Original Research Article

Effect of Eosin Solution Exposed to Sunlight on *Anopheles* Mosquito Larvae

Abstract

Mosquitoes are a menace for millions of people around the world, they are vectors for destructive parasites which cause diseases like Malaria, Dengue fever and Lymphatic filariasis, affecting people in developing countries and areas with tropical climates. *Anopheles species*, a predominant species transmits malaria, and the World Health Organization have shown that 350,000 lives especially children and pregnant women are lost annually by it. The aim of this study is to determine the effect of eosin solution exposed to sunlight on larvae of *Anopheles* mosquito. The use of control method was aid at eliminating the larva stages of the mosquito life cycle. A total of one hundred and twenty (120) *Anopheles* mosquito larvae were harvested using dipper with handle and net from drainages at Eagle Island and Rivers State University both in Port Harcourt. Five different concentrations of Eosin solutions were prepared in volumes of 1000 microlitre (µl), 800µl, 600µl, 400µl, 200µl after a stock solution of 1gram(g) in 100ml and a control, the physicochemical parameters of the solutions were determined using Extech model DO700 measuring instrument. Twenty (20) mosquito larvae, were carefully introduced into each of the concentrations, exposed to sunlight and observed for 24 hours(hrs) for a period of six (6) days for susceptibility. A hundred percent (100%) mortality was recorded in eosin volume of 1000µl and 800µl. The separate solutions of eosin showed significant effects of their concentrations on the *Anopheles* mosquito larvae of P-value 0.017 at $P<0.05$. The result obtained for the physicochemical parameters were; pH 5.24, temperature 30.4^{o}C, conductivity 168µS/cm, salinity 0.08‰, total dissolved solids 118 milligram per litre(mg/L) and dissolved oxygen was 6.5mg/L for the control. Changes occurred in the values of the dissolved oxygen before and after exposure to sunlight in all the dilutions. The results obtained showed that after 24 hrs, the mortality rate of the larvae increased, indicating that *Anopheles* mosquito larvae expose to concentrations of eosin solutions results in their mortality within 48 hrs. It may be concluded that this study has provided some evidence of larvicidal effect of eosin solution exposed to sunlight on larvae of *Anopheles* mosquito.

Key words: Mosquito larvae, Eosin solution, Sunlight, Susceptibility

Introduction

The transmission of fatal diseases causes major public health problems resulting in large morbidity and mortality each year globally [1] among the populace from the bites of mosquitoes. The revelation in the late 1800s that arthropods transmit a number of protozoan diseases to humans, prompted the development of different techniques to control the arthropod vectors and thereby minimize disease transmission [2]. Several mosquito management strategies have been developed by the early 1900s [3]. Chemical pesticides, environmental management to minimize or remove arthropod vectors, and the use of biological control agents were among the methods used to reduce mosquito vector contact with vertebrate hosts, such as window and door screens, insect repellents and bed nets [4]. At the beginning of the 20th century, mosquito management techniques were crude by current standards, and lacked many of the materials and approaches now available. New discoveries in arthropod physiology and microbiology lead to the production of more efficient and safer materials on the understanding concerning mosquitoes and its

environment. Greater knowledge of the ecology of vector-host-pathogen systems have improved approaches to vector management [5]. After a century or more of research, a wealth of information is available on the life cycles of both disease, organisms and their vectors, as well as the many variables that influence disease prevalence and incidence [6].

Conventionally, vector control methods have centred on killing adult mosquitoes using a variety of insecticides. In locations where mosquito borne diseases were endemic the environmental management is frequently utilized alongside chemical or biological ovicides, larvicides, and pupicides [5]. The National Malaria Strategies also known as the Nigeria National Malaria Elimination Programme (NMEP) developed by the Federal Ministry of Health has included mosquito larvae control as a means of reducing the adult multplication [7]. This includes the larval source management (LSM) which is redefining the long-term vector control strategies in Nigeria, the NMEP incorporated LSM as a component of IVM. Pilot larviciding has been carried out in five locations in Nigeria which are Rivers, Nasarawa, Ogun, Lagos and Jigawa States and is sparingly implemented in Lagos and Rivers States with minimal spread [7].

