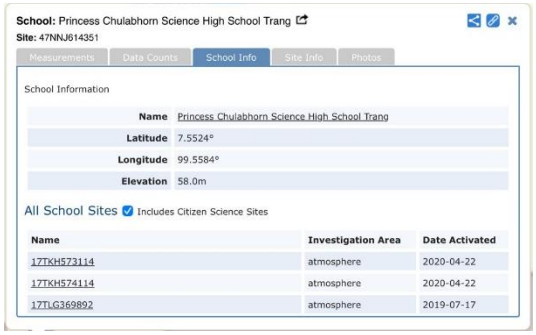
4.1 Boraphet extract solutions were prepared at different concentrations: 2.5, 5.0, 7.5, and 10 ppm. For each concentration, solution volumes of 10, 20, and 30 milliliters were prepared to compare the effects of different concentrations and volumes.

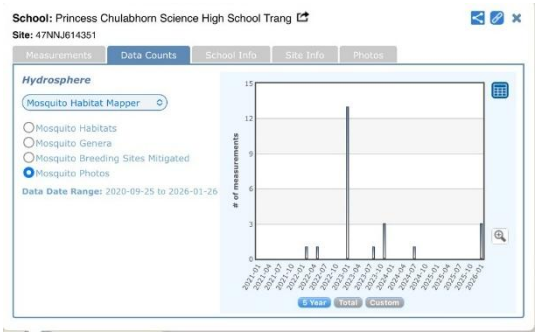
4.2 Each extract solution was poured into experimental containers and placed in the testing area. The timing was then initiated to observe the effects of the extract on mosquitoes.

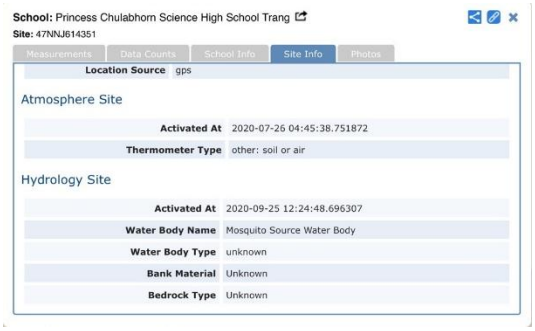
4.3 The number of dead mosquitoes and the time required for the extract to exert its effect at each concentration were recorded. The collected data were used to analyze the effectiveness of Boraphet extract in repelling or eliminating mosquitoes.

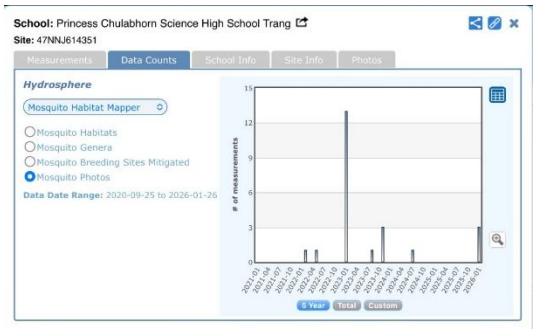
4.4. Conducting measurements three times in accordance with GLOBE measurement principles, and calculating the mean and standard deviation.

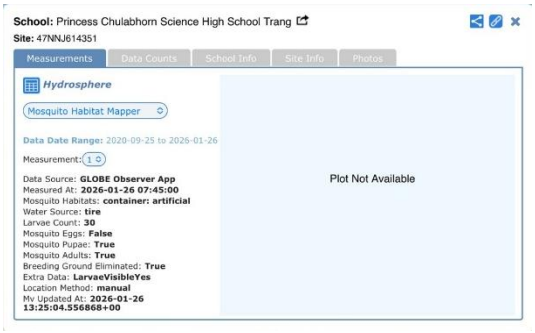
4.5. Submit the collected data to the GLOBE Data Entry system.











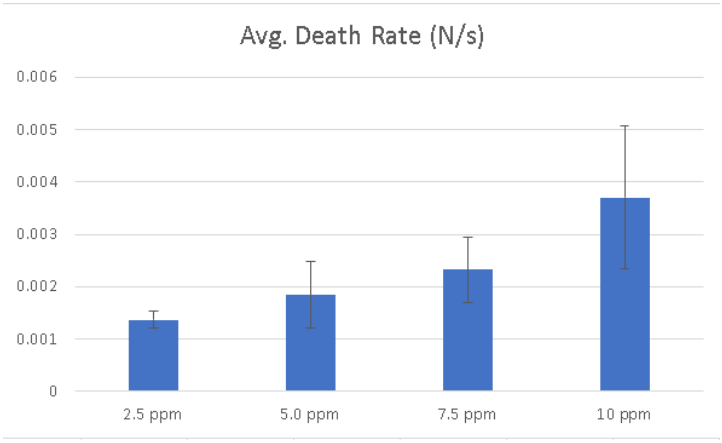
5. Data analysis

The data were analyzed using the following methods:

