DO THE INCANDESCENT AND LED LIGHTS ALTER THE BIOLOGY OF *Aedes aegypti* (DIPTERA: CULICIDAE)?

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ABSTRACT

The invention of the light bulb brings darkness into the light in the sky. This study aims to understand the impact of light types and exposure duration on altering the biology and development of *Aedes aegypti*. A total of 100 eggs in triplicate were exposed to two types of light: light produced by the light-emitting diode (LED) light bulb and the incandescent light bulb. The eggs were exposed to the light at three different duration (1) 12h light: 12 h dark, (2) 6h light: 18h dark, and (3) 1h light: 23h dark. The results revealed that the hatching rate of *Ae. aegypti* eggs increased in mosquitoes exposed to incandescent light with a longer duration of light exposure, but not significantly different ($p>0.05$). The same pattern has been observed for mosquitoes exposed to the LED light bulb. However, when comparing the 6h light: 18h dark, a significantly more hatching rate occurred after exposure to LED light ($p=0.016$). More days was required by *Ae. aegypti* to reach adulthood when reared under incandescent light at 12D: 12L duration than LED light ($p<0.05$). Exposure to both types of light did not seem to alter the sex ratio of adult mosquitoes emerged. In conclusion, neither incandescent nor LED light has a significant effect in altering the biology and development of *Ae. aegypti*.

Keywords: *Aedes aegypti*, development, incandescent, LED, light, mosquito

ABSTRAK

Rekaan bola lampu membawa kegelapan ke dalam cahaya di langit. Kajian ini bertujuan untuk memahami kesan jenis lampu dan masa pendedahan dalam mengubah biologi dan perkembangan *Aedes aegypti*. Sejumlah 100 biji telur dengan replikasi sebanyak tiga kali telah didedahkan kepada dua jenis lampu; cahaya yang dihasilkan oleh diod pemancar cahaya (LED) dan lampu pijar. Telur telah didedahkan kepada cahaya pada tiga tempoh cahaya iaitu (1) 12 jam cahaya: 12 jam gelap, (2) 6 jam cahaya: 18 jam gelap, dan (3) 1 jam cahaya: 23 jam gelap. Keputusan mendedahkan bahawa kadar penetasan telur *Ae. aegypti* meningkat dalam nyamuk yang telah didedahkan kepada lampu pijar dengan tempoh pendedahan, tetapi tidak berbeza secara signifikan ($p>0.05$). Corak yang sama telah diperhatikan pada nyamuk yang telah didedahkan kepada lampu LED. Walaubagaimanapun, jika dibandingkan dengan 6L:18D, lebih banyak kadar penetasan berlaku secara signifikan selepas terdedah kepada lampu LED ($p=0.016$). Lebih banyak hari diperlukan oleh *Ae. aegypti* untuk mencapai peringkta dewasa semasa dipelihara di bawah lampu pijar pada tempoh masa 12L:12D berbading lampu LED

($p<0.05$). Pendedahan kepada kedua jenis lampu dilihat tidak mengubah nisbah jantina secara perbandingan. Kesimpulannya, secara fakta samada lampu pijar atau LED mempunyai kesan signifikasi dalam mengubah biologi dan perkembangan *Ae. aegypti*.

Kata kunci: *Aedes aegypti*, perkembangan, pijar, LED, cahaya, nyamuk

INTRODUCTION

Aedes aegypti also known as a primary vector of the yellow fever mosquito in the tropic region and few in the temperate regions (Gibbons & Vaughn 2002), with identification feature of visible four white stripes with violin-shaped on the dorsal (top) part of the thorax region (Zettel & Kaufman 2013). They become endemic due to urbanization, lack of sanitation, global warming, and insecticide resistance (Walker 2001). *Aedes aegypti* (Linnaeus) is known as a cosmopolitan mosquito and was given considerable attention due to its medical importance as the major vector of dengue transmission in subtropical and tropical regions the world (Zhou et al. 2004). It has also been known to carry Chikungunya virus in Malaysia which been detected since 1998 (Rozilawati et al. 2016).