In order to plan and manage a treatment program, mosquito control requires an awareness and knowledge of the behavioural and environmental distinction among species. The adult stage of the mosquito is the most significant stage for vector control, and it has been known to man for a long time. Mosquito control has traditionally focused on killing the adult mosquito using synthetic insecticides containing compound like organochlorines, carbamates, organophosphates and pyrethroids [8]. The use of these insecticides has to be regulated given that the development of insecticide resistance is widespread [9]. And there is concern with respect to its damage to the environment and effect on non-target organisms. As a result of these, other methods are being developed and tried for mosquito control such as the elimination of breeding places, use of mosquito nets, biological control with parasites such as fungi [10] or predators such as fish [11], and introduction of large sterile male mosquitoes [12]. However, insecticide resistance is now widespread in a number of mosquito species [13] and there is need for new affordable, cheaper and reliable mosquito control strategies [5]. Hence, environmentally friendly alternatives are being researched into to reduce the selection pressure created by insecticide resistance. The alternatives can be biological and non-biological that encompasses chemical and environmental (physical) control.

There are different authorities that have put up the argument that mosquito control should include the application of larvicides as a technique, and this has been accepted into the management of disease transmission by vectors. The use of photosensitizers in the control of larvae of mosquitoes has become necessary since their products are friendly. The Photosensitizers produce singlet oxygen ($^1O_2$) on exposure to sunlight and possess the ability of penetrating soft tissues. Photosensitizers undergo different reaction pathways in reaching its final product stage, by either donating an electron to the substrate or abstracting a hydrogen atom from the substrate, as the photosensitizers triggers a physicochemical shift in a nearby molecule [14]. The photosensitizer gradually returns to its ground state, where it stays chemically

unchanged until more light is absorbed. Singlet oxygen ($^1O_2$) can be generated by photosensitizer molecules that absorb ultraviolet (UV) and visible light through photochemical reactions. The singlet oxygen $^1O_2$ is an oxidizing agent that can cause oxidative damage to biomolecules when it reacts with them. rose bengal, eosin, and methylene blue are excellent examples of photosensitizers since they have triplet states with sufficient energies for oxygen sensitization. Methylene blue is a phenothiazinium dye with a high absorbance in the 550-700 nm range and a high quantum yield [15]. Xanthene dyes, such as rose Bengal and Eosin, have strong absorption bands in the visible spectrum's green region (480-550 nm) and produce substantial yields of singlet oxygen [16].

Eosin is an organic molecule that is a brilliant red dye. It's a fluorescein derivative composed of eosin Y and eosin B, two closely related molecules. The most extensively used is eosin Y. It is a tetrabromo fluorescein derivative with a tinge of yellow (so is also known as Eosin Yellowish). Eosin Y comes in two varieties: water-soluble and ethanol-soluble. Eosin B is a bluish-coloured dibromo dinitro fluorescein derivative. Eosin B is nearly as effective as eosin Y and can produce a stronger red colour. These two dyes can be mixed and matched. It's possible that using one or the other is only a matter of taste or tradition. Eosin is capable of photodynamic generation of singlet oxygen $^1O_2$ and is a very effective photosensitizer, as it possesses triplet states of appropriate energies for sensitization of oxygen [17]. Biological dysfunction induced by $^1O_2$ includes cell death, membrane damage and enzyme inactivation [18].

The sunlight is responsible for the development and continued existence of life on earth. Radiation is fully responsible for the harmful effects of sunshine on biological systems. It is usually assumed that of all the environmental elements, light has the biggest impact [19]. While light plays a vital function, there have been few studies that look at how light interacts with other factors [20]. Natural sunlight has wavelengths as short as 300 nm, but not wavelengths between 250 and 260 nm (as ozone layer blocks these wavelengths from reaching the atmosphere). Bacterial spores and crystals have been affected by incident solar radiation [19]. In the case of pesticide degradation or photo degradation of Bacillus species, the influence of sunlight has been thoroughly examined with regard to the control of aquatic mosquito larvae. The aim of this study is to see the effect of eosin solution exposed to sunlight on mosquito larvae.