1. Two-Factor Analysis of Variance (ANOVA) with replication.
2. Standard Deviation (S.D.).
3. Mean ($\bar{x}$).

Results

The effectiveness of Heart-leaved moonseed*(T. crispa)* extract at different concentrations on the mortality rate of mosquito larvae was investigated by dividing the experiment into four concentration levels: 2.5, 5.0, 7.5, and 10 ppm, with three replications for each concentration. The experimental results are presented in **Picture 3**.

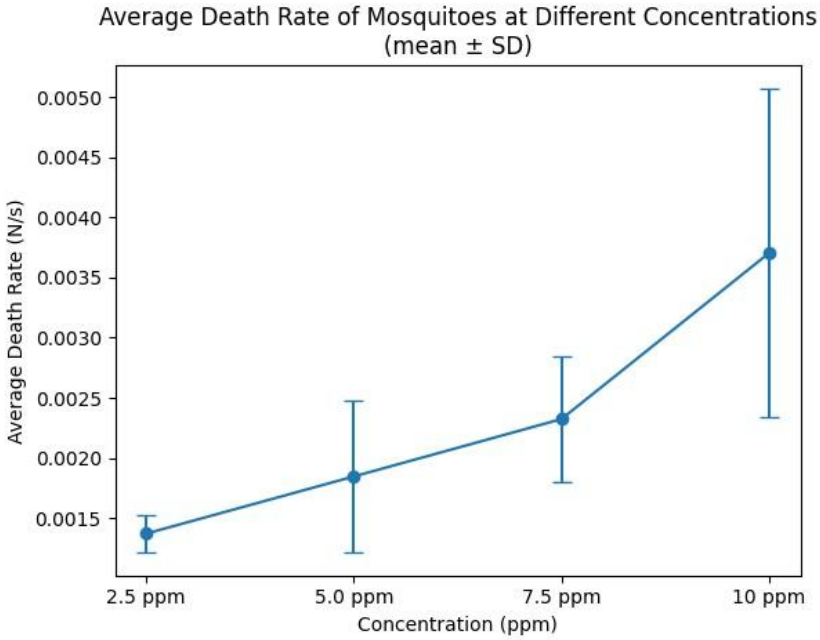


Picture 3 A graph showing the mosquito mortality rate within one day.

The result from the study of the concentration of *Tinospora crispa*

The results showed that as the concentration of Heart-leaved moonseed*(T. crispa)* extract increased, the average time required for mosquito larvae to die tended to decrease, while the mortality rate increased markedly. The experimental group treated with Heart-leaved moonseed*(T. crispa)* extract at a concentration of 2.5 ppm exhibited a mean mortality rate of 0.00137 ± 0.00015 N/s, followed by the 5.0 ppm treatment with a mean mortality rate of 0.00185 ± 0.00063 N/s, and the 7.5 ppm treatment with a mean mortality rate of 0.00232 ± 0.00064 N/s, respectively.

The effectiveness of Heart-leaved moonseed*(T. crispa)* extract on the mortality rate of mosquito larvae was investigated by applying three different extract volumes, namely 10, 20, and 30 mL, with three replications for each volume. The experimental results are presented in **Picture 5**.



Picture 5 A graph showing the mosquito mortality rate at different volumes within one day

The result from the study of the mortality rate of mosquito larvae

The results showed that the mortality rate of mosquito larvae increased throughout the experimental period, and the volume of Heart-leaved moonseed*(T. crispa)* extract had a clear effect on larval mortality. The experimental group treated with 10 mL of Heart-leaved moonseed*(T. crispa)* extract exhibited the highest mean mortality rate, with an average value of 0.00371 ± 0.00137 N/s. This was followed by the group treated with 20 mL, which showed a mean mortality rate of 0.00232 ± 0.00064 N/s. The lowest mean mortality rate was

observed in the group treated with 30 mL, with a value of 0.00185 ± 0.00063 N/s, respectively.

Discussion

According to the findings of the study on the effectiveness of Heart-leaved moonseed*(T. crispa)* extract in controlling mosquito larvae, the results demonstrate that the mortality rate of mosquito larvae increased with increasing concentrations of the extract. The larvae exposed to higher concentrations of Heart-leaved moonseed*(T. crispa)* extract showed significantly higher mortality compared to those in the control group.

