The Impact of Outer and Inner Pomelo Peel on Mosquito Larvae

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Abstract

This study primarily investigates the effects of different concentrations of pomelo peel solutions on mosquito larvae. Pomelo peels were separated into outer and inner peels, and varying concentrations were added to water. Changes in mosquito larvae's heart rate, survival rate, wriggling frequency, and breath-holding time were observed. Additionally, pH levels and dissolved oxygen levels in the mosquito larval habitat were measured to assess the impact of different concentrations of pomelo peel solutions on mosquito larvae. Results indicated that the outer peel solution affected breath-holding time and survival rate. The wriggling frequency increased more in response to the outer peel solutions than to the inner peel solutions. Outer peel solution also increased heart rate. In summary, the outer peel solution had more pronounced effects on mosquito larvae in various aspects compared to the inner peel solution. Based on the experiments, we recommend using a 0.45% concentration of pomelo outer peel solution for mosquito larvae control.

Keywords : *Aedes albopictus*, *Culex*, pomelo outer peel, pomelo inner peel

Introduction

Pomelo

The pomelo (*Citrus paradisi*) is a fruit belonging to the Rutaceae family of the citrus genus and is originally from tropical Asia. The pomelo tree is an evergreen species with a round or oval-shaped crown, deep green leaves. The fruit is typically large and spherical with a thick peel that ranges from orange-yellow to green-yellow. The peel of the pomelo contains abundant essential oils, giving it a distinctive aroma, especially when rubbed or cut, enhancing the fragrance.

Research indicates that pomelo essential oil has effective mosquito-repelling properties against adult *Aedes albopictus* mosquitoes. Other substances such as orange, onion, tangerine, and garlic also exhibit mosquito-repelling effects, but pomelo essential oil demonstrated the most significant effectiveness. It was found that limonene is the main component in pomelo peel essential oil (Visakh, 2022).

These components may be used in perfumes and skincare products to blend fragrances, enhance product stability, provide antimicrobial and antibacterial effects, and offer moisturizing and skin-softening effects. Hence, we are curious about which part of the pomelo peel is responsible for the excellent mosquito-repelling effect on mosquito larvaes.

Aedes albopictus

The *Aedes albopictus* mosquito, commonly known as the Asian tiger mosquito, is characterized by five white spots on each leg and white spots on the thorax, abdomen, and tail. A wide, straight white line is present in the middle of the thorax and back.

Culex

Adult mosquitoes in the household are approximately 4 mm in length, with a brown body and dark brown wings. Their mouthparts and antennae are roughly equal in length, and there are no white markings on the thorax or back.

Prevention and control methods in Taiwan

Health authorities recommend installing screens on windows and doors at home and using mosquito nets during the dengue fever epidemic. Insecticides should be used. Additionally, mosquito traps can be used in dark areas indoors during the day for mosquito control.

Research objective

This study aims to investigate the impact of the outer and inner peels of pomelo to mosquito larvae. The objective is to determine whether incorporating these pomelo peels can effectively serve as a method for mosquito larvae control.

(1) The species of mosquito

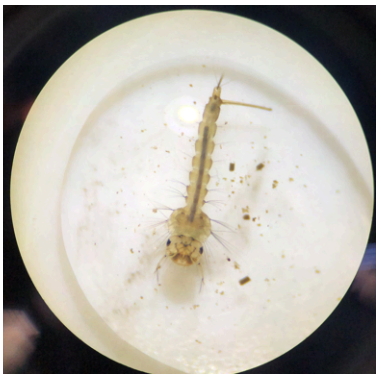
(2) pH and dissolved oxygen levels in the mosquito larvae habitat