Materials and Methods

Study Area

The study was conducted at Rivers State University's Medical Microbiology Laboratory, which is part of the Department of Medical Laboratory Science. Rivers State is located at the Southern Region of Nigeria popularly known as the Niger Delta Region. Geographically, the state is surrounded by Abia, Akwa Ibom, Bayelsa and Imo State. Occupationally, her citizens are mainly

involved in Farming, Fishing, hunting, traders and civil servants. On an average, the standard of living is between 40-60% with moderate level of literacy. The high rate of poor environmental sanitation as well as poor hygienic environment encourages the breeding of most vector of diseases especially Mosquito as there are lot of blocked drainages, stagnant water and massive dumpsite of used tyres around the State thereby causing the endemicity of most vector borne diseases. This has however denied the state its beauty as it is called the Garden city

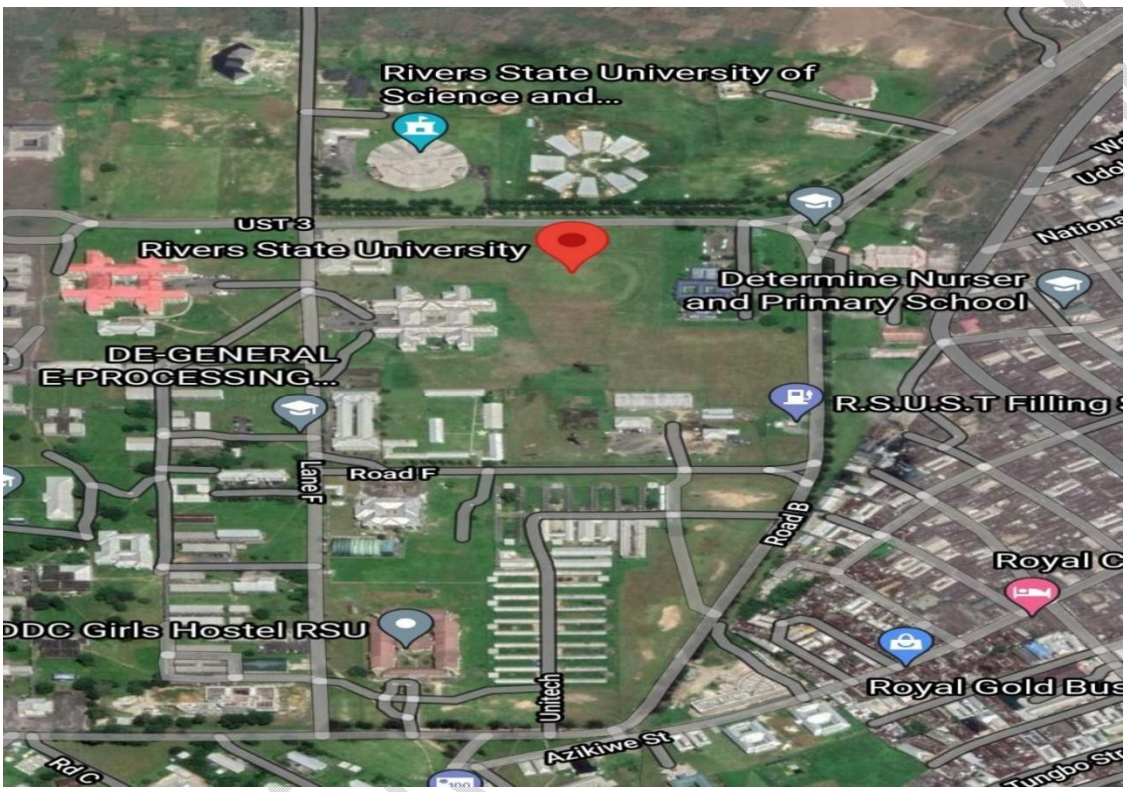

Figure 1: Location of Rivers State University, Port Harcourt, Rivers State. Source: [21]

Collection of samples

The mosquito larvae were collected using randomised sampling from a natural stagnant water in an open environment using the standard dipping method with a standard dipper attached to a handle. Sampling of the water was done using the dipper. Larvae were collected by conventional ladle spoon, pipette and dropper from different spot. While dipping, care was taken so that shadow was cast away from the habitat. The dipper was gently lowered in an angle of 45^{o} just below the surface. Proper care was taken not to disturb the water too much to avoid the larvae diving downwards.

Sample transportation: A total of 15 dips were taken per spot from the habitat where mosquito larvae is suspected. The collected larvae were transported in transparent plastic jars containing clean tap water. The jars were labelled (date, name of sampling habitat) and then transported to the Medical Microbiology Laboratory in the department of Medical Laboratory Science, Rivers State University.