Statistical analysis using one-way ANOVA revealed that the mortality rates of mosquito larvae in the experimental groups treated with *Tinospora crispa* extract were significantly different from the control group ($P < 0.05$). In particular, the highest concentration of Heart-leaved moonseed*(T. crispa)* extract exhibited a statistically significant difference when compared with all other treatment groups, indicating a strong concentration-dependent effect.

The variation in larvicidal effectiveness among different concentrations may be attributed to the presence of bioactive compounds in Heart-leaved moonseed*(T. crispa)*, such as diterpenoid lactones and alkaloids, which are known to interfere with the digestive and nervous systems of insects. These compounds may disrupt physiological processes, leading to reduced survival and increased mortality of mosquito larvae.

Therefore, the results of this study suggest that Heart-leaved moonseed*(T. crispa)* extract has significant potential as a natural larvicidal agent. Its effectiveness increases with concentration, supporting its possible application as an environmentally friendly alternative to synthetic chemical larvicides for mosquito control.

Conclusion

The study on the effectiveness of Heart-leaved moonseed*(T. crispa)* extract in controlling mosquito larvae can be summarized and discussed as follows.

The experiment was divided into four treatment groups: Group 1, without Heart-leaved moonseed(*T. crispa)* extract (control group); Group 2, treated with Heart-leaved moonseed*(T. crispa)* extract at a concentration of 2.5 ppm; Group 3, treated with Heart-leaved moonseed*(T. crispa)* extract at a concentration of 5 ppm; Group 4, treated with Heart-leaved moonseed*(T. crispa)* extract at a concentration of 7.5 ppm; and Group 3, treated with Heart-leaved moonseed*(T. crispa)* extract at a concentration of 10 ppm. The experiment was conducted over a period of 24 hours.

The results showed that the mean mortality rate of mosquito larvae in the control group was 12.3 ± 1.52%, while Group 2 exhibited a mean mortality rate of 46.7 ± 2.08%. Group 3 showed a higher mean mortality rate of 73.5 ± 1.76%, and Group 4 demonstrated

the highest mean mortality rate at 91.2 ± 1.34%. These results clearly indicate that an increase in the concentration of Heart-leaved moonseed*(T. crispa)* extract leads to a significant increase in mosquito larval mortality.

Statistical analysis using two-way ANOVA with replication revealed that the mortality rates of mosquito larvae in the treatment groups receiving Heart-leaved moonseed*(T. crispa)* extract were significantly different from the control group ($P < 0.05$). In particular, the group treated with 10 ppm Heart-leaved moonseed*(T. crispa)* extract showed statistically significant differences compared to all other experimental groups.

The findings of this study are consistent with the research conducted by Sureeporn et al. (2017), who reported that bitter-tasting herbal extracts can disrupt the digestive and nervous systems of mosquito larvae, leading to larval death within a short period of time. Furthermore, the results are in agreement with the study by Somchai (2019), which investigated the effects of Heart-leaved moonseed*(T. crispa)* extract on agricultural insect pests and found that bioactive compounds in Heart-leaved moonseed*(T. crispa)*, such as diterpenoid lactones, exhibit toxic effects on insects during the larval stage.

In conclusion, Heart-leaved moonseed*(T. crispa)* extract is effective in controlling mosquito larvae, particularly at higher concentrations, and has the potential to be developed as an alternative method for mosquito control in place of synthetic chemical larvicides. This approach may help reduce environmental impacts while increasing safety for humans and other living organisms.

Acknowledgements

We would like to express our sincere gratitude to our teacher, **Mrs. Salamiyah Kittibunyatiwakorn**, **Mrs. Pacharee Chaipetch** for her valuable guidance, encouragement, and continuous support throughout the research process. Her advice and supervision greatlycontributed to the successful completion of this study.We would also like to thank all individuals and organizations who provided assistance, facilities, and resources necessary for conducting this research on the effectiveness of Heart-leaved moonseed*(T. crispa)* extract in controlling mosquito larvae.

Finally, we would like to express our appreciation to everyone who contributed, directly or indirectly, to the completion of this research.

