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INVESTIGATION OF *HIBISCUS SABDARIFFA* AS A NEW ECO-FRIENDLY PHOTO-LARVICIDAL NATURAL PRODUCT FOR THE CONTROL OF MOSQUITOES

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ABSTRACT

The Culex spp. is a vector of several disease-causing organisms including filarial parasites, encephalitis virus and the West Nile Virus; the control of these diseases is hinged on controlling the vector. Synthetic insecticides have previously been applied in the control of Culex spp. which poses a danger to the environment. In the present research, the use of light and a naturally occurring product, methanol extract of Hibiscus sabdariffa calyces (HSME), in controlling the breeding and spread of Culex tritaeniorhynochus was investigated. Third instar larvae of C. tritaeniorhynochus were fed with HSME and exposed to light for 18 hours. Results indicate over 80% mortality of the test sample with LC_{50} of 528.44 ppm; also increase in percentage mortality with corresponding increase in HSME concentration and longer irradiation time was established. The trend observed in light reactions was not seen in the control and dark reactions, implying that the presence of light and the photosensitizer (HSME) is required to induce mortality in Culex tritaeniorhynochus larvae population.

Keywords: *Culex*, Larvicide, Mosquito, Photosensitizer, Photo-larvicidal, *Hibiscus Sabdariffa*, Insecticide

INTRODUCTION

The quest for environmentally friendly larvicides and insecticides to combat the threat of mosquito-borne diseases, especially in the tropics, has accelerated in recent years. A lot of synthetic insecticides have been in use as they are very efficient in insect control. Many of them, however, have been shown to be detrimental to the environment and human health. They cause ecosystem disruptions, are harmful to a wide variety of non-target species and have a high proclivity for accumulating in the environment (Szymon *et al*., 2014). Insecticides can be classified by their mechanisms of operation as they interact with different target and non-target sites, including receptors, enzymes, and many other known and unknown molecules. Photo-insecticides can therefore be defined as those chemicals (dyes) that can exert toxicity on insects through the absorption of light of an appropriate wavelength.

When visible light encounters photosensitizers, it produces substances called photo toxins that are damaging to living cells. According to Tardivo *et al*. (2005), a photosensitizer is a substance that absorbs energy directly from a light source and then transfers it to molecular oxygen to create an activated form of oxygen called singlet oxygen ($^1O_2$). John & Jason (2000) evaluated the toxic effect of photoactive dyes, rose Bengal and phloxine B, on American and migratory grasshoppers at various concentrations. Both were found to be effective at inducing mortality of grasshoppers when applied at 2

and 5% to bran bait. Lima *et al*. (2018) used eosin-methylene blue (EMB) as a photosensitizer for photodynamic control of *Aedes aegypti* larval populations. They subjected third instar *A. aegypti* larvae to different EMB concentrations in different light doses and found EMB as an effective photoactive compound to control larval populations.

Ulva lactuca seaweed extract was used as a reducing and capping agent in the synthesis of zinc nanoparticles (NPs) by Ishwarya *et al*. (2018) and the larvicidal activity of the *U. lactuca* fabricated ZnO NPs was tested on fourth instar *A. aegypti* larvae at 50 µg/ml within 24 h. Thandapani *et al*. (2018) fabricated TiO_2 nanoparticles by treating *P. hysterophorus* leaf extracts with TiO_4 solution and the larvicidal activity of TiO_2 NPs was studied against fourth instar larvae of *A. aegypti* and *C. quinquefasciatus.* These studies reported over 80% mortality in the populations.

Patil *et al*. (2015) used dyes from *Beta vulgaris* subsp. *Vulgaris* (beetroot) to activate the *Bacillus thuringiensis* (Bt) toxin to improve its efficiency as an insecticide. This preparation was evaluated on fourth instar *Anopheles stephensi* and *Aedes aegypti* and found to improve the larvicidal activity of the Bt toxin more than 60%. Other dyes that have been studied for their photo-insecticidal activity as enumerated by Robinson (1983) are eosin, erythrosine, and rose bengal, among others. These have been found to be effective larvicides.