Identification of larval mosquito sample: Some common characteristics of the mosquito larvae samples was identified using standard keys in species identification at the Department of Animal and Environmental biology, Rivers State University. The mosquito larvae are dorsoventrally flattened, lay horizontal to the water surface and the body divided into three parts: Head, thorax and abdomen.

Eosin preparation procedure: A stock solution of eosin was prepared by weighing 1.0g of the eosin powder (Vetec®) with the use of a weighing balance and placed in a beaker containing 100ml of distilled water, the mixture was swirled gently and left standing for 30 minutes until all powder were dissolved. After which the solution was filtered using the Whatman filter paper, and was transferred into an amber coloured bottle and cocked properly. This was stored away from sunlight and in a cool place.

A precision pipette was used to measure 200µl of the stock solution and added to 100 ml of distilled water, 400µl was measured from the stock and added to 100ml of distilled water, 600µl, 800µl and 1000µl were also measured from the stock solution and added to 100ml of distilled water separately to attain the various concentrations needed except the control containing just 100 ml of distilled water. The physicochemical parameters of the resultant dilutions of the solutions were taken and recorded at day one.

A spatula was used to pick twenty (20) larvae of mosquito of various instar stages and were carefully introduced into each of the test solution and control containers respectively and exposed outside under direct sunlight at normal atmospheric temperature and pressure for six (6) days and results taken every 24 hours The physicochemical parameters were taken on day one (1) and day six (6).

Determination of Physicochemical properties

The physicochemical parameters determined were Temperature, Electrical conductivity, Hydrogen ion concentration, Salinity, Total Dissolved Solids and Dissolved oxygen. These parameters were measured *in situ* using Extech DO 700 after calibrating the instrument with the necessary standard solutions and rinse in distilled water before and after each test or measurement.

Temperature: The sensitive part of the Extech DO 700 thermometer (probe) was immersed directly into the routine distilled water without any reagent or solution and allowed to stabilize. At stability, the temperature value was read and recorded. Three separate readings were taken

and the mean values of the three were calculated and recorded as the water temperature before the addition of the solutions. The same procedure was done for the test solutions of varying concentrations.

Hydrogen ion concentration: The instrument was first calibrated with the standard Extech DO 700 pH calibration solution. The measurement was done immediately after standardization by dipping the pH probe directly into the water or solution. The switch button of the instrument was put on while the arrow key will be moved to pH command displaying the values. After the value had stabilized, the reading was taken. The process was repeated three times and the average value was taken. The same process was applied to all the solutions.

Electrical Conductivity: The same procedure used to measure pH was adopted but the arrow key was positioned at electrical conductivity parameter. When the instrument stabilization was completed, the value will be taken and recorded after the calculation of the mean value.

Salinity: The same procedure used to measure pH was adopted but the arrow key was adjusted to salinity parameter. The instrument was rinsed properly several times with distilled water at each test before measurements were taken, this ensured accurate readings. The instrument was allowed to standardize for about 20 minutes before salinity readings were taken, calculated and recorded in parts per thousand (ppt) or percentage (%).

Dissolved Oxygen: The sensitive part of the Extech 700 DO Probe was immersed directly into water or solution and allowed to stabilized. At stability, the reading was recorded. Three readings were then taken and the mean value of the three were calculated and recorded as the DO levels of the solution (test).

Total Dissolved Solids: Total dissolved solids of the solution and control was carefully measured with the use of the same instrument after the instrument had been standardized with reagent and distilled water then rinsed with the test solution of the particular samples to be measures. The probe was dipped directly into the solution (test) and allowed to stabilize at turbidity parameter before the value would be taken and recorded.

Data analysis: The data generated were tested with one-way analysis of variance (ANOVA) by using Microsoft Excel. The recorded and observed values were used to determine the corrected mortality using Abbott's formula as well as $LC_{50,}$ the generated data provided values used for the log probit scales representing concentrations versus percentage of mortality to establish regression lines.

Results

The findings from this study shows the effect of the Eosin on the larva susceptible to various concentrations of the solutions. A high mortality rate of the larva occurred generally within day 2 to day 5 after their exposure to direct sunlight in the solution containing highest concentrations in

the three solutions and a moderate concentration in others. Some of the smaller (L3) larva in the control were observed to increase in size into the mature larva (L4). The table 1 shows that as the concentration of the eosin increased, so also did the observed percentage mortality of the *Anopheles* mosquito larvae increase from 50%, 70%, 85% and 95% for the 200µl, 400µl, 600µl and 800µl respectively while the 1000µl concentration had a 100% mortality of the larvae. The percentages observed mortality were corrected with the aid of the control using the Abbott's formula. The probit and log concentrations were used to determine the LC_{50} as 2.39µl/ml.