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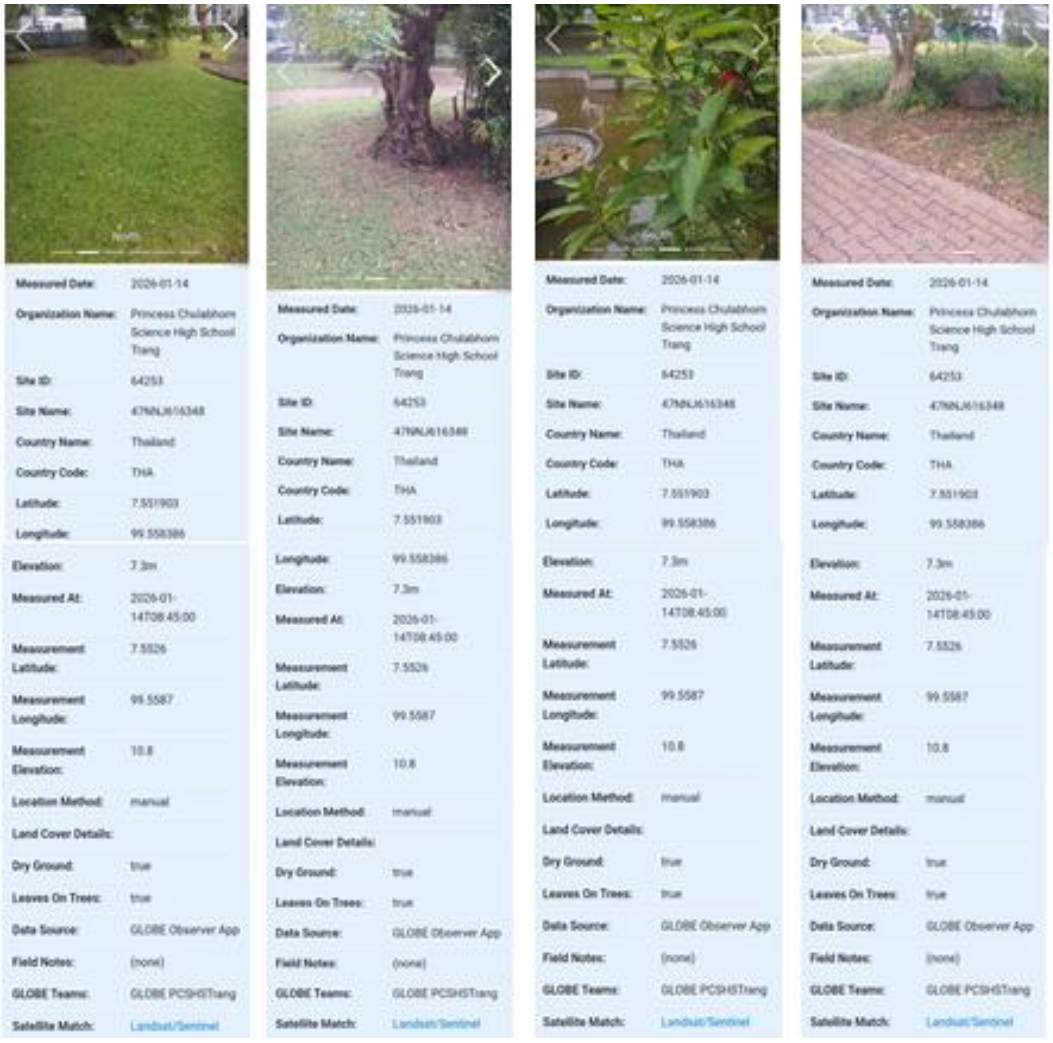
GLOBE's Database



Badge Descriptions/Justifications

I AM A PROBLEM SOLVER

This report was developed from recognizing the problem of mosquito outbreaks in the community, which are a major cause of dengue fever. Students analyzed the problem and designed an experiment to find a solution by studying the appropriate concentrations of Heart-leaved moonseed*(T. crispa)* extract for eliminating mosquito larvae. The experiment enabled students to solve problems systematically and apply the results of the study to real-life situations.

I AM A DATA SCIENTIST

This report applied processes of data collection and analysis from real experiments. Students determined several concentration levels of the extract and recorded the number of mosquito larvae that died within a specified period of time. The data were then calculated as percentages, compared across different concentrations, and presented in tables and graphs. Students also discussed the limitations of the data, using evidence-based information to draw conclusions and answer the research questions logically.

I MAKE AN IMPACT

This report originated from the issue of mosquito-borne disease outbreaks in the community, which negatively affect public health. Students applied scientific knowledge to investigate and experiment with solutions by using Heart-leaved moonseed*(T. crispa)*, a local herbal plant, as an alternative to chemical substances. The results demonstrate that this approach can be practically applied to reduce mosquito breeding sites and create a positive, sustainable impact on community health and the environment.