Artificial light is currently being researched on its potential in influencing animal and insect's behaviour, physiology and even psychology (Kehinde et al. 2019). Various experiments had been done and showed that a particular insect is attracted to a specific region of the light spectrum and different light intensities, including the aspect of behavioural or electrophysiological to prove the positive reaction of insect eye toward ultraviolet irradiation (Reverte et al. 2016; Shimoda & Honda 2013). The impact of light on insects is mainly due to spectra specific to the light (Masatoshi et al. 2014). Nakashima et al. (2017) reported that the animal would generate reactive oxygen species (ROS) in response to artificial light. Thus, the build-up of ROS in the cell may lead to RNA and DNA damage and may cause cell death. This might have a good impact on mosquito management. It has been proved by Taniyama et al. (2021) that the light within 400-500nm, which emitted the blue-light irradiation and produced a toxic wavelength, impacted the development of *Culex pipiens*. We have been seen the increasing trend of the mosquito control device innovation using light; however, the light impact on mosquito development and the life cycle is not yet fully understood.

Incandescent derives from the Latin word, "*incandescere"* means "to glow white" using thermal radiation to produce visible light. Incandescent light bulbs produce light by heating the filament at a fraction of radiation that falls into the visible light spectrum with electrical current until glowing occur. An improved version incandescent light bulb is with the addition of a small amount of halogens such as bromine or iodine. Incandescent light produces red to near-infrared red emission ranging from 630-950nm (Turon et al. 2018). However, the incandescent light bulb is considered a less efficient light bulb as it is only able to convert less than 5% of the energy received into visible light, while another energy is released in heat energy (Keefe 2007).

In time the incandescent light bulb is replaced with other more efficient light bulbs such as a fluorescent lamp and light-emitting diode (LED), especially in the industrial lighting field (Schubert & Kim 2005). LED is a type of semiconductor light source and white LED emitted hight proportion of blue light at 450nm (Abdel-Rahman et al. 2017). It was first introduced as an electrical component, and the colour of light produced is dependent on the energy bandgap of the semiconductor. Due to the advantages of LED, which is durable, higher efficient of illumination, longer life-span compared to other types of light, it is widely used in all field such

as industrial application, vehicle lighting, and even housing decoration lighting. The invention of LED cause increasing of insects in the urban region as LED lure at least 48% more compared to other lights such as High-pressure sodium lamp (HPS) in a broad range of insect families, including Trichoptera, Coleoptera, Hymenoptera, Hemiptera, and Diptera (Pawson & Bader 2014).

Not much study has been done in looking at possible changes on *Ae. aegypti* mosquito biology and their development caused by these lights. This study is critical to understand the impact of light used on promoting the expansion of *Ae. aegypti* mosquito due to the light used in the surrounding. In which, the existence of various light types together with different spectrum and wavelength might influence the life-history of normal mosquitoes' cycle. The increasing number of *Ae. aegypti* mosquitoes might increase the potential of dengue and dengue haemorrhagic infection among human. Thus, this study aims to study the effects of light produced by incandescent and LED light bulbs over the different durations of light exposure on mosquito development.

MATERIAL AND METHODS

Colonization of *Aedes aegypti*

In this study, the susceptible strain of *Aedes aegypti* was used and obtained from Vector Control Researches Unit (VCRU), Universiti Sains Malaysia. This strain was used due to the ability to survive under the laboratory conditions successfully and may reduce the mortality due to the unsuitability of laboratory condition. All of the eggs for each set of experiments were using the same generation of eggs. Plastic containers measure 16.5cm x 11.5cm x 7.0cm were chosen during the experiment filled with tap water, and the water must be left overnight for at least 24 hours to reduce the chlorine content. The chlorine content in the water may affect the hatching rate of the eggs. Throughout the experiment, the larvae that hatched were fed daily with 0.5g of larvae food made of dog biscuit, beef liver, yeast, and milk powder at the specific ratio of 2:1:1:1. The larvae culture was maintained under the standard laboratory conditions of 70%-80% humidity, 26±2°C of temperature.

Study Design

Two types of light bulbs were used in this study: (1) the light-emitting diode (LED) light and (2) the incandescent light bulb. LED light bulb is chosen because it is currently being used widely, and the invention of LED has caused a massive impact on the current society. This LED has been used in most of the buildings, housing and streetlight nowadays. While the incandescent light bulb is invented before the LED light. Although it is being replaced by LED bulb, it is still mainly being used in rural areas.