Table 1: Percentage mortality and Probit values of *Anopheles* Mosquito Larvae in Eosin concentrations exposed to sunlight

Concentration	Log Concentration	Percentage observed Mortality	Percentage corrected Mortality	Probit
200µl	2.30	55	50.00	5.00
400µl	2.60	70	66.67	5.41
600µl	2.78	85	83.33	5.95
800µl	2.90	95	94.44	6.55
1000µl	3.00	100	100.00	7.33
Control	--	10	--	--

Lethal Concentration 50 (LC_{50}) —2.39µl/ml

The tables 2a and 2b are showing the obtained results of the physicochemical properties for the 1000µl concentration and the control water used as the source of solvent and eosin solutions before and after exposure to sun light. The values in table 2a is before exposure to sun light and has pH 5.24, dissolved oxygen 6.5mg/L, temperature 30.4^{O}C, whereas the conductivity value was168µS/cm and total dissolved solids was 118 mg/L, while the physicochemical parameter values obtain for control after exposure to sun light in table 2b remained the same just like that recorded in table 2a when they were compared. The obtained values for the eosin solutions are as presented in tables 2a and 2b, and are showing that there were different values obtained for pH, conductivity, salinity, temperature, total dissolved solids and the dissolved oxygen before and after exposure to sunlight.

The tables 3a, 4a, 5a and 6a are indicating the various physicochemical properties for the 800µl, 600µl, 400µl, 200µl concentrations of eosin solutions and control water before exposure to sun light, while tables 3b, 4b, 5b and 6b are the eosin solution and control water after their exposure to sunlight and introduction of the mosquito larvae. The determined and obtained results are as presented in the tables showing that the values of the parameters increased in the different

concentrations of the eosin solution after exposure to the sunlight. It was noted that the values obtained for the dissolved oxygen was low after the eosin solution was exposed to sunlight just like other parameters

Table 2a: Physicochemical Parameters of Control Water and 1000µl concentrations at Day One (1) Before Exposure to Sun Light

	Parameters	pH	Conductivity (µS/cm)	Salinity ($^{o}/_{oo}$)	Temperature (^{O}C)	TDS (mg/L)	Dissolved Oxygen (mg/L)
1	Eosin	5.98	65.2	0.03	29.3	46.2	6.5
2	Control	5.24	168	0.08	30.4	118	6.5
3	WHO References	6.5-8.5	200-400	0.05-0.09	33	>600	6.5-8.0

Key:

WHO- World Health Organization

TDS- Total Dissolved Solids

Table 2b: Physicochemical Parameters of Control Water and 1000µl Concentrations at Day Six (6) After Exposure to Sunlight

	Parameters	pH	Conductivity (µS/cm)	Salinity ($^{o}/_{oo}$)	Temperature (^{O}C)	TDS (mg/L)	Dissolved Oxygen (mg/L)
1	Eosin	6.35	74.5	0.06	30.6	49.5	3.9
2	Control	5.24	168	0.08	30.4	118	6.5
3	WHO References	6.5-8.5	200-400	0.05-0.09	33	>600	6.5-8.0

Key:

WHO- World Health Organization

TDS- Total Dissolved Solids

Table 3a: Physicochemical Parameters of Control Water and 800µl concentration at Day One (1) Before Exposure to Sun Light

No.	Parameters	pH	Conductivity (µS/cm)	Salinity ($^{o}/_{oo}$)	Temperature (^{O}C)	TDS (mg/L)	Dissolved Oxygen (mg/L)
1	Eosin	5.92	63.4	0.03	29.3	44.7	6.3
2	Control	5.24	168	0.08	30.4	118	6.5
3	WHO References	6.5-8.5	200-400	0.05-0.09	33	>600	6.5-8.0

Key:

WHO- World Health Organization

TDS- Total Dissolved Solids

Table3b: Physicochemical Parameters of Control Water and 800µl concentrations at Day Six (6) After Exposure to Sun Light