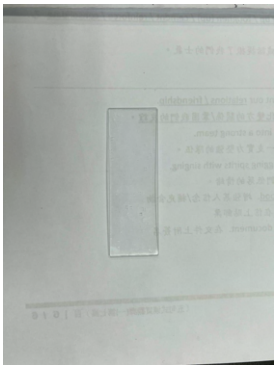
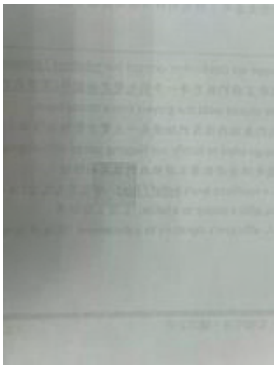
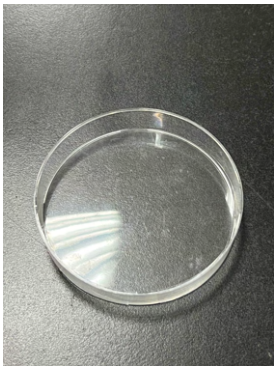
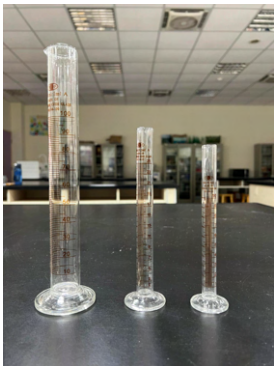
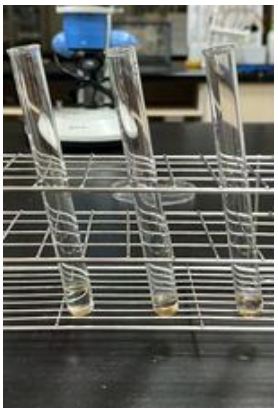
(3) Effects of pomelo outer peel on *Aedes albopictus* larvae breath-holding time, survival rate, heartbeat rate, wriggling frequency

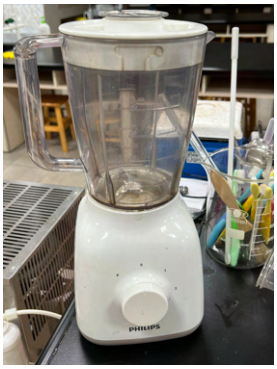
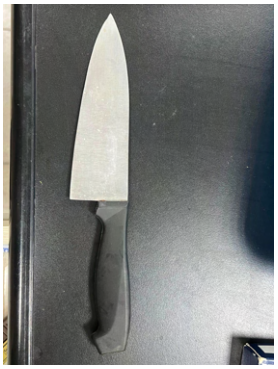
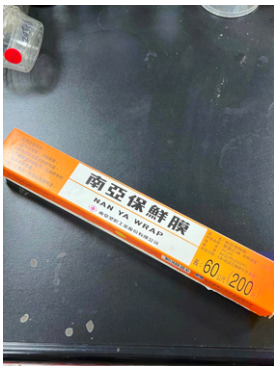
(4) Effects of pomelo inner peel on *Aedes albopictus* larvae breath-holding time, survival rate, heartbeat rate, wriggling frequency

(5) Effects of pomelo outer peel on *Culex* larvae - breath-holding time, Survival Rate

Research equipment and apparatus

		
1.Pomelo	**2.*Aedes albopictus* larvae (20x)**	**3.*Culex* larvae (20x)**

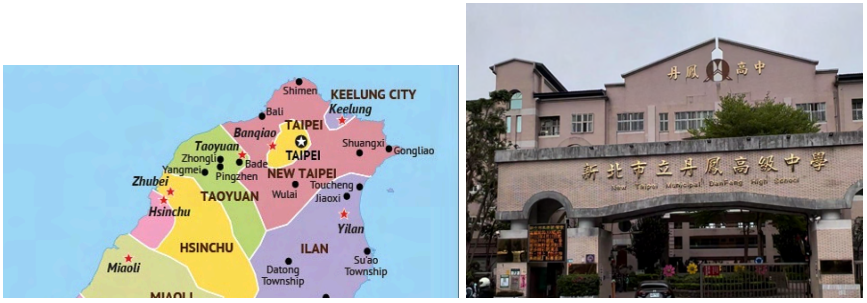
			
4.Compound microscope	**5.Dissecting microscope**	**6.Microscope slide**	**7.Cover slip**
			
8.C*f*oncave slide	**9.Petri dish**	**10.Cylinder**	**11.Dropper**
			
12.Beaker	**13.Test tube rack**	**14.Test tube**	**15.Dissolved oxygen test reagent**

			
16.pH meter	**17.Juicer**	**18.Knife**	**19.Scissor**
			
20.Filter mesh	**21.Mesh basket**	**22.Plastic wrap**	**23.Scale**

Study sites

This study was conducted at No. 72, Long'an Rd., Xinzhuang Dist., New Taipei City 242 , Taiwan (R.O.C.).(25.020764”N, 121.415369”E) during 5 October to 16 February 2024.