During the experiment, a total of 100 eggs in triplicate was placed into the plastic container sized 16.5cm x 11.5cm x 7.0 cm containing 215 ml of chlorine-free water. The container contained eggs were exposed for three different duration of times for each type of light bulbs with the ratio of Light: Dark (L:D); (1) 12 h L: 12 h D, which is the normal day times experience in our country and be served as a control for the comparison, Malaysia, (2) 6 h L: 18 D which similar to the period of time of the light switch on for those rarely stay in-home, and (3) 1 L: 23h D as the minimum light exposure for comparison. Containers contained eggs were positioned on the rack and placed 40cm below the light sources.

The exposure to these lights started from the egg stage until it develops into the adult stage. Larvae were fed with a sufficient amount of larvae food daily at 0.5 gm throughout the

experiment. The position of the containers was exchange daily to ensure all of the containers received the same amount of light and avoid biasedness. The experiment was conducted in the laboratory under artificial light without the source of natural light with the temperature of 25 ± 2°C and relative humidity (RH) 70-80%. The water temperature under the exposure of lights was taken daily.

Data Collection and Analysis

At the interval of 24 hours, the data for the percentage of hatching rate, the number of first instar larva survivorship rate, development days, and the sex ratio of the adult mosquitoes were recorded. Data were subjected to a normality test. A Log_{10} transformation was subjected to normalize the data to fulfill the assumption of parametric tests. One-way ANOVA was used to examine for comparison between duration of light exposure for; (1) hatching rate, (2) survival of first instar larvae, (3) development days for each stage. Further analysis of post hoc multiple comparisons were run to determine means using Tukey's test at $\alpha = 0.05$. To compare the effects of two different lights types; incandescent and LED, an independent t-test was performed for all data. For the sex ratio, the chi-square test was performed. All analyses were performed using SPSS analysis version 26.0.

RESULTS

Hatching Rate and Survival Rate of *Ae. aegypti* after Exposure to Different Lights and Durations

The hatching rate of the *Ae. aegypti* eggs and their survival into the 1st instar larvae under two different type of light sources with different duration of exposure are shown in Figure 1. The results revealed a hatching rate of *Ae. aegypti* that were reared under the incandescent light at the exposure period of less light at 1L:23D caused only 50% of the eggs to hatch but not significantly different than those exposed to 12L:12D and 6L:18D (one-way ANOVA, p=0.86). In comparison with incandescent, the hatching rate was found higher for LED light except for 12L:12D. The results from T-test revealed that the hatching rate in LED is significantly higher than incandescent light at 6L:18D exposure duration (T-test, p=0.016).

The survival rate was quantified base on the percentage of the hatched individual from eggs that survive the 1st instar stages. Roughly, from Figure 1, it can be seen that the survival percentage reared under the incandescent light were higher than those under LED light at all duration of exposure. However, no significant differences in survival rates were found between all duration of exposure between incandescent light versus LED light (T-test, p>0.05).

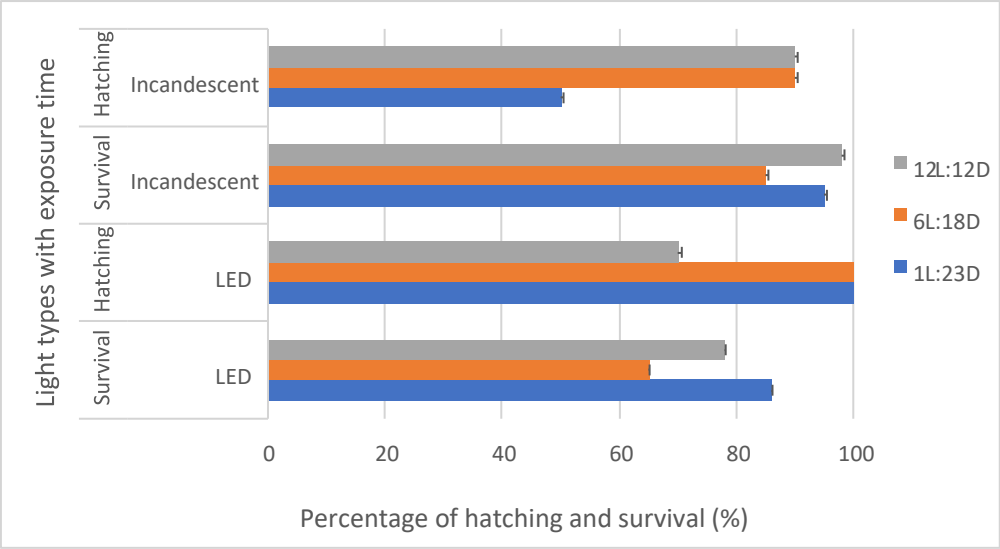

Figure 1. The percentage of hatching and survival rate of *Aedes aegypti* under the different duration of exposure to incandescent and LED lights

