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The Effect of the Visible Light Spectra on the Phototaxis of Yellow Fever Mosquito (A. aegypti) Larvae as an Alternative Pest Control System

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Abstract –Yellow fever mosquitoes (A. aegypti) are the primary vectors of dengue, chikungunya, yellow fever and Zika virus. The larvae and pupae of A. aegypti are entirely aquatic, are short and stout, and have a breathing tube containing tufts. A. aegypti lays eggs in any container and can withstand long periods of dryness ("The Editors of Encyclopaedia Britannica," 2023). The rising cases of mosquito-borne diseases urge the development of new and sustainable pest control systems to help curb mosquito populations, with an emphasis on avoiding larvae growth in prominent mosquito breeding sites. This study aims to create an alternative pest control system by determining the effect of the visible light spectra on the phototactic behavior of A. aegypti, targeting the species' larvae to ensure that adults do not arise. Phototaxis assays tested the following colors for their ability to effectively attract and make A. aegypti larvae linger: red, green, blue, orange, yellow, cyan, indigo, and purple. The experiment revealed that purple (7.95 > 4.96; 8.71 > 4.96) was the color that showed the most efficacy in attracting and producing a lingering effect on the larvae. Placed in an aquatic light trap, purple light effectively lured and trapped the mosquito larvae. Therefore, the study showed that the visible light spectra is a viable way of attracting and trapping, through the use of an aquatic light trap, A.aegypti mosquito larvae. However, modifications to increase the effectiveness of the light trap is still needed.

Keywords: *aegypti, visible light spectra, phototaxis, pest control system, light trap*

I. INTRODUCTION

1.1 Mosquito Borne Diseases

Mosquitoes (Culicidae) are flying insects inhabiting almost all parts of the globe (CDC, 2022). They are known as vectors that transmit pathogens, contributing to the toll of morbidities worldwide. Arboviruses are diseases transmitted through vectors such as mosquitoes ("New York State Department of Health," 2017). Mosquito-borne diseases affect the quality of life of individuals, with conditions like Zika, Dengue, Yellow fever, and Chikungunya continually affecting almost 700 million people yearly. (Mistica et al., 2019; "World Mosquito Program," 2023; "Mosquito-borne Diseases," 2018).

The World Health Organization (WHO) reported that Dengue cases have increased from 505,430 in 2000 to 5.2 million in 2019. Additionally, the Pan American Health Organization (PAHO) recorded about 3 million cases of Dengue, 324,000 cases of Chikungunya, and 27,000 cases of Zika virus.

In the Philippines, arboviruses like Dengue are major public health issues (Lubrica et al., 2023). WHO reported 80,318 cases of Dengue in the Philippines, with 299 casualties. The Department of Health — CAR (DOH-CAR) data shows that from January 1st to June 4th, 2022, a 366% increase in dengue cases was observed, with 2,312 cases recorded. Moreover, from January 1st to June 4th, 2022, 264 dengue cases were recorded in Baguio City, while Benguet had 665 cases and one (1) death, an increase of 118% and 123%, respectively.

The cases of mosquito-borne diseases in Baguio prompted the researchers to consider SDG 3 - Good Health and Well- Being, adopted by the United Nations in 2015. The current agenda emphasizes a systematic approach to achieving sustainability for all. SDG 3 focuses on ensuring healthy lives and promoting well-being for all ages. Furthermore, the present research targets this specific SDG as it represents a significant stride toward developing innovative and sustainable pest control solutions. This research aligns with SDG 3's objective of combating diseases, enhancing access to healthcare, and promoting overall well-being. The study addresses public health concerns by contributing to developing environmentally friendly methods to curb mosquito populations. It supports the broader agenda of creating a healthier and more sustainable world for all.

1.2 A. aegypti Life Cycle

Aedes aegypti, or Yellow fever mosquitoes, are white-banded mosquitoes living in tropical environments. A. aegypti is the vector for several diseases, such as Yellow fever, Dengue, Zika, and Encephalitis. The larvae and pupae of A. aegypti are entirely aquatic, are short and stout, and have a breathing tube containing tufts. A. aegypti lays eggs in any container and can withstand long periods of dryness (“The Editors of Encyclopaedia Britannica,” 2023).

According to the CDC and Clemons et al. (2010), the mosquito’s life cycle lasts for 8-10 days, marked by instar phases. After feeding on the blood of animals, adult female mosquitoes (Aedes) lay eggs on the inner walls of water containers and hatch after 1- 4 days when the water level rises. The first instar comprises the egg hatching and the initial larvae stage, which lasts 1-4 days. Larvae feed off the microorganisms and nutrients in the water to grow. Instars mark the development of mosquito larvae and the phases of molting the larvae go through before entering the pupal stage. There are four instar phases, marked by gradually increasing molt size. The fourth instar, which marks the last larval phase, happens on days 7-8 or 8-9 for male and female larvae, respectively. On days 7-9, pupae will develop. Day 9 (males) or 10 (females) marks when pupa emerges from the water as adult Aedes mosquitoes.

