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Development of *Aedes aegypti* L. under the action of light radiation at different wavelengths

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ABSTRACT. *Aedes aegypti* is a holometabolous insect, vector of medical importance for arboviral transmission, and has shown the ability to develop chemical larvicides resistance, which are the worldwide used to control mosquitoes' population because of their low cost. Due to the well-known photophobia, a striking characteristic of the behavior of *A. aegypti* larvae, this study aimed to observe the development of this insect in its larval stage under the action of certain ranges of light radiation and its possible biological effects. For that, the experiments used larvae in L1, observed during seven days under the action of LEDs (light-emitting diode) that emitted light at different wavelengths, with six different colors, one for each experiment. Some were tested with a light-dark interval every 10 minutes and others every two minutes, with three repetitions. At the end, mosquitoes, pupae and larvae were counted and the data submitted to statistical evaluation. The experiment showed a significant difference between the control and the different wavelengths used, when exposed at two-minute intervals. LEDs that emitted blue (λ = 457.9 nm) and white (λ = 448.58 nm) wave frequencies were the most promising for the development of equipment that could act synergistically with other forms of control in order to improve its efficiency.

Keywords: dengue; photophobia; vector mosquito.

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Introduction

Aedes aegypti mosquito (Linneau, 1762) is the main transmitter of dengue, zika, and chikungunya infectious diseases caused by arboviruses that affect millions of people per year in Brazil (Donalisio, Freitas, & Zuben, 2017). This vector is a dipteran, culicid that has complete development (Holometabolus), with the stages of egg, larvae (L1, L2, L3 and L4), pupa and adult (Rey & Lounibos, 2015). It presents dark coloration with white stripes on the legs and a lyre-shaped design on the mesonotum. They live approximately 45 days and are very well adapted to the urban environment, preferring places where people are present (Ndenga et al., 2017). Males and females of *A. aegypti* feed on nectar from flowers and fruits, however, only the female becomes hematophagous, as it needs a blood meal to ingest proteins that will serve for the maturation of its eggs (Consoli & Oliveira, 1998). It is during this process that *A. aegypti* can become infected and transmit arboviruses. Usually, this feeding is done in the morning and in the late afternoon. After the meal, the female lays its eggs, preferably in places where there is clean and still water and the larvae can hatch in four hours when in favorable conditions. However, in the absence of these conditions, they may still remain viable for more than a year (Ndenga et al., 2017). After hatching, the larvae feed intensively, passing through four larval stages, until they become pupae.

Dengue was considered eradicated in Brazil in the 1950s, but was reintroduced in Pará in 1967 from where it spread throughout the national territory (Araújo, Bezerra, Amâncio, Passos, & Carneiro, 2017), but for Barreto and Teixeira (2008), the first evidence of dengue epidemic in our country was in 1982, when the DENV-1 and DENV4 serotypes were found in Boa Vista (RO). After that, the country recorded several outbreaks and epidemics of dengue. That's why some measures were taken to try to reduce cases of the disease. As well as the use of chemical insecticides to eliminate outbreaks and potential mosquito breeding sites, as well as the elimination of larvae (Zara, Santos, Fernandes-Oliveira, Carvalho, & Coelho, 2016). However, several studies have already shown that populations of *A. aegypti* are becoming resistant to the

main insecticide used in the country (Góes, Góes, Góes, Moreira, & Quemel, 2021). Although some forms of control have been developed by several researchers, such as the use of plants and biological insecticides as an alternative to chemical insecticides, a resistance monitoring plan, development of models of the propagation of epidemics and more studies are still needed to obtain a more effective form of control (Zara et al., 2016), and knowledge of the genetic structures that determine the variability of populations (Hiragi et al., 2009).

Another very striking feature of the *A. aegypti* larva is its photophobia, that is, the mosquito is sensitive to light, fleeing when illuminated, and this feature is one of the ways to identify this vector (Bermudi et al., 2017). Vezzani and Albicócco (2009) carried out a study where they found that a greater amount of A. aegypti larvae survived until the adult stage, when deposited in shaded places compared to larvae deposited in a very bright place. Adults also prefer to live in shady places in the intra or peri domiciliary regions.

Due to these photophobic characteristics, the present study aimed to evaluate the development and possible biological effects in *A. aegypti* under the influence of light radiation of different wavelengths in the light and dark cycles, different from the natural environment.