The Development Days of Each *Ae. aegypti* Stage

As featured in Figure 2, it seems that under the incandescent light at the duration of exposure of 6L:18D, the development times for each instar of *Ae. aegypti* was found shorter as compared to other durations of light exposure, but no significant differences were found between each stage (one-way ANOVA, P>0.05). Only the adult stage showed a significantly shorter development time when reared under 6L:18D as compared to 12L:12D duration of exposure (one-way ANOVA, p=0.09). Whereas, under LED light the development time for all stages was found to have no significant differences between all duration of light exposure (p>0.05, Figure 3). Independent t-test revealed no significant differences were found when comparing each stage between incandescent and LED lights (p>0.05).

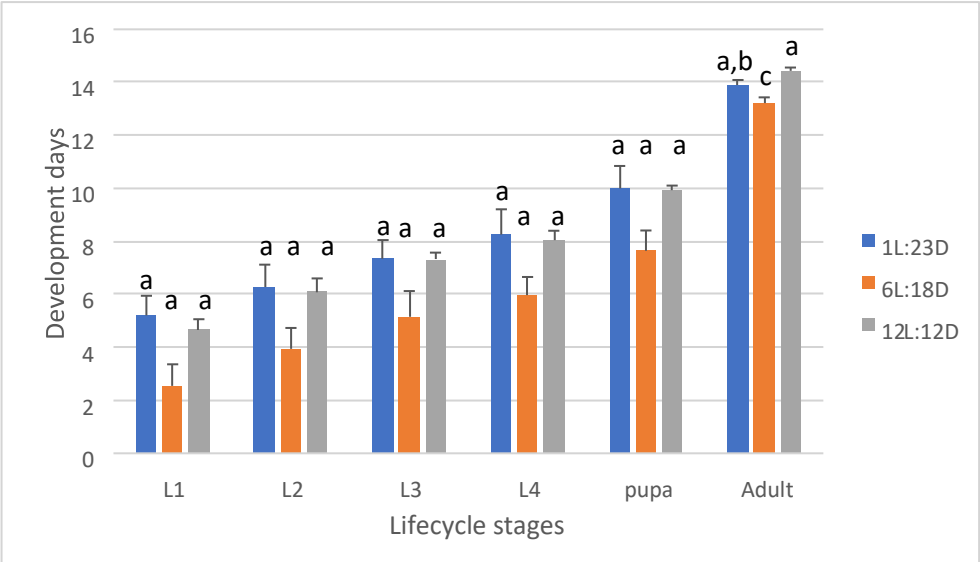

Figure 2. Number of days required by *Aedes aegypti* for its development in each life stage under the exposure of incandescent light. The same letter indicates no significant differences