1.3 Mosquito Larvae Movement

Larvae movement is essential in the survivability of the larvae during its developmental stages. A study by Strickman (1989) focused on the biosystematics of larval movement of Central American Mosquitoes and its use for field identification. The description of movement for each species was divided into four components, and one of them was according to path.

The path is the line of movement of the larva about its external environment. The path was horizontal, vertical, or erratic when reduced to a simple scheme. Horizontal paths were common for species that remained principally at the water surface (e.g., Anopheles) or near the bottom of the flask (e.g., Sabethes). However, some species that occupied more of the water column also tended to move horizontally.

For instance, Culex peus (formerly thriambus, Strickman 1988) took a vertical course, as opposed to the surface dwellers and bottom dwellers, which restricted their use of vertical paths to ascents and descents. Like other genera of the subgenus Culex, Culex peus made quick ascents and descents in planes that were either oblique to the surface or perpendicular to the bottom. The most frequently seen erratic path was in species that did not move constantly or energetically (e.g, Cx. pilosus). These species flexed abruptly in response to stimuli, creating closed loops with abrupt direction changes on their route.

Another study by Nelson (1986) on the biology and ecology of A. aegypti states that A. aegypti larvae swim with a distinct serpentine movement. In comparison, Culex larvae are characterized by jerky side-to-side movement of the abdomen through the water column.

1.4 Mosquito Larvae Light Perception

Larvae eye morphology has been studied by McDonald et al. (2022), wherein they averred that larval eyes are developmentally distinct from their adult counterparts and lack the visual pigment diversity and morphological specializations in adult eyes. However, recent studies proved that larval eyes are more complex than previously thought and warrant closer investigation.

According to a study by Rocha et al. (2015), mosquito larval eyes contain five larval ocelli placed laterally on each side of the head. The stemmata contain two photoreceptors, rhodopsin Aaop3 and rhodopsin Aaop7. Both photoreceptors are long- wavelength rhodopsins that possess similar spectral properties. Moreover, the adult compound eye starts to develop during the larvae’s 1st instar, adding to the larvae’s ability to sense specific light spectrums.

In a study by Liu et al. (2022) on Lakeside, A. Opsin3 and Opsin7 are expressed in different clusters of photoreceptors in aegypti larvae. Opsin1 is found in R1-6 and R8 cells in compound eyes, Opsin2 and Opsin8 are only found in R7 cells, and Opsin9 is found in some R7 and some R8 cells in the dorsal area. Opsins' light-induced expression and mobility have been understood, but their functional roles still need clarification. Opsin1 has been found to play a crucial role in regulating the intrinsic attraction towards light.

1.5 Visible Light Spectra and Phototaxis

The visible light spectrum is the section of the electromagnetic radiation spectrum visible to the human eye. This results from the bending of white light, splitting into visible spectra. It ranges in wavelength from approximately 400 nm (violet) to 700 nm (red). It is also known as the optical spectrum of light or the spectrum of white light (Jones, 2020).

In line with this, the ability of light to influence animal behavior has only been recently researched. Falcon et al. (2020), Thompson et al. (2019), and Dickerson et al. (2023) discovered that light can disrupt the circadian cycle of animals, hinder movement, and shift animal acoustic communication.

In addition, several studies have investigated the effect of the visible spectra on mosquitoes. Taniyama and Hori (2022) and Kehinde et al. (2018) studied the lethal effects of light on mosquitoes. The latter found that yellow light was more fatal to mosquito larvae, while the other claimed that blue light was most effective in killing larvae and adult mosquitoes.

Phototaxis is a behavior in which organisms move towards (positive phototaxis) or away (negative phototaxis) from a light source (Ueki & Wakabayashi, 2017). According to Arietta et al. (2017), phototaxis is a phenomenon in which mosquito larvae exhibit behavioral changes based on light colors. Staff (2021) states from their theory that bugs and other insects are easily attracted or repelled by light for them to find their food. Furthermore, the extent to which an insect's phototactic response to light occurrence is influenced by various factors, including the attributes of the light source, such as color and intensity, as well as external conditions like weather and the insect's physiological state (Kim et al., 2019; Park & Lee, 2017).

The phototactic behavior of mosquitoes has been tackled by studies like Jhaiaun et al. (2021), Oriyomi and Babalola (2020), and Wilson et al. (2023). UV light, purple, and white lights made by combining narrow-banded blue and yellow LEDs proved effective in attaining positive phototaxis on mosquitoes used in their experiments.

1.6 LEDs and Light Traps

Mosquitoes serve as the primary carriers of arboviruses; therefore, managing mosquito populations is key to managing these diseases effectively. Mapossa et al. (2021) indicated that light-emitting diodes (LED) have gained momentum following the discovery of their impact on animal physiology, psychology, and behavior. Mosquitoes are found to be highly sensitive to light intensity, direction, contrast, and color.

Moreover, luminous efficacy measures lumens per watt. It shows light output per energy input, thus signifying that higher efficacy means efficient lighting, which helps to attract or repel the mosquito because of the eye's sensitivity to the different colors of LED lights such as red LED (610 nm-760 nm), green LED (500 nm- 570 nm), or blue LED (450 nm- 500 nm) (Fernandez., 2022).