	Parameters	pH	Conductivity (µS/cm)	Salinity ($^{o}/_{oo}$)	Temperature (^{O}C)	TDS (mg/L)	Dissolved Oxygen (mg/L)
1	Eosin	6.30	70.2	0.04	32.4	47.7	4.0
2	Control	5.24	168	0.08	30.4	118	6.5
3	WHO References	6.5-8.5	200-400	0.05-0.09	33	>600	6.5-8.0

Key:

WHO- World Health Organization

TDS- Total Dissolved Solids

Table 4a: Physicochemical Parameters of Control Water and 600µl concentration at Day one (1) Before Exposure to Sun Light

	Parameters	pH	Conductivity (µS/cm)	Salinity ($^{o}/_{oo}$)	Temperature (^{O}C)	TDS (mg/L)	Dissolved Oxygen (mg/L)
1	Eosin	5.96	54.6	0.02	29.6	45.8	6.5
2	Control	5.24	168	0.08	30.4	118	6.5
3	WHO References	6.5-8.5	200-400	0.05-0.09	33	>600	6.5-8.0

Key:

WHO- World Health Organization

TDS- Total Dissolved Solids

Table 4b: Physicochemical Parameters of Control Water and 600µl concentrations at Day Six (6) After Exposure to Sun Light

	Parameters	pH	Conductivity (µS/cm)	Salinity ($^{o}/_{oo}$)	Temperature (^{O}C)	TDS (mg/L)	Dissolved Oxygen (mg/L)
2	Eosin	6.26	69.8	0.04	31.0	47.7	4.0
4	Control	5.24	168	0.08	30.4	118	6.5
5	WHO References	6.5-8.5	200-400	0.05-0.09	33	>600	6.5-8.0

Key:

WHO- World Health Organization

TDS- Total Dissolved Solids

Table 5a: Physicochemical Parameters of Control Water and 400µl concentration at Day one (1) Before Exposure to Sun Light

Key:

WHO- World Health Organization

	Parameters	pH	Conductivity (µS/cm)	Salinity ($^{o}/_{oo}$)	Temperature (^{O}C)	TDS (mg/L)	Dissolved Oxygen (mg/L)
1	Eosin	5.83	65.2	0.03	28.8	44.8	6.3
2	Control	5.24	168	0.08	30.4	118	6.5
3	WHO References	6.5-8.5	200-400	0.05-0.09	33	>600	6.5-8.0

TDS- Total Dissolved Solids

Table5b: Physicochemical Parameters of Control Water and 400µl concentrations at Day Six (6) After Exposure to Sun Light

Key:

	Parameters	pH	Conductivity (µS/cm)	Salinity ($^{o}/_{oo}$)	Temperature (^{O}C)	TDS (mg/L)	Dissolved Oxygen (mg/L)
1	Eosin	6.30	57.6	0.03	31.2	48.9	4.1
2	Control	5.24	168	0.08	30.4	118	6.5
3	WHO References	6.5-8.5	200-400	0.05-0.09	33	>600	6.5-8.0

WHO- World Health Organization

TDS- Total Dissolved Solids

Table 6a : Physicochemical Parameters of Control Water and 200µl Concentration at Day one (1) Before Exposure to Sun Light

Parameters	pH	Conductivity (µS/cm)	Salinity ($^{o}/_{oo}$)	Temperature (^{O}C)	TDS (mg/L)	Dissolved Oxygen (mg/L)

	Parameters	pH	Conductivity (µS/cm)	Salinity ($^{o}/_{oo}$)	Temperature (^{O}C)	TDS (mg/L)	Dissolved Oxygen (mg/L)
1	Eosin	6.39	50.3	0.06	32.0	49.2	4.8
2	Control	5.24	168	0.08	30.4	118	6.5
3	WHO References	6.5-8.5	200-400	0.05-0.09	33	>600	6.5-8.0

Key:

WHO- World Health Organization

TDS- Total Dissolved Solids

Table 6b: Physicochemical Parameters of Control Water and 200µl concentrations at Day Six (6) After Exposure to Sun Light

1	Eosin	5.92	65.2	0.05	29.3	45.2	6.4
2	Control	5.24	168	0.08	30.4	118	6.5
3	WHO References	6.5-8.5	200-400	0.05-0.09	33	>600	6.5-8.0

Key:

WHO- World Health Organization

TDS- Total Dissolved Solids

Discussion

The results of the study reported in this work clearly indicates that, Eosin exposure to sunlight has a lethal effect on the larvae of *Anopheles* mosquito. This showed