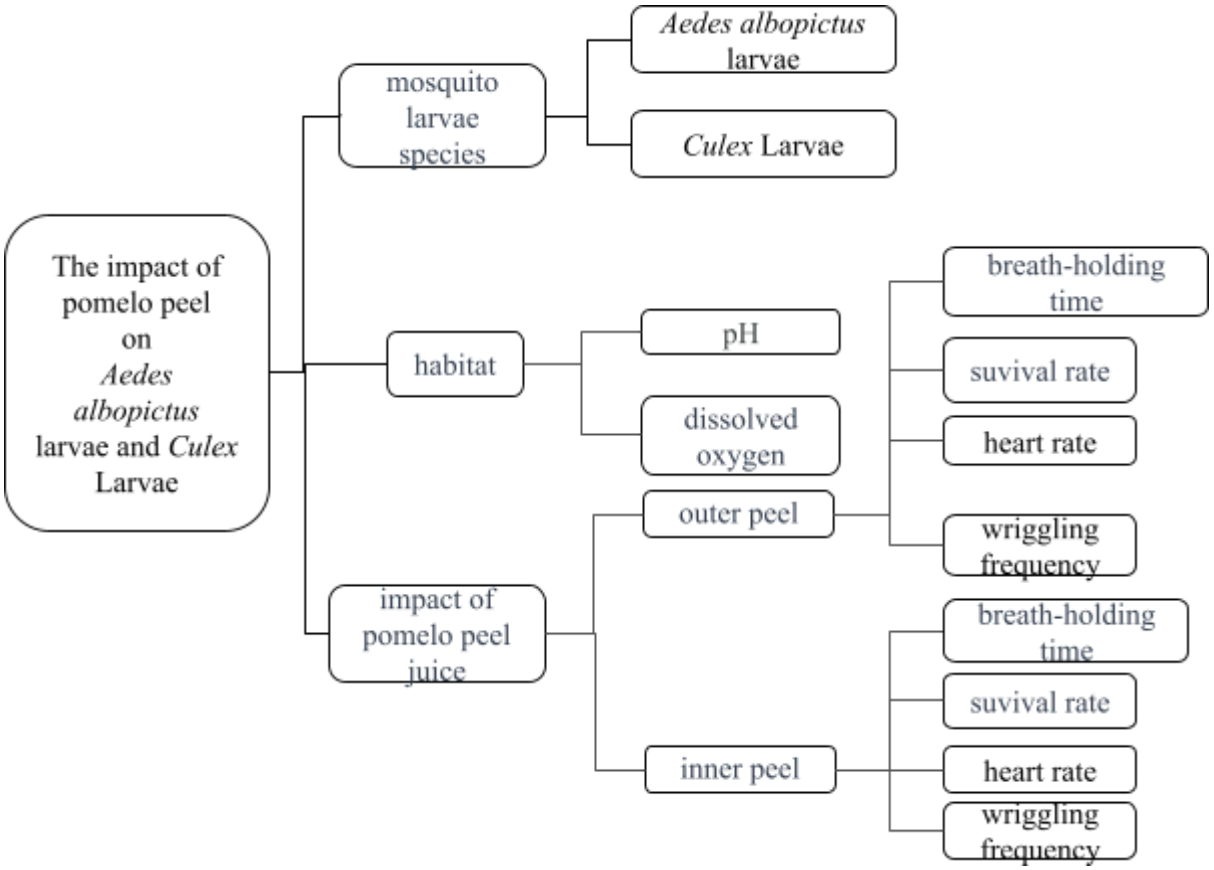
(a)



(b) (c)

Figure 1: Map of Taiwan (R.O.C) and study site at Long'an Rd., Xinzhuang Dist., New Taipei City. (a)Taiwan (R.O.C) (b)New Taipei City (c)New Taipei Municipal DanFeng High School.

Research outline



Research methods

Data collection

Mosquito larva survey was conducted during the period from October 2023 to February 2024, we went to the water storage barrels at grandma's vegetable garden in Xinzhuang every week (see Figure 2) to collect our sample. We select larvae with a length between 0.4cm and 0.6cm for the following experiments.

Figure 2: Larvae collection site.

Measuring the pH of the larvae habitat water

Using a calibrated pH meter to measure the pH value of the collected water.

Measuring the dissolved oxygen levels in the larvae habitat water

1.Begin by using the first test cup to measure 10 mL to 15 mL of the sample water.

2.Sequentially add 5 drops of test reagent #1 and test reagent #2 into the sample water (be careful not to shake the sample water; a light yellowish precipitate will form after this step).