In previous studies, CDC-light traps replaced the incandescent bulbs with white, blue, green, and red LED lights to attain higher trapping efficiencies (Mwanga et al., 2019). Green LEDs excel at capturing various sand fly species in Brazil. Elsewhere, red LEDs lure more sand fly species than blue, green, or incandescent bulbs in Egypt. Lastly, Florida woodland mosquitoes show varied responses to different-colored LEDs. This shows that external factors such as species, environment, and location affect the efficacy of light-trapping devices in an area.

Disease-carrying insects like mosquitoes, biting midges, and sandflies are among the most often examined for human and animal health reasons (Ricciuti et al., 2017). Light traps have been the standard method for capturing active insects at dusk and dawn for over a century. These traps are widely employed to monitor and control various flying insects effectively and for laboratory research purposes, such as virus isolation (Holah et al., 2013; Brandenburg, 2022).

Likewise, in mosquito investigations, the choice of light trap is often made based on the requirement to capture as many mosquitoes of the target species as feasible. For many years now, populations of mosquito vectors have been observed using light trapping techniques instead of live bait cues. It is a method that eliminates the need for human interaction with mosquitoes and is generally safe (Jhaiaun et al., 2021).

Research Gaps and Statement of the Problem

Numerous research studies have been done regarding the effect of visible spectra on insect phototactic behavior. However, few have focused on larval phototaxis, as papers concentrate on the phototactic behavior of adult insects. Previous research focused on red, blue, green, violet, and UV lights. The effects of all the different colors of the visible spectra have yet to be compared against each other for differences in efficacy. Lastly, emphasis was put on larvae's mortality and development rates instead of larval phototactic behavior.

Therefore, the present study aims to investigate the effects of the visible light spectra on the phototactic behavior of mosquito larvae, specifically A. aegypti larvae, as they are the primary vectors of Dengue. The various colors of the visible spectra will be considered to further the study results.

Research Questions

To accomplish the study's objectives, the researchers have constructed the following research questions:

1. What are the effects of the visible light spectra on the phototaxis of mosquito larvae?

2. What color of light is most effective in changing the phototactic behavior of mosquito larvae?

3. Is there a significant difference among the various colors of light on the phototaxis of mosquito larvae as

determined by:

  a. *The farthest phototaxis zone (P zone) the larvae stay in*
  b. *Duration of time larvae spent swimming towards farthest P zone*
  c. *Duration of time the larvae stay in the farthest P zone*

4. Can lights from the visible light spectra become a pest control method?

5. How can the visible light spectra become an effective pest control system?

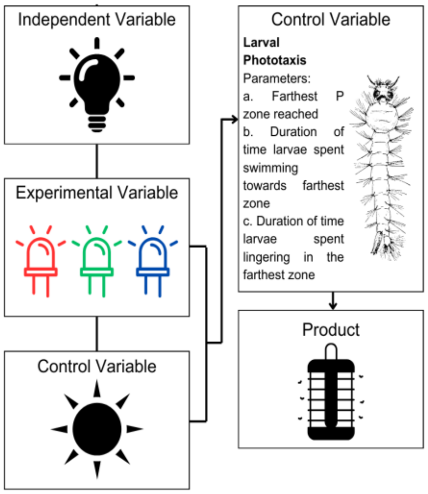
Two hypotheses were formulated to aid in answering the research questions formulated. The null hypothesis states that the lights do not significantly affect mosquito larval phototaxis. Meanwhile, the alternative hypothesis states that lights significantly affect mosquito larval phototaxis.

An independent and dependent variable were identified as a standard for experimental research. Furthermore, at the end of phase 1 of the research, a product was created as a light trap to be tested during phase 2.

The independent variable of the current study is light, with varying colors as the experimental variable and natural light as the control group. The dependent variable is the larval phototactic response of mosquito larvae. From there, the results of phase one influenced the making of the pest control system during phase two (light trap experiment).

Figure 1

Conceptual framework presenting the independent and dependent variables and the product of the study



Significance of the Study

As the study aims to create a new way to control mosquito populations, it can provide evidence that can be used to manufacture sustainable light traps that will help manage and control mosquito populations. Moreover, the research will benefit the following:

Researchers: The findings will help future researchers understand the effects of LED light on mosquitoes' phototaxis and build upon the results of this study through the recommendations.

Community: The study results will benefit the community by reducing the increase of mosquitoes in their area using sustainable methods. It will also help establish a cleaner and healthier community environment.

Department of Agriculture: The light traps created may be implemented in rice fields for a more sustainable way of removing larvae bred in the rice paddies. Similarly, light traps may be implemented in farms so mosquito bites and mosquito-borne diseases do not continually torment livestock animals. The mosquito larvae collected by the traps may serve as fish food in fisheries.