Material and methods

Biological resource: *Aedes aegypti* Larvae

Aedes aegypti eggs of the Rockefeller lineage were acquired at the Entomology Department of the Aggeu Magalhães Research Center/Fiocruz, Recife-PE and the larvae were placed to hatch in plastic trays with a capacity of 2 liters, at a temperature of 26 ± 2ºC and 70% humidity (Figure 1 A).

Structural preparation

Seven wooden boxes (22 x 30 x 20 cm) with lids were made (Figure 1 B). They were previously numbered, and in each one a light circuit board with LEDs (Light Emitting Diode) was placed (Figure 1 C). Each box had a LED board made up of 20 diodes, with different wavelength, being I for the negative control (NC), that is, without light; II for ultraviolet (λ = 365.7 nm); III for blue (λ = 457.9 nm); IV for white (λ=448.58 nm); V for red (λ = 631.92 nm); VI for yellow (λ = 598.88 nm) and VII for green (λ = 517.41 nm) (Figure 2).

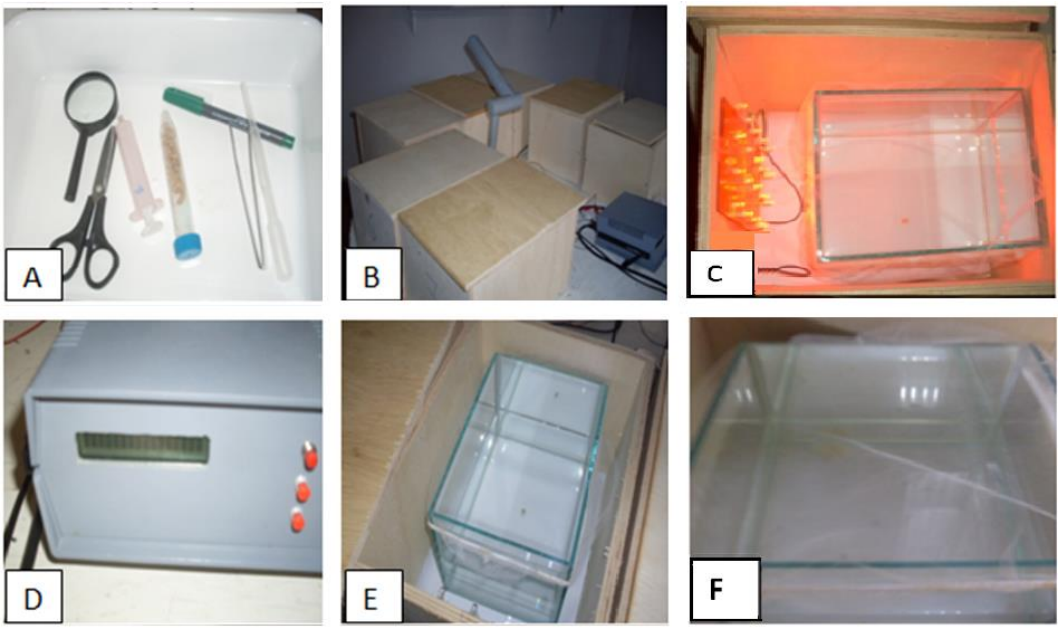

Figure 1. A. Tray and other objects used in handling eggs, larvae and pupae – B. boxes of experiments with coupled LED light, connected to the automatic ascending device, containing aquariums with larvae – C. LED board inside the box – D. light control equipment – E. aquarium with larvae in L1 – F. Tulle fabric covering aquarium to prevent contamination by strange Culicidae.

Experimental assays

The boxes were connected to a device developed in the laboratory GIAQ (Group of Instrumentation in Analytical Chemistry) (Figure 1 D) at the Academic Unit of Serra Talhada - UAST – *Universidade Federal*

Rural de Pernambuco - UFRPE, which controlled the time the LEDs should stay lit. An aquarium (15 x 21 x 15 cm) was placed inside each box (Figure E) with a mirrored face inwards in order to increase the light intensity, with 2 liters of distilled water and about 20 1st instar larvae (L1) of the Rockefeller strain, in addition to a portion of Whiskas® cat food for larvae feeding, provided daily until the pupa stage. The aquariums were then covered with a tulle screen to prevent other insects from laying eggs, contaminating the experiment and the boxes were closed with a wooden lid (Figure 1 F).