The *Culex* spp. has been found to be one of the vectors for filarial parasites (Sabatinelli *et al.*, 1994), *encephalitis virus* and the West Nile Virus (Paul *et al.,* 2014). *Lymphatic filariasis* commonly called elephantiasis is a tropical, parasitic disease that affects the lymph nodes and lymph vessels, causing swelling in the legs, arms and the genitalia. In Nigeria, it is estimated that 80 to 120 million people are at risk of the disease (Hotez *et al*., 2012). Viral encephalitis is an inflammation of the brain parenchyma that is indicated by fever, headache and in extreme cases, seizures (fits), confusion, drowsiness, and loss of consciousness, and even coma (Saema & Michael, 2020). The West Nile Fever is a flavivirus (a group of single stranded RNA viruses that cause severe endemic infections and epidemics on a global scale) that has similarities with the viruses that cause St. Louis encephalitis, Japanese encephalitis, and yellow fever and infects humans, horses, and a variety of bird species. Most infected people show no symptoms, but a small percentage of them develop severe neurological disease that can be deadly.

The Illinois Department of Public Health (2017) describes the *Culex* as medium-sized mosquitoes that are brown with whitish markings on the abdomen. These are the house mosquitoes (*C. pipiens* and *C. quinquefasciatus*) that develop in urban areas, and the western encephalitis mosquito (*C. tarsalis*) that is frequently seen in rural communities. Typically active in the evenings, they bite at dusk and after dark and rest by day in and around structures and vegetation. They have a life cycle of between 10 and 4 days, or longer in chilly weather with four stages in their life cycle. These include the egg which hatches mostly within 48 hours, the larva that lives in water and develops into pupa within 5 days. In 2 to 3 days, the pupa develops into an adult.

Substances introduced directly to the body of water in which *Culex* mosquito larvae develop can be used to suppress them. These substances may be organisms that consume

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them such as larvivorous fish (Chandra *et al.*, 2013) poisonous biological substances that can cause illnesses peculiar to them and can kill them (Ramírez-Lepe & Ramírez-Suero, 2012), compounds that interfere with their development or physiology, such as methoprene -an insect growth regulator (Lawler & Lanzaro, 2005); or suffocating oils and films (Bukhari *et al*., 2011). However, oils and films also suffocate non-target aquatic life. While the insects can develop resistance to chemicals applied for their control, biological methods can be expensive to sustain.

Hibiscus sabdariffa commonly called Roselle (also known as 'zobo' in Nigeria) belonging to the family *Malvaceae*, and is widely grown in Central and West Africa, Southeast Asia and elsewhere (Cahlíkováa *et al*., 2015), was evaluated as a viable photo-larvicide to control the spread of *Culex* spp. in this study. The red calyces of the flower are consumed for their health benefits; the juice or concoction prepared from the plant is taken as a preventive and curative measure against diabetes and hypertension (Owoade *et al*., 2019).

MATERIALS AND METHODS

Reagents

Methanol and ethyl acetate used for extraction were products of Guangdong Guanghua Sci-Tech Co. Ltd, China. Distilled water was used to dissolve the extract.

Plant Material

Dried calyces of *Hibiscus sabdariffa* was purchased from the Fruit Garden Market in Port Harcourt, Nigeria. The plant was identified by Dr. J. O. Elemchukwu (Taxonomist), Department of Plant Science and Biotechnology, Rivers State University, Nigeria. It was prepared by careful separation of unwanted particles from the plant material and drying to a constant weight of 90.9 g.

Organism Used

Photo-larvicidal tests were conducted with third instar larvae of *Culex tritaeniorhynochus* harvested from natural environment by Mr. I. D. Ekerette of Malaria Vector Surveillance and Insecticide Resistance Monitoring Laboratory, Department of Animal and Environmental Biology, Rivers State University, Nigeria.