II. METHODS

A. Research Design

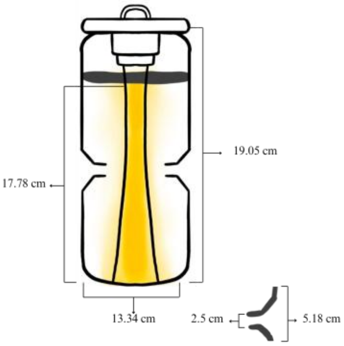
A quasi-experimental research design was employed. It is an experimental type of research whereby the researchers often do not have control over the treatment but instead study pre-existing groups that receive different treatments after the fact. This type of research aims to establish a cause-and-effect relationship between an independent and dependent variable. (Thomas, n.d).

A quasi-experimental research design aligns with the study's practical constraints and ethical considerations. It provides a valuable opportunity to investigate the causal relationship between the independent and dependent variables in a methodologically rigorous and ecologically relevant manner. Quasi-experimental is the better fit for the study since it allows the researchers to work with naturally occurring groups without imposing experimental conditions, making it more ethically acceptable. Lastly, quasi-experimental methods allow the observation of variables in a more authentic environment.

B. Subject and Locale

The study was conducted at Saint Louis University Basic Education School Laboratory Senior High School in Baguio City, Philippines.

Phase 1 of the study was conducted at Dontogan, Baguio City, wherein the setup of the phototaxis was readied and conducted in a controlled environment. Phase 2 of the study was conducted at the innovation lab of SLU-BEDs Senior High School.



Aedes aegypti larvae were the subjects for experiments.

C. Instrumentation

Light-emitting diodes (LEDs)

The researchers examined the impacts of different light colors on mosquito larvae activity. The primary type of light the researchers used contained a variety of colors: red (625–750 nm), orange (590–625 nm), yellow (565–590 nm), green (520–565 nm), cyan (between 500–520 nm), blue (435–500 nm), indigo (425-450 nm), and purple (between 380–435 nm) (Jones, 2020).

Aquarium

Four (4) transparent aquariums with measurements of 12x6x8 inches were used during phase 1 of the experimentation. Black panels were placed on three sides of the aquarium to control the amount of natural light passing through. The front of the aquarium stayed uncovered as it served as a viewing window so that the ruler at the back of the aquarium was visible. The back panel had 7 marked lines measuring 1 inch apart to indicate P zones. The left panel had a hole to let natural light through in the control tank, while the other three (3) let LED light through for the experimental tanks.

Thermometer

Two (2) aquarium thermometers were used during the mosquito larvae rearing, wherein the water temperature was closely monitored to ensure optimal larvae rearing.

Devised Light Trap

A light trap was designed by the researchers as an alternative pest control system that uses LED lights to trap mosquito larvae. The light trap had a height of 19.05 cm and a diameter of 13.34 cm. The inner light source is composed of a strip of LED light and is contained in a plastic bottle that is 17.78 cm in height and 6.60 cm in diameter. A water-tight seal ensures the light will not come into contact with the water when the trap is submerged. The funnel entrance for the mosquito larvae measured 5.18 cm in diameter at the opening and 2.5 cm at the end.

Figure 2

Prototype design of the aquatic light trap

Larval rearing setup

The sight of larvae rearing was an incubation setup made of a cardboard box, four plastic containers filled with matured water and a lamp with a 20-watt bulb.

Figure 3

Rearing Setup for A. aegypti

D. Data Gathering Procedure

There were two phases during the data-gathering process: the phototaxis assay phase (phase 1) and the trap experiment phase (phase 2).

Before phase 1, larvae of A. aegypti mosquitoes were reared in a larval rearing setup per the protocol of Imam et al. (2014). Rearing the larvae eliminates species, nutrient level, and gestation period as potential intervening variables.

A. aegypti mosquito eggs were sourced from the University of the Philippines Los Baños Institute of Weed Science, Entomology and Plant Pathology (UPLB-IWEP). About 1,000 mosquito eggs, placed on two filter paper strips, were availed in the institution and stored in a microwavable plastic container until used.

Figure 4

A.aegypti Egg Strip

Note. Two egg strips were availed from UPLB-IWEP. Each egg strip contained an estimated 1,000 A. aegypti eggs to be used for the experimentation.

Four plastic containers and a jug filled with stagnant water were left in the sunlight for 24 hours to mature. The four plastic containers were placed in an incubation setup made of a heated lamp with a 20-watt yellow bulb and a cardboard box. A water temperature of 27°C was maintained for the larvae to develop properly. The jug of stagnant water continued to mature until it was used during the phototaxis assays.

Figure 5

Egg Strip in First Primary Container

On November 26, 2023, the egg strip was placed in the first plastic container. After 6 hours, 1st instar larvae were identified. The egg strip was removed and placed in the second container to prevent overcrowding of larvae. The process was repeated until the egg strip reached the fourth plastic container. The larvae were fed ¼ brewer yeast pellets to keep the larvae growing optimally. The water was changed every two days to prevent food waste accumulation and larvae from dying.

Figure 6

Primary Container with 1st Instar Larvae

Two days after initial hatching (November 28, 2023), larvae molted into the 2nd instar. On the 3rd day of gestation (November 29, 2023), larvae molted into the 3rd instar, characterized by an increase in size and the distinguishing of several parts of the larvae, like the head and the hair-like filaments in its body. 3rd instar larvae were used for phase 1.