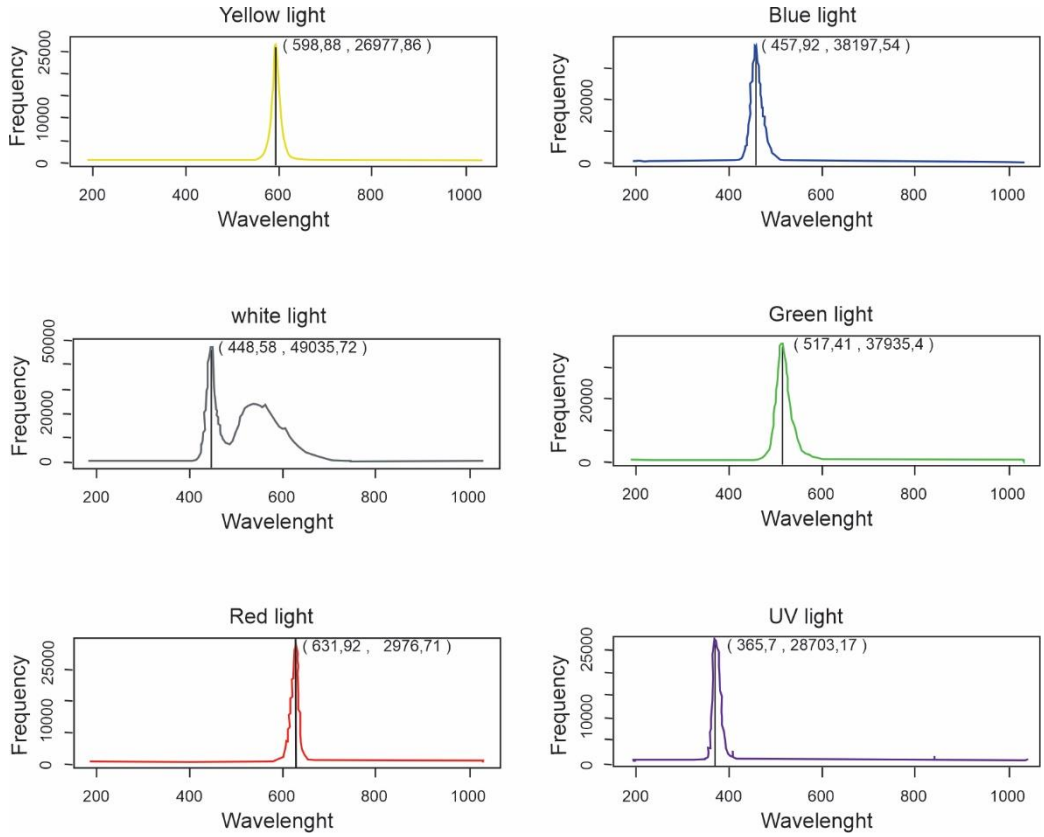

Figure 2. Demonstration of the wavelength values of the lights used in the exposure experiments of *A. aegypti* larvae.

Data analysis

The device to control the lighting time of the LED lighting system of the boxes was programmed in the first experiment for a time of 2:2 (2 minutes light and 2 dark), and in the second experiment at 10:10 (10 minutes light and 10 minutes dark), both totaling 12 hours of light and 12 hours of dark at the end of every 24 hours. For each time, three repetitions were performed, and the individuals were exposed from L1 onwards, for about seven days, until they reached the pupal stage.

Larvae were observed and counted daily in early morning and at the end of the afternoon, and food replacement was done whenever necessary. Upon reaching the pupa stage, they were counted and transferred to transparent pots with lids and numbered according to the box. At the end of the seven-day period, the remaining larvae were counted, as well as pupae and mosquitoes that had emerged, the survivor individuals were euthanized by freezing, and then discarded.