3.Immediately use the second test cup to scoop a full cup of sample water, pouring it along the cup wall of the first test cup until it reaches the brim, with a total volume of approximately 27.5mL (ensure no air bubbles are generated when pouring into the test cup, then carefully cover the cup to prevent air from entering).

4.Allow the sample water to stand for about 10 minutes, allowing the precipitate to settle at the bottom of the cup, and the precipitate will turn light brown due to a chemical reaction.

5.After 10 minutes, open the cup lid, pour off the clarified liquid layer above the precipitate, reducing the total volume to 15mL (be careful not to pour out the precipitate).

6.Immediately add 5 drops of test reagent #3. shake for 30 seconds to dissolve the precipitate (if not completely dissolved, an additional drop may be added).

7.Begin adding test reagent #4, gently shake the test cup after each drop, carefully observing the color change until the water color changes from yellow-brown to colorless. Note the total number of drops of test reagent #4.

8.For result analysis, each drop of test reagent #4 corresponds to a concentration of 0.5mg/L of dissolved oxygen. The dissolved oxygen concentration can be calculated by multiplying the total number of drops of test reagent #4 by 0.5mg/L.

Preparing the outer and inner peels of pomelo solutions

1.Peel off 10g of the green outer peel and the white inner peel of the pomelo (Figure 3).

2.Add 100mL of water and blend them in a juicer to obtain pomelo peel juice solutions of different concentrations.

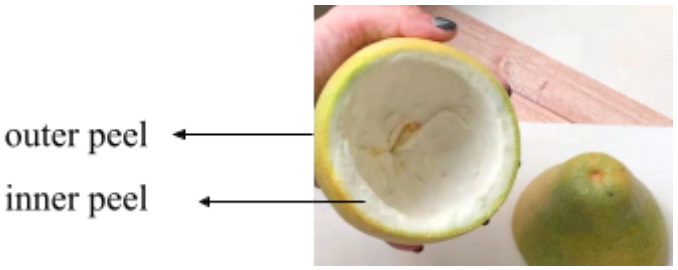


Figure 3: Pomelo outer peel and inner peel.

Observation of breath-holding time, survival rate, heart rate, and wriggling frequency of mosquito larvae in different concentrations of pomelo peel juice

Breath-holding time and survival rate experiments are conducted in test tubes, with three mosquito larvae tested for each concentration to obtain an average value (Figure 4). Heart rate and wriggling frequency experiments are performed on glass slides, with three mosquito larvae tested for each concentration to obtain an average value (Figure 4).

Breath-holding time: pomelo peel juice concentrations of 0.9%, 0.45%, 0.22%, and a control group constitute four groups. Calculate the longest breath-holding time within five minutes for mosquito larvae in each concentration.

Survival Rate: pomelo peel juice concentrations of 0.9%, 0.45%, 0.22%, and a control group constitute four groups. Calculate the percentage of survivors after the experiment.

Heart rate: Adjust pomelo peel juice concentrations to 9%, 2.2%, 1.1%, and a control group, comprising four groups. Place the mosquito larvae on a slide, let them rest for five minutes, and observe their average heartbeat.

Wriggling frequency: Adjust pomelo peel juice concentrations to 9%, 4.5%, 2.2%, and a control group, constituting four groups. Calculate the number of times the larvae touch their tails to their heads within ten seconds.

Figure 4: Experiment conducted in a test tube (Left). Experiment conducted on a concave slide (Right).

Results

The species of mosquito larva

From October 2023 to December 2024, we collected a total of 126 *Aedes albopictus* and 56 *Culex* larvae. The *Aedes albopictus* larvae developed into adults over time, whereas no Culex larvae matured into adult mosquitoes. Therefore, we only observed the Aedes albopictus adults. The *Aedes albopictus* has a white line in the middle of the thoracic dorsum, a black body, and legs that are black and white alternating. To distinguish between mosquito genders, we observed their antennae; the antennae of female mosquitoes are thread-like, while those of males have hairs (Figure 5).

Figure 5: Anatomical microscopic view of *Aedes albopictus* of different genders (20x). The left side of the image depicts a female mosquito, while the right side depicts a male mosquito.