Figure 7

Comparison of Larval Instars

Note. Larva of the 1st instar is seen at the very left, characterized by a semi-transparent body. The 2nd instar larvae show a growth in size and a semi-translucent body. The 3rd instar larvae to the right show growth in size and a brown body with tufts of hair all over the segments. The breathing tube is evident in this instar.

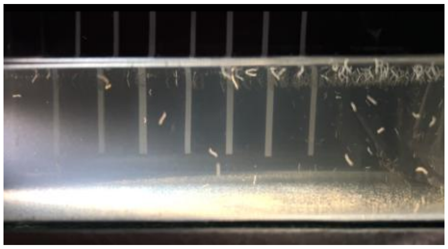
During the phase 1, the lights and their different colors were tested to determine if there was an effect on the phototaxis of the mosquito larvae. The efficacy of the lights to attract mosquito larvae was tested through a phototaxis assay corresponding to the method of Muppala et al. (2022).

Four 2.5-gallon fish tanks were used, three of which were assigned as the experimental tanks and one assigned as the control tank. The tanks were filled with 2 inches of matured water, and larvae from three plastic containers were split equally and placed in the tanks.

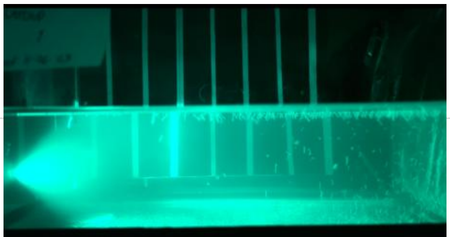
During the phototaxis assays, the tanks were placed in a dark room where larvae were subjected to 5 minutes of dark time before exposure to LED light. The control tank had a black cloth draped over it to simulate dark room conditions for the control variable. The control tank, assigned as tank 4, was exposed to natural light in the morning. The rest of the tanks numbered 1, 2, and 3, were placed in a dark room before the LEDs were turned on.

Figure 8

Phototaxis Assays



Note. The image above shows the control tank testing larval phototactic response to natural light. The image below shows the experimental tank testing larval phototactic response to



green LED light.

The phototaxis assays lasted for three days, where a total of eight colors were tested: red, green, blue, yellow, orange, cyan, indigo, and purple. Each color had two trials per tank that lasted 5 minutes each. Between testing of each color, a 1-minute dark period was observed to allow the larvae to disperse once more before starting another trial. In a day, the four established groups were tested for their phototactic behavior.

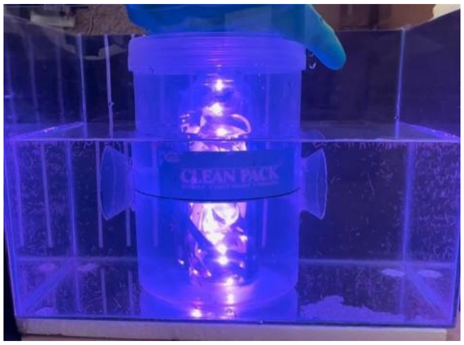
The larvae's phototactic behavior was observed through the following parameters: the farthest phototaxis zone most larvae reached (A), the time it took to reach the zone (B), and how long the larvae lingered in that zone (C). Variable A was measured through the ruler at the back panel of the aquarium numbered from 1-7, an increase in number indicating an increase in phototaxis. Variables B and C were measured in seconds by timing footage of each phototaxis essay.

After conducting the phase 1, the proper biological waste disposal protocols were followed. The larvae from each tank were returned to their primary container and drained of all water. They were sealed before being placed in a -20˚C freezer. Following a 24-hour cold treatment period, the insects are taken out of the freezer and disposed of in a plastic bag adequately labeled as biological/insect waste along with their primary container. Throughout phase 2, the results of the laboratory experiment (phase 1) were tested for its effectiveness by creating the light trap.

The trap was tested in the laboratory using the larvae from the fourth plastic container. The light trap was submerged in an aquarium that contained stagnant water and the mosquito larvae. The trap was left for 10 minutes before retrieving and observing the results. The light trap underwent the first two trials, wherein modifications were made to make the trap more effective. A third trial was performed wherein the trap was submerged in the same test aquarium for 12 hours.

Figure 9

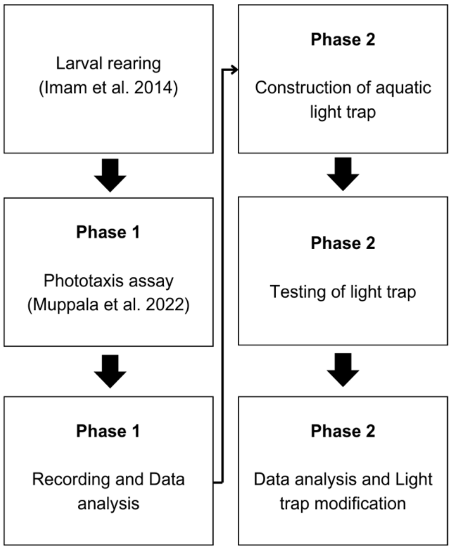
1st Light Trap Prototype Testing



Note. The light used for the light testing is the light that will show the most efficacy in attracting the mosquito larvae.