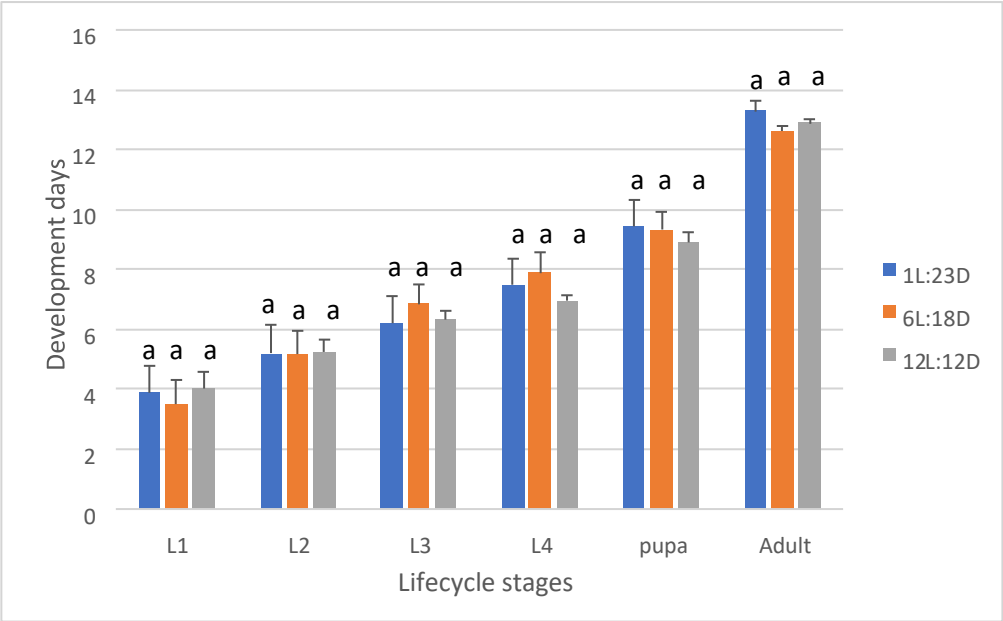

Figure 3. Number of days required by *Aedes aegypti* for its development in each life stage in response to LED light. The same letter indicates no significant differences

The Effect of Lights on The Sex Ratio

After the adult emergence, the mosquitoes were separated based on their sex. More females emerged as compared to males for all light types and durations with a ratio of male to female, which is between 0.88:0.97 (Figure 4). The overall ratio between male and female is closed to 1. Chi-square test revealed that no significant differences were found between the number of male and female *Ae. aegypti* emerged when exposed to all light types and duration of exposure ($p>0.05$).

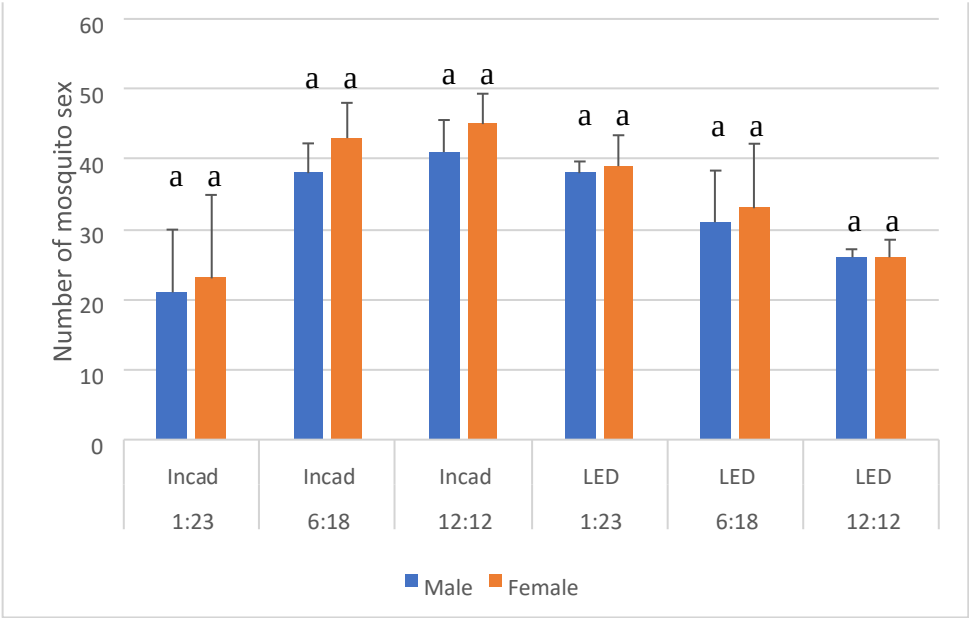

Figure 4. Number of males and females *Aedes aegypti* emerged after exposure to different light. The same letter indicates no significant differences

DISCUSSION

Our study indicated that the hatching rate in incandescent light bulb condition increases as the duration of light exposure increases. This probably due to the heat released from the incandescent light bulb. The longer period of light exposure from incandescent light bulb creates a constant slightly higher temperature than normal room temperature, which is around 27-35°C.

According to Impoinvil et al. (2007), *Anopheles gambiae* egg with different constant temperature and different period of times, at a temperature around 22 °C and 27°C it has the higher number of overall mean egg hatched, at the temperature is at 12°C and 42°C the number of egg count hatched will decrease drastically.