Distinguishing between mosquito larvae species primarily involves observing their tails. The respiratory siphon of *Aedes albopictus* is shorter, and when they breathe at the water surface, their bodies are oriented vertically (Figures 6 and 7). In contrast, the respiratory siphon of *Culex* larvae is longer and, when they breathe at the water surface, their bodies are more inclined (Figures 6 and 7). *Culex* larvae have longer antennae on their heads, and they possess more abundant hair compared to *Aedes albopictus* (Figure 8).

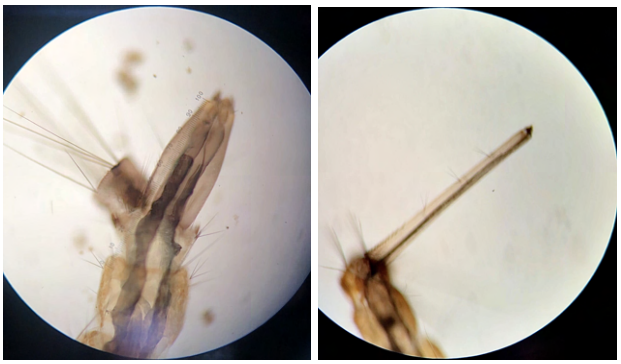


Figure 6: Respiratory siphons of *Aedes albopictus* and *Culex* larvae (40x), with *Aedes albopictus* on the left and *Culex* on the right.

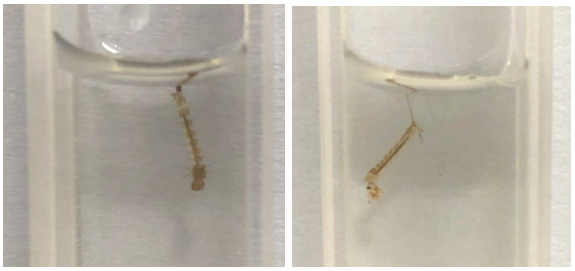


Figure 7: Larvae of the *Aedes albopictus* and *Culex* breathing on the water surface, with *Aedes albopictus* larvae on the left and *Culex* larvae on the right.

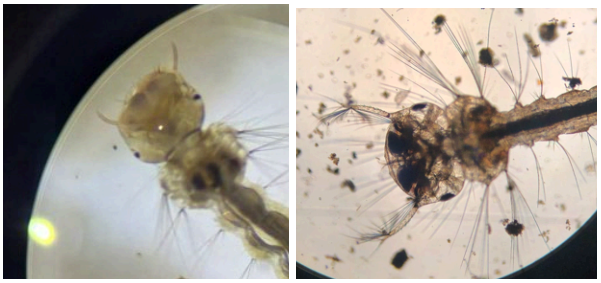


Figure 8: Heads of *Aedes albopictus* and *Culex* larvae (40x), with *Aedes albopictus* on the left and *Culex* on the right.

The pH and dissolved oxygen levels in the mosquito larvae habitat

It can be observed that the pH and dissolved oxygen levels in the water storage barrels at the guishan grandma's vegetable garden fall within the range of 7 to 9, indicating the survival environment for mosquito larvae (Table 2).

Table 2: pH values and dissolved oxygen levels in the sample area.

Time	**10/26**	**11/2**	**11/9**	**11/16**	**11/23**
pH	8.93	8.22	7.66	7.61	8.02
Dissolved oxygen (mg/L)	9	8	9	7	7

The impact of pomelo outer peel and inner peel on *Aedes albopictus* larvae.

Breath-holding time

Based on (Figure 9, left), it can be observed that outer peel increases the breath-holding time of mosquito larvae, with significant differences between all three concentrations and the control group. On the other hand, inner peel reduces the breath-holding time of mosquito larvae (Figure 9, right).

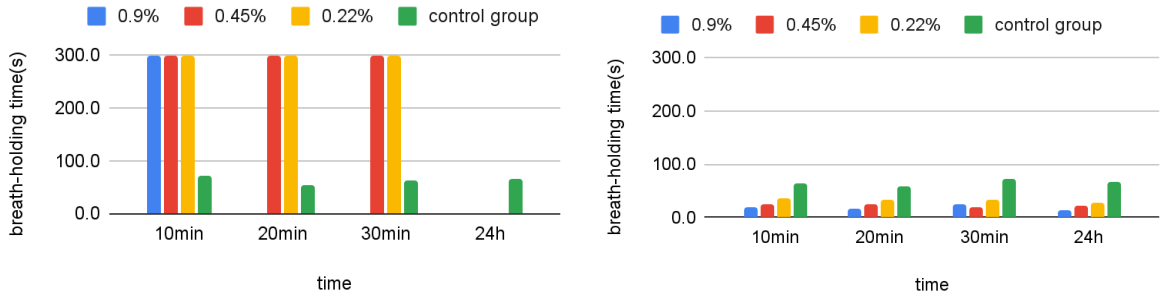


Figure 9: The impact of pomelo outer peel on the average breath-holding time of *Aedes albopictus* larvae (left). The impact of pomelo inner peel on the average breath-holding time of *Aedes albopictus* larvae (right).