Figure 10

Flowchart of the procedure



E. Data Analysis

Inferential statistics was utilized to answer the formulated research question. Analysis of Variance (ANOVA) with a significance level of 0.5 was used to analyze the significance of the means of the collected data. ANOVA was employed to identify the differences between the means of each color used during phase 1.

Tukey's post hoc test was used to make pairwise comparisons between group means. The post hoc test was utilized to compare specific light colors to identify each color's efficacy against another. Moreover, the post hoc test aided the researchers in determining which colors are most effective in manipulating larval phototactic behavior.

These statistical methods were employed by the researches of Kehinde et al. (2018), Inacio et al. (2020), Muppala et al. (2022), Oriyomi and Babalola (2020), and Sheppard et al. (2017).

F. Ethical Consideration

The proper laboratory and safety protocols were followed throughout the laboratory and field experiment.

The researchers ensured that the proper personal protective equipment, like laboratory gowns and gloves, is used during the laboratory experiment.

Adequate disposal of insect waste used in the research (A. aegypti) was strictly adhered to after conducting the experiments.

III. RESULTS AND DISCUSSIONS

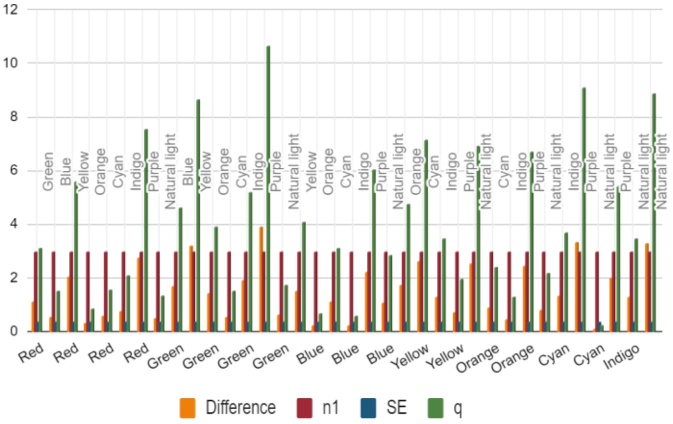
There were three variables to consider: the farthest zone larvae reach (a), the time larvae spend swimming towards the zone (b), and the time larvae linger at the most distant area (c). There was a significant difference when it came to variables a (0.007 < 0.05) and b (0.01 < 0.05). However, there was no significant difference regarding the time larvae swam towards the light.

Observation of the assays reveals that larvae have an initial adverse phototactic reaction, wherein larvae proceed to the dropping zone before the phototactic zone (P zone) 1. However, after a specific time, larvae moved to positive phototactic behavior, wherein larvae started swimming towards the P zone. Furthermore, observation reveals that short-wavelength light like indigo and purple caused immediate attraction and lingering of the larvae in the farthest P zone, while long-wavelength lights like red and orange caused larvae to stay from P zones 1-4, indicating mild attraction towards the light color.

Larvae phototaxis had a significant difference (P < 0.05) regarding the phototactic zone reached. A P-value of 0.007 implies a considerable difference in the phototactic area. The result indicates substantial evidence of a significant impact on the larvae's behavior in response to light stimuli.

Figure 11

Tukey's Post Hoc Test results of Variable A



Note. Q-score set to 4.96.

Significant differences in the furthest phototaxis zone most larvae reached between various wavelengths of light have been shown using Tukey's post hoc test. The comparisons between specific light colors such as Red and Green (3.09 < 4.96), Green and Blue (4.61 < 4.96), Blue and Yellow (4.07 < 4.96), Yellow and Orange (4.75 < 4.96), Orange and Cyan (2.41 < 4.96), Cyan and Indigo (3.69 < 4.96), Indigo and Natural Light (3.46 < 4.96), etc. showed no significant differences, rejecting the alternative and accepting the null hypothesis.

On the contrary, Red and Yellow (5.58 > 4.96), Red and Purple (7.54 > 4.96), Green and Yellow (8.68 > 4.96), Green and Purple (10.64 > 4.96), Blue and Purple (6.03 > 4.96), Yellow and Cyan, (17.7 > 4.96), Yellow and Natural Light (6.94 > 4. 96), Orange and Purple (6.71 > 4.96), Cyan and Purple, (9.13 > 4.96), Indigo and Purple (5.43 > 4.96), and Purple and Natural Light (8.90 > 4.96) showed that the phototaxis of A. aegypti mosquitoes is significantly impacted by varying visible light spectrums, thereby rejecting the null hypothesis and accepting the alternative.

These results indicate that purple and yellow LEDs most effectively attract the larvae to the farthest phototaxis zone. Previous studies have mentioned that larval eyes are morphologically distinct from their adult counterparts. However, following the results of Oriyomi and Babalola (2020), purple light effectively attracts not only adult mosquitoes but also mosquito larvae. In addition to their results, yellow light shows attractiveness, aside from increased mortality rate, as stated by Kehinde et al. (2018).