Data referring to the change of larval stage and behavior were recorded, placed in a spreadsheet and analyzed in the statistical software R Development Core Team (2011). The proportion of mosquitoes after the seventh day was used to characterize the samples, in relation to the number of larvae initially placed in the aquariums. One experiment was chosen to be destined to the exposure to all wavelengths (λ) for a time interval (2 mM and 10 mM) and the treatments as each specific wavelength represented by each box. The percentage of individuals that reached the pupal stage was calculated. Statistical comparison tests were performed between each treatment within each of the experiments, with respect to the control (which was not subjected to light), and then among the experiments. For that, the tests applied were chi-square (χ^2) for proportion, with a confidence interval of 5% ($p < 0.05$).

Results

At the end of the 10-minute experimental activities, it was observed that in box I (control), 87% of mosquitoes had emerged after 7 days, in relation to the initial number of larvae placed in the aquarium, which is in accordance with number the standard result for the Rockefeller strain used in the experiment. The results of the other boxes containing artificial lights with different wavelengths were compared with the control: box II had an emergence of 74%; box III, 72%; box IV, 86%; box V, 79%; box VI, 86% and box VII, 85% of the individuals among adult mosquitoes (Table 1). Comparing the boxes of each 10-minute treatment with the control box (Figure 2), no statistical difference was obtained, at the 5% level of significance, in any of the wavelengths, with ($p > 0.05$) in all the boxes, a result also obtained when all treatments of the 10-minute experiment were compared with each other (Table 2). During this 10-minute experiment, the larvae showed typical movement, feeding normally, with a slight tremor when the lights turned on or off. At the end, 3 dead individuals, 2 larvae in L2 and 1 pupa were counted.

Table 1. Number of larvae and mosquitoes, and percentage of Adult Individuals, after seven days of light exposure of the 10:10 minute treatment (light and dark), and after seven days of light exposure.

Type of light	Larvaes	Mosquitoes	Death	Percentage
Box I (NC)	68	59	9	87%
Box II (Ultraviolet)	43	32	11	74%
Box III (Blue)	62	43	19	72%
Box IV (White)	59	51	8	86%
Box V (Red)	68	54	14	79%
Box VI (Yellow)	64	56	8	86%
Box VII (Green)	59	50	9	85%

For the 2-minute experiments, 90% of adult mosquitoes were observed at the end of 7 days on the control group. From the boxes submitted to treatment, we obtained: box II, 77% of adult individuals; box III, 58%; box IV, 61%; box V, 66%; box VI, 63% and box VII, 67%, respectively (Table 2). The proportional comparison of the boxes with a 2-minute treatment with the control box showed that there is a difference at the level of 5% of significance with $p < 0.05$, between the negative control (I) and boxes V, VI and VII. There was no difference between I and Ultraviolet (II) boxes, which showed $p = 0.057$. All treatments of the 2-minute experiment were compared with each other and showed a difference between some treatments: II x III and II x IV, all with $p < 0.05$ (Table 3). Larvae moved and fed normally during the 2-minute experiments. They showed a slight tremor when turning the lights on and off.

Table 2. Number of larvae and mosquitoes, and percentage of adult individuals, from the 2:2 minutes treatment (light and dark), after seven days of light exposure.

Type of light	Larvaes	Mosquitoes	Death	Percentage
Box I (NC)	72	65	7	90%
Box II (Ultraviolet)	80	62	18	77%
Box III (Blue)	76	44	32	58%
Box IV (White)	77	47	30	61%
Box V (Red)	74	49	25	66
Box VI (Yellow)	73	46	27	63%
Box VII (Green)	74	50	24	67%

Table 3. Comparisons between all 2-Minute treatments. Data: p-value ($p>0.05$ there was no difference; $p < 0.05$ there was a difference at the 5% significance level).

Boxes	II	III	IV	V	VI	VII
I	0.05707	1.844e-05	8.202e-05	0.0009212	0.000234	0.00162
II		0.01424	0.03895	0.1677	0.074	0.2295
III			0.817	0.378	0.378	0.2911
IV				0.6229	0.9356	0.5048
V					0.8154	1
VI						0.6842

The total percentages of the experiments of 10 and 2 minutes were compared. It was found that there was a significant variation at the level of 5% (p = 6,237e-06) between them (Figure 3). Therefore, considering two groups of experiments, one of ten minutes of light and ten minutes of dark, another two minutes of light and two minutes of dark, the totals showed a statistically significant difference.