Extraction

Dried calyces of *H. sabdariffa* (90.9 g) were pulverized and sequentially extracted with ethyl acetate (300 ml) and methanol (300 ml) at room temperature (Ndukwe *et al*., 2020). The methanol extract was filtered through cotton wool and concentration to dryness using a rotary evaporator at 45 ℃ afforded 9.6 g of HSME. HSME (8 g) was dissolved in 4 L of distilled water and was considered as stock solution (2000 ppm). From the stock solution, different concentrations were prepared.

Photo-Larvicidal Test

The test was conducted (using third instar larvae of *Culex tritaeniorhynochus)* according to WHO (2005) test guidelines. The test comprises of the light and dark reactions, each done in duplicates containing 30 third instar larvae in each test jar containing 300 ml of test photosensitizer (HSME) dissolved in distilled water and 3 ml of dissolved cabin biscuit as food. From the stock solution, different concentrations (25, 50, 100, 250 and 500 ppm) of the HSME were made for both reaction conditions as well as the control (0 ppm). Irradiation was done using a sodium-tungsten lamp (500 W) for 18 hours with temperature maintained between 28 ℃ and 30 ℃. The experiment was repeated to determine the LC_{50} with expanded concentrations of 200, 400, 600, 800 and 1000 ppm for the light set up and 200, 400, 600, 700, 800, 900 and 1000 ppm for the dark set up. The number of dead larvae was

counted after 18 hours for the first test and hourly for 18 hours of exposure for the second test and expressed as percent mortality (equation 1). Larvae were considered dead when motionless and showed no response to any form of mechanical stimulus.

$$\text{Mortality}(\%) = \frac{\text{Number of dead larvae}}{\text{Number of larvae tested}} \times 100 \quad (1)$$

Data Analyses

All experiments were performed in duplicates and the percentage mortalities were calculated as an average of the duplicates. Data obtained were analyzed by one-way analysis of the variance (ANOVA) on Microsoft excel. Probit analysis was used to determine the LC_{50} value.

RESULTS AND DISCUSSION

Results as presented in Figures I, II and III show that exposure of third instar larvae of *Culex tritaeniorhynochus* to HSME and light induces mortality in the larvae. Percentage mortality of the test sample increased with increase in concentration of the photosensitizer, HSME (Figures I and II) and time of exposure to light (Figure III), which agrees with previously reported works by Souza *et al*. (2017) and Mezzacappo *et al*. (2021). This trend is absent in the control as well as in the dark reactions. The implication is that even with the presence of a photosensitizer, light is required to induce mortality in the population. It also implies that even when light is present, a photosensitizer is also required for mortality to be induced in the population. The lethal concentration at which 50 % of the test samples die (LC_{50}) was determined using probit analysis to be 528.44 ppm. The number of deaths recorded in the dark reaction can be attributed to handling and preparation as Tielong Xu *et al*. (2014) reported that mosquito sample collection and preparation methods significantly affected mortality rates in the standard WHO tube resistance bioassay. Because sunlight is one of the most crucial factors in its effectiveness, employing photosensitive materials like HSME to eliminate *Culex* spp. larvae can be regarded as safe and low-cost alternative, especially given the abundance of *Hibiscus sabdariffa* calyces within the environment at photo-chemically active doses. According to Fabris *et al*. (2012), photogenerated cytotoxic intermediates often act through a multi-target method, causing damage to a wide variety of cell constituents (such as proteins, unsaturated lipids, and steroids) at the same time. As a result, unlike most chemical insecticides, HSME and visible light work together to limit the chances of the larvae developing resistance.