In insects, photoreceptors are displayed as slender, cylindrical, and clustered into species-specific combinations (Carlson et al. 1984). Receptors contain rhodopsin, the functional molecule of photoreceptor cells in organelles in the cell's distal region. The expression of photoreceptors in specific cells in larval compound eyes may explain this. As Liu et al. (2022) state, varying opsins are found in certain cells, creating specific photoreceptor clusters. A. Opsin3 and Opsin7 are expressed in different clusters of photoreceptors in aegypti larvae. Opsin1 is found in R1-6 and R8 cells in compound eyes, Opsin2 and Opsin8 are only found in R7 cells, and Opsin9 is found in some R7 and some R8 cells in the dorsal area. The specific arrangement of the photoreceptors may explain why larvae are attracted to short-wavelength lights like purple and mid-wavelength lights like yellow but are unattracted by mid-wavelength lights like green and long-wavelength lights like red.

Throughout the experimentation, it was observed that larvae avoided direct exposure to green-colored light. The larvae's tendency to avoid green may be linked to the expression of two rhodopsins, Aaop3 and Aaop7. Rocha et al. (2015) discussed that Aaop3 and Aaop7 expressions are characterized by distinct color discrimination within the blue-green range of light. Color perception with the blue-green range increases sensitivity to such color, leading to mosquito larvae actively avoiding green light.

Ultraviolet light, like purple, has a range of 354-468 nanometers and is particularly attractive because of its specialized photoreceptor cells that are sensitive to UV light. When exposed to UV light, these photoreceptor cells stimulate the larvae to swim towards the light source. (Oriyomi and Babalola, 2020) In practical terms, UV light can be used to attract and trap mosquito larvae. the specialized photoreceptor cells have yet to be identified by researchers.

The expression of certain photoreceptor arrangements has yet to be intensively studied by researchers. Only those of Aaop3 and Aaop7 have been functionally identified by researchers. The current results of the paper suggest that A.aegypti larvae have a photoreceptor combination that allows distinguishing the colors yellow and purple.

There is no sufficient statistical evidence to conclude that there are significant differences among the group means of Variable B (P < 0.05). Therefore, the absence of adequate statistical evidence to establish significant differences among the groups means that the observed data do not support rejecting the null hypothesis, suggesting similarity or equality between the groups. Hence, opting not to perform a post hoc test signifies a cautious approach, as further pairwise comparisons were considered unnecessary due to the lack of identified overall group differences. Although A. aegypti larvae are attracted to certain colors, this does not significantly affect their movement towards such light.

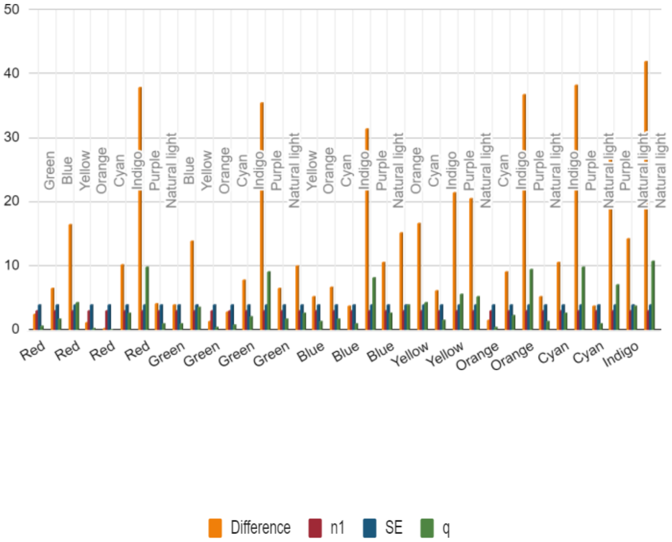
Given the diversity in the appeal of various mosquito species to light-baited traps, it is plausible to anticipate variations in wavelength attraction among individual species. As evidenced by behavioral responses, the tendency for attraction to specific wavelengths may or may not align with their respective spectral sensitivities (Burkett & Butler, 2005).

According to a study by Hu et al., 2012, researchers described how rhodopsins in Aedes larval photoreceptors express themselves and migrate in response to light. Five stemmata are composed of these photoreceptors, which are arranged laterally on the head. The majority of stemmata photoreceptor cells express the long-wavelength Aaop3 rhodopsin. Aaop3 localizes to cytoplasmic vesicles in the photoreceptor cell body in a lit environment, but it travels into the rhabdom in a dark environment. It has been suggested that this widespread light-dark translocation of rhodopsin, Aaop1, in adult Aedes mosquitoes represents an adaptive mechanism for optimizing the light sensitivity range of photoreceptors.

There was a significant difference (P<0.05) between the groups regarding the larvae's time lingering. Evidence suggests that larvae were attracted to certain colors of light.

Figure 12

Tukey's Post Hoc Test results of Variable C



Note. Q-score set to 4.96.