Survival rate

It can be observed that, except for the control group of mosquito larvae, all others treated with pomelo outer peel juice died within one day (Figure 10, left). Within the first 30 minutes, it is evident that higher concentrations of pomelo peel correlate with lower survival rates. On the other hand, we observed an almost 100% survival rate under inner peel treatment even after one day (Figure 10, right).

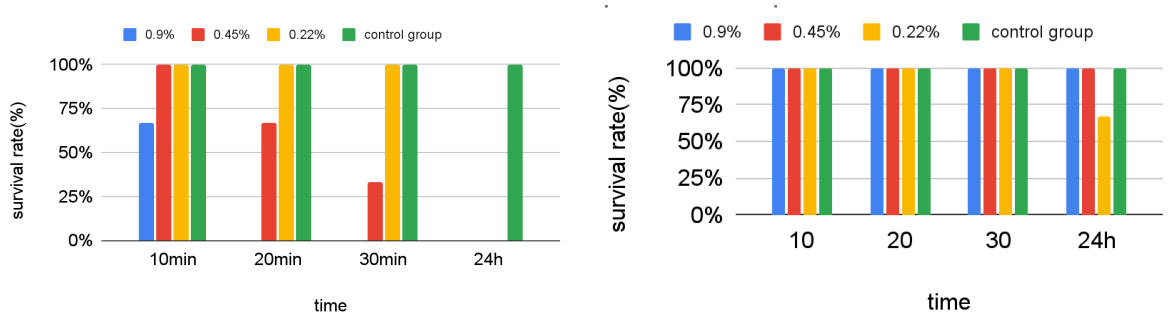


Figure 10: The impact of pomelo outer peel on the survival rate of *Aedes albopictus* larvae (left). The impact of pomelo inner peel on the average survival rate of *Aedes albopictus* larvae (right).

Heart rate

We tested four concentrations (9%, 2.2%, 1.1%, 0%) of pomelo peel solutions, but the mosquito larvae in the 2.2% solution moved too quickly for us to count. It can be observed that at 9%, the heart rate is significantly higher than the control group, while at 1.1%, there is no significant impact (Figure 11, left). On the other hand, there are no significant differences in heart rate between the various concentrations and the control group (Figure 11, right).

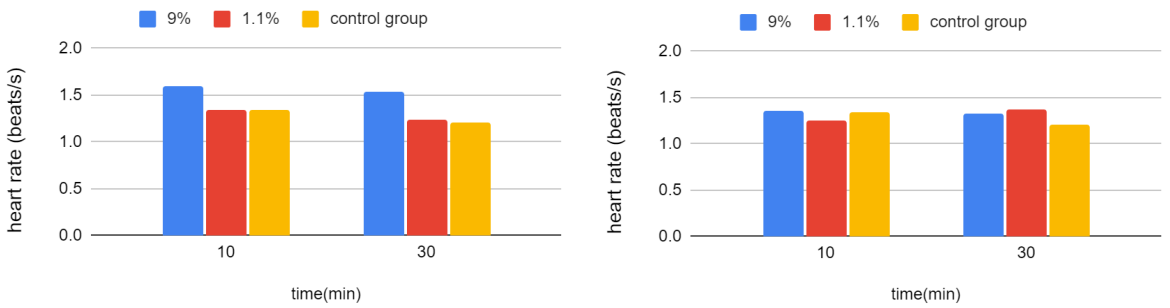


Figure 11: The impact of pomelo outer peel on the average heart rate of *Aedes albopictus* larvae (left). The impact of pomelo inner peel on the average heart rate of *Aedes albopictus* larvae (right).

Wriggling frequency

Based on (Figure 12, left), it can be observed that at the beginning of the experiment, all three concentrations of outer peel resulted in a higher wriggling frequency compared to the control group. As the experiment progressed for 20 minutes, the wriggling frequency decreased but remained higher than the control group. The addition of inner peel also increased the wriggling frequency, but only at the initial stages, with no significant differences observed at 10 and 20 minutes among the four groups (Figure 12, right).