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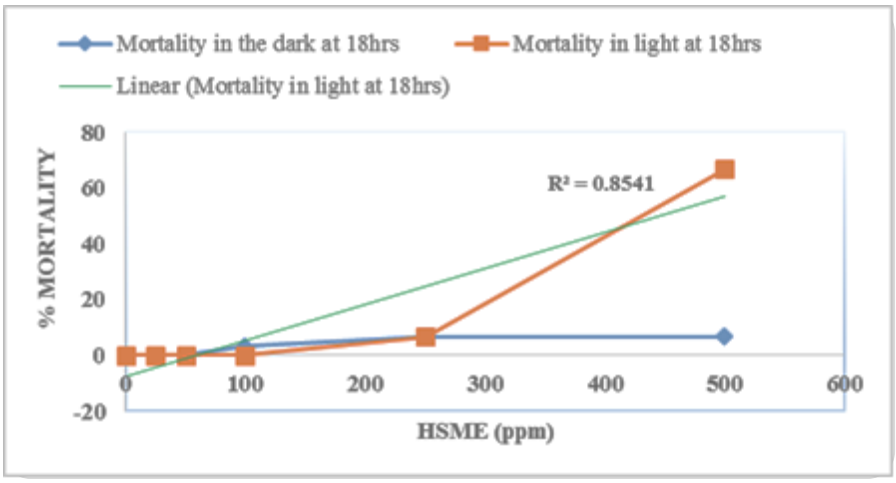

Figure I: Photo-larvicidal Activity of HSME

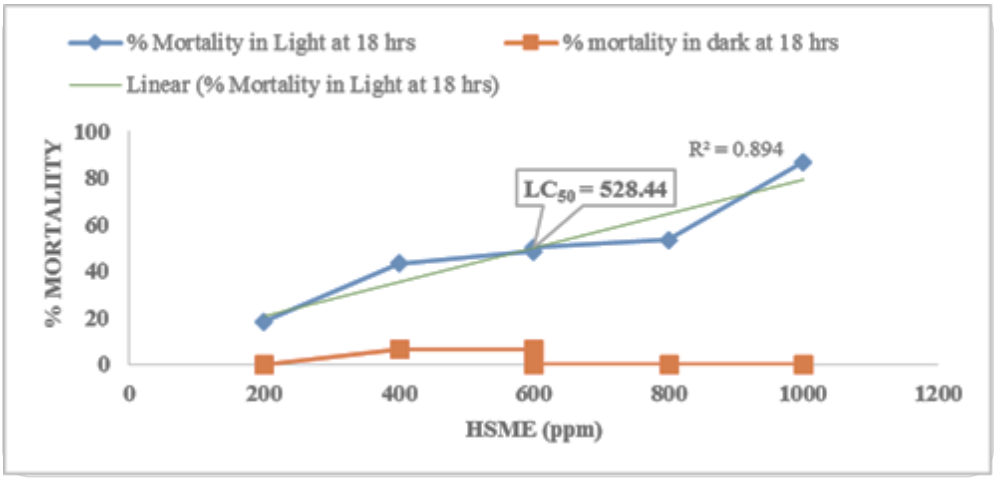

Figure II: Dependence of Mortality on Concentration of HSME

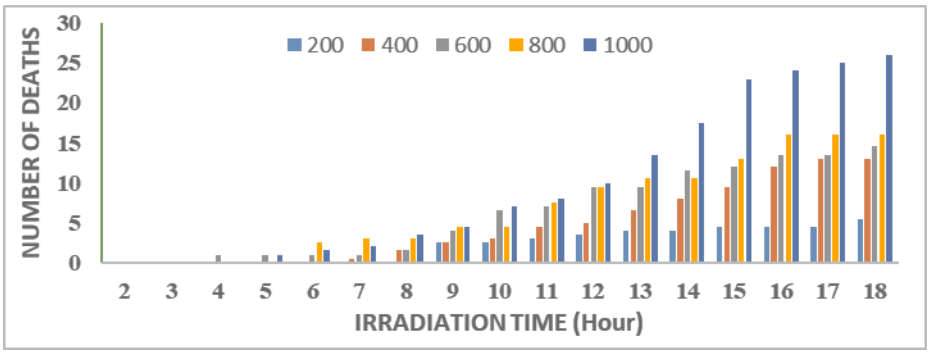

Figure III: Mortality Dependency on Irradiation Time and HSME Concentration

CONCLUSION

The findings of this study have shown the effectiveness of *Hibiscus sabdariffa* calyces as a good photo-larvicide. Using plant extract such as *Hibiscus sabdariffa* to control mosquito population at the larval stage in the environment can have a wide range of benefits for vector control and management.

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