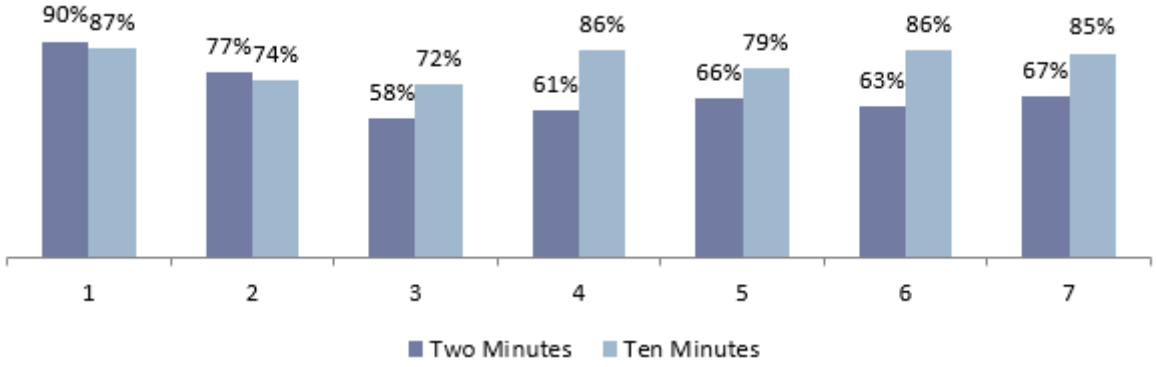

Figure 3. Comparative between 2-minute and 10-minute treatments showed differences in number of mosquitoes emerged in relation to the initial number of larvae.

Discussion

The results obtained in the control boxes corroborate the results usually observed for the Rockefeller strain (*A. aegypti*), for emergence on the seventh day of 80 to 95% of the mosquitoes. In the 10-minute experiment, no significant variation was found between treatments, indicating that neither the 10-minute time alternating between light and dark, nor the wavelengths themselves, had any biological effect on the larval development of *A. aegypti*.

For the 2-minute experiment, the results showed a significant variation in the development of larvae in boxes III, IV, V, VI and VII in relation to the negative control. It was observed that the exposure of larvae to the same wavelengths, for a longer time (10 minutes), had no effect on the present generation of *A. aegypti*. This variation is probably due to factors other than the effect of a particular wavelength. A likely explanation could be interference with the insect's circadian rhythm. This rhythm is regulated by several genes (Meireles-Filho & Kyriacou, 2013) that have already been extensively studied in *Drosophila melanogaster*. Several studies have already shown that changes in this cycle can modify the behavior and physiology of different organisms, as in the studies by Gomes, Sciavico, and Eiras (2006) and Iseki, Negrão, and Castrucci (2010). However, it could not be established whether these 2-minute experiments were sufficient to alter this cycle. Another factor that may have occurred is the stress caused by the constant going up and down of the lights, generating a difficulty in feeding and consequently in the proper development of the larvae, since they present negative phototaxis.

Despite the confirmed action of ultraviolet radiation on several organisms, in this study, no significant difference was observed between this radiation and the control box. It may have occurred due to the wavelength value used, which was 365.7 nm, corresponding to the UV-A range. According to Owens and Lewis (2018) the harmful effects of wavelengths longer than 320 nm are minimal. Also, the brightness emitted by the UV light used was not enough to strongly interfere with the development of larvae and pupae, making it similar to the white light effect.

Comparison of treatments within the same experiment showed no difference between wavelengths in the 10-minute experiments. In the 2-minute experiments, there was a variation between the boxes that contained blue and white light in relation to UV. This is due to the fact that the ultraviolet light obtained a very similar result to the negative control. However, despite the similarity, the ultraviolet radiation showed lower adult hatching proportions than the control, which led to the non-occurrence of a significant difference between this and the red, yellow and green lights.

The total percentages of the 10-minute and 2-minute experiments compared to each other obtained p = 6.237e-06, which revealed a significant difference between them, revealing the effective difference between the two treatments.

Conclusion

The present study did not show changes in the larval development of *Aedes aegypti*, resulting from the action of different wavelengths, including ultraviolet (UV-A). The alterations that were observed were due to the modification of the photoperiod, which may have altered the circadian rhythm of this vector or caused a stress that hindered its feeding and consequently its proper development. However, it was not possible to determine interferences in the insect's reproductive cycle or changes in subsequent generations due to logistical limitations.

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