The post hoc test, as indicated by figure 15, reveals that differences between Red and Green (0.63 < 4.96), Green and Blue (1.02 < 4.96), Blue and Yellow (2.58 < 4.96), Yellow and Orange (2.58 < 4.96), Orange and Cyan (0.37 < 4.96), Cyan and Indigo (2.72 < 4.96), Indigo and Purple (3.69 < 4.96) etc., are not significant. On the contrary, Red and Purple (9.80 > 4.96), Green and Purple (9.16 > 4.96), Blue and Purple (8.14 > 4.96), Yellow and Purple (9.16 > 4.96), Yellow and Natural light (5.29 > 4.96), Orange and Purple (9.50 > 4.96), Cyan and Purple (9.87 > 4.96), and Purple and Natural light (10.85 > 4.96) are statistically significant. These pairs reject the null hypothesis, suggesting that yellow and purple LEDs make A. aegypti larvae linger most effectively.

Substantial research has yet to be done regarding larval photoreceptor expression that can influence light perception of A.aegypti larvae. Only certain opsin arrangements, such as Aaop3 and Aaop7, have been discovered to influence light perception of the larvae. In agreement with the statement of Liu et al. (2022), it is clear that species-specific opsin arrangements exist within larval eyes; however, the functions of these species-specific photoreceptors have yet to be identified. Moreover, McDonald et al. (2022) also stated that larvae eyes are morphologically unique to those of an adult mosquito and warrant closer investigation into the expression of photoreceptors in larvae eyes.

The lack of literature regarding photoreceptor expression in A.aegypti larvae eyes leads to the further discovery of photoreceptor combinations that may influence the light perception of the mosquito larvae.

Considering all three variables measured throughout phase 1, a light trap was created after the phototaxis assays revealed that purple is the most effective color in attracting and making A. aegypti larvae linger.

Figure 13

First Aquatic Light Trap Prototype

Note. A round plastic container was used as the primary trapping chamber. The bottle inside contained the color shifting LEDs used during the experimentation.

Light traps designed by the researchers were tested in the laboratory, undergoing two trials that lasted 10 minutes each, with a collection period between wherein larvae trapped were counted. Of an estimated 200 larvae in the test tank, 30% were trapped in the light trap.

The tests ensured that the researchers' design was adequate; however, modifications were needed to make the trap more effective.

Modifications were done from the results of the first set of trap tests. The trap was modified as it was transparent in color, making the light visible through the plastic, possibly affecting the mosquito's movement as they were observed to be attracted to the walls rather than the funnel entrance into the trap. Furthermore, no drainage system was present in the trap, forcing water out from the funnel entrance, where larvae were observed to escape through the escaping water.

Figure 14

Second Aquatic Light Trap Prototype

The trap was modified so that the trap's walls were painted black to prevent the light from escaping from areas other than the funnel entrance, where the larvae would go to enter the trap. At the bottom, a drainage hole with a double screen was created to drain the water once the trap was ready for collection.

The prototype was tested once again for its effectiveness. This time, it was submerged for 12 hours before the collection period. During the collection period, it was observed that as the trap was being collected, larvae could escape through the drainage hole at the bottom. Larvae were no longer counted during this trial, as many ran through the drainage hole.

Another modification was made in response to the 3rd trial. The drainage hole was covered in double screen mesh so water could drain while the larvae remained inside.

Phototaxis using visible spectra is a viable method for trapping mosquito larvae and can be implemented into an aquatic light trap to trap mosquito larvae. The results directly coincide with Mapossa et al. (2021), who stated that LEDs impact animal behavior. Moreover, the design of the light trap took advantage of the path A. aegypti larvae take through serpentine movements that aid in the horizontal movement of the larva (Nelson, 1986). As stated by Jhaian et al. (2021), light trapping significantly decreases human interaction with mosquitoes, contributing to the safety and sustainability of this trapping method.

IV. CONCLUSIONS AND RECOMMENDATIONS

The study aimed to determine if the visible light spectra affected A. aegypti larval phototaxis. Moreover, making an effective pest control system was targeted as an application of the results of the first objective.

Ultimately, it was concluded that purple and yellow light have the most significant effect in attracting A.aegypti larvae, with purple showing more efficacy when implemented in the light trap. In addition, the light trap designed by the researchers attracted and trapped mosquito larvae, leading to an effective pest control system that can benefit communities with identified mosquito breeding sites.

With these conclusions, several recommendations can be made based on the outcome of the present study: A study on different mosquito species larvae may be conducted, as unidentified species-specific photoreceptor combinations may affect the larvae’s light perception.

Further studies on the morphology of mosquito larval eyes may be conducted following the study's results. This may lead to new photoreceptor combinations that cause specific reactions to different colored lights. This will contribute to the knowledge regarding insect phototactic behavior and add to the current knowledge of mosquito light perception, a field yet to be studied intensively by researchers.

Modifications regarding water drainage and buoyancy control are still needed, as the trap continues to float even when fully submerged. Weights may be installed at the bottom of the trap to keep it fully submerged. In addition, regarding the modifications, consideration should be given to how the funnel entry is positioned to optimize the efficacy of the light trap.

Lastly, a field test for the light trap can be done as the light trap was only tested under laboratory conditions. A field test will aid in improving the light trap's effectiveness and also help develop it for future commercial use.

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