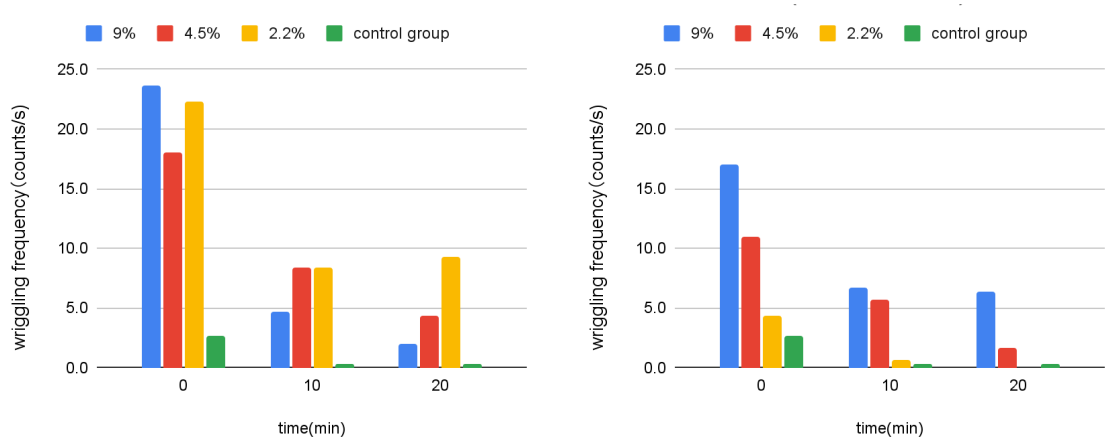


Figure 12: The impact of pomelo outer peel on the wriggling frequency of *Aedes albopictus* larvae (left). The impact of pomelo inner peel on the wriggling frequency of *Aedes albopictus* larvae (right).

The effect of pomelo peel juice on *Culex* larvae

Breath-holding time

It can be observed that outer peel juice prolongs the breath-holding time of *Culex* larvae. The absence of data for 0.9% and 0.45% concentrations is due to the complete mortality of *Culex* larvae within the first 10 minutes at those concentrations (Figure 13).

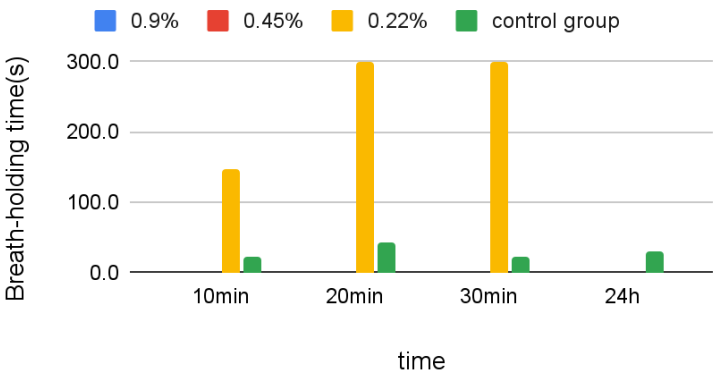


Figure 13: The impact of pomelo outer peel on the breath-holding time of *Culex* larvae.

Survival rate

It can be observed that all other *Culex* larvae treated with pomelo outer peel died within one day. The absence of data for 0.9% and 0.45% concentrations is due to the complete mortality of *Culex* larvae within the first 10 minutes at those concentrations (Figure 14).

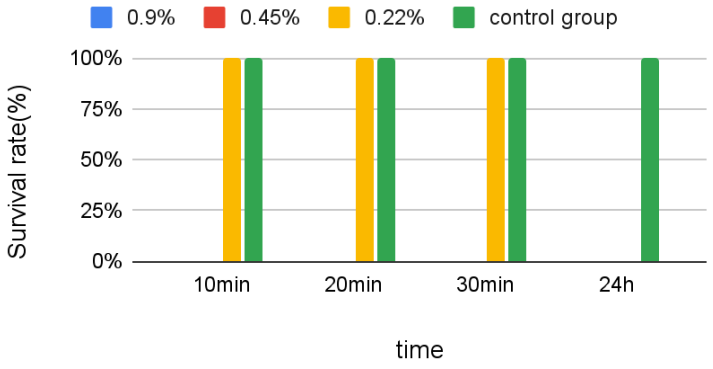


Figure 14: The impact of pomelo outer peel on the survival rate of *Culex* larvae.