In the case of LED light bulb, a higher hatching rate was observed compared to the incandescent light bulb. As heat is not produced from the LED light bulb, unlike the incandescent light bulb, which releases most of its energy in heat (Keefe 2007). The temperature experienced by *Aedes* eggs was less fluctuated, which is closer and constant to room temperature of 27 to 28°C compared to those reared under the incandescent light bulb. In which this temperature range is the optimum temperature suitable for egg hatching. The sudden decrease in hatching at the 12 light: 12 darkness periods could be explained by the room temperature was at a lower range, plus it is during the rainy season when the study was conducted. However, the effect of duration of exposing light to *Ae. aegypti* mosquito eggs had no significant difference between LED and incandescent light bulb.

Generally, colony reared under incandescent light bulb has a higher survival rate than those in LED light bulb. This may occur due to the different amount of larvae food intake. Mosquito larval are poikilothermic as their body temperature is affected by the temperature of the surrounding (Heinrich 1981). We hypothesize, response to heat from the incandescent light bulb will indirectly cause a high metabolic rate on the larvae and consumption rate would be faster with more food is needed compared to those undergo LED light bulb condition. The larvae food will also produce a single layer of fat easily if it leaves a certain time, and that could cause the death of the larvae during the development period due to the blockage on siphon. As suggested by Merritt et al. (1992), both *Ae. aegypti* and *Anopheles* spp. mosquito prefer clean water for breeding. However, differences in their feeding behaviour allow *Anopheles* spp. to have a higher survival chance with the presence of a fatty layer on the water surface or so-called biofilm consist of organic material, whereas *Ae. aegypti*'s feeding behaviour is classified as shredding compare to *Anopheles* spp. which more to a collecting – filter behaviour (Merritt et al. 1992).

The Natural selection theory by Fisher (1930) suggested that surviving of individual may contribute to future generations, and each life stage may experience mortality. The trends in the development rate of *Ae. aegypti* found against our original hypothesis which in the short duration of light exposure, the development rate of the mosquito larvae will increase due to mosquito larvae will behave just like their adult stage where they tend to be more active during the dark period and tend to eat more with faster development rate or it is affecting the insect photoperiodism. The study by White & Sundeen (1967) found that under darkness, the polysome formation is happening in mosquito's helical eyes and require high protein metabolism. Thus, the longer development days especially mosquitoes reared under minimal photoperiod of one hour, which requires more energy for polysome formation. Based on the study by Zani et al. (2005), thermal tolerance follows two nonexclusive patterns; (1) life stages

that expose to thermal more than the stable environment tends to tolerate the thermal, (2) thermal tolerance tends to be greater in life stages that are sessile or are captive in the restricted habitat than those are mobile or at open habitat. In this study, the *Ae. aegpti* mosquitoes were found capable of tolerating the thermal environment caused by the light, which proved by nonsignificant differences between light exposure durations.

Comparing between the duration of 1: 23 and 12: 12, the shorter light period required a longer time to reach the adult stage compared to a more extended light period in both incandescent and LED conditions. The exposure to longer light exposure increases the water temperature where the immature stages on mosquito live. This supported by the thermal equilibrium hypothesis, that stated that insects' that facing optimal thermal regime will be maximized in body size of adults, fecundity and abundance (Ward & Stanford 1982). Furthermore, the photosensitivity of insects may completely change the structure of body on holometabolism via metamorphosis (Shibuya et al. 2018) which prolong the development process depending on the development stages. According to Shelton (1973), the time required for the immature cycle tended to increase when the temperature decrease, and this condition apply to this study. This situation only being observed at the early stage of the larvae, and as closer to the adult stage, the pattern changed as the influence from the light does give a more negligible effect on the pupa to the adult stage.

Most of the animals follow the approximate 1:1 Fisher's ratio of sex allocation, and that includes the *Ae. aegypti* in the study. A pair of the allele exists in the heterozygous organism, and this equal allele is a product of gametogenesis (Craig & Hickey 1966). Thus, the possibility to lead to 1:1 sex ratio is very high unless the natural pressure had occurred. From this study, even though the mosquito undergoes a different type of light bulbs and even different durations, it does not cause any severe effect that would alter the sex of the mosquito skewed toward one side.

CONCLUSIONS

The exposure to incandescent and LED light at different duration has no impact on a different aspect of the *Ae. aegypti* behaviour such as hatching rate, survival rate, the time required for the development of immature stages, the sex allocation between males and females except in development of the adult stage in incandescent light exposure.

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