Discussion

Pomelo outer peel resulted in an increase in the breath-holding time and a decrease in the survival rate of *Aedes albopictus* larvae

In the experiment with outer pomelo peel, higher concentrations of the pomelo peel solution significantly increased the breath-holding time of mosquito larvae, leading to a reduced survival rate. We hypothesize that the reason behind this outcome is the higher

concentration of essential oils within the outer peel , making ventilation more challenging. Under microscopic observation of the solution from outer and inner peels, it was evident that outer peels contained more oil droplets, while inner peels contained fewer oil droplets but more fibrous structures (Figure 15). Limonene and related essential oils possess various insecticidal properties (Ling et al.,2022, Sevigan et al.2020). Furthermore, limonene disrupts the insect cuticular wax layer, increasing the porosity of the wax, leading to water loss. The cuticular disruption causes fluid to be expelled, blocks spiracles, interferes with oxygen exchange, ultimately resulting in insect death. Therefore, we infer that the varying component is a key factor contributing to the differences between outer and inner pomelo peels**.**

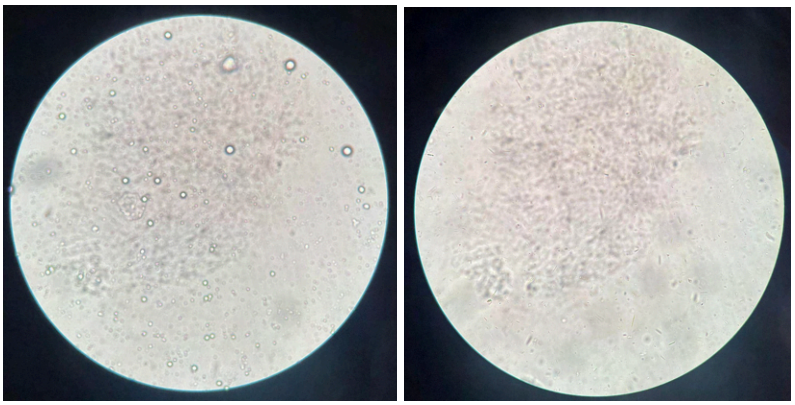


Figure 15: Outer peel solution containing oil (Left), inner peel solution (Right).

Pomelo outer peel resulted in an increase in heart rate and wriggling frequency

Our experiment results suggest that mosquito larvae in high-concentration outer peel may exhibit increased movement and struggling behavior, possibly due to discomfort. In contrast, at lower concentrations, the wriggling frequency decreases. We speculate that the larvae become more stable after an adaptation period since lower concentrations are not lethal.

In the experiment with inner peel solution, high concentrations of inner peel have a certain impact on the larvae's movement behavior, although less pronounced compared to outer peel juice. We hypothesize that this effect may be due to the larvae's initial discomfort upon entering a new environment.

Aedes albopictus exhibit a higher tolerance to pomelo peel compared to *Culex*

Regardless of whether exposed to outer or inner peels, the survival rates of *Aedes albopictus* larvae were higher than those of *Culex* larvae. From our experiments, it is evident that *Aedes albopictus* larvae exhibit a higher tolerance to pomelo peel juices compared to *Culex* larvae. Therefore, if the goal is to completely eliminate mosquito larvae, the use of outer peel is a better choice. As our experiments indicate that inner peel juice does not have a lethal impact on *Aedes albopictus* larvae. We selected 0.45% as it represents the minimum concentration that is lethal to both *Aedes albopictus* and *Culex* larvae. We opted against using a 0.9% concentration due to concerns about potential bad effects on other coexisting aquatic organisms, disrupting the overall ecosystem rather than specifically targeting mosquito larvae.

Conclusion

1. *Aedes albopictus* can be found in the vegetable gardens of New Taipei City, Taiwan, during November to December.
2. *Aedes albopictus* live in habitats with neutral pH and moderately high dissolved oxygen levels.
3. The outer peels of pomelos have a more significant impact on Aedes albopictus larvae than the inner peel.
4. Pomelo outer peel can increase the breath-holding time, heart rate, and wriggling frequency but decrease the survival rate.
5. *Aedes albopictus* exhibits a higher tolerance to pomelo peel compared to *Culex*.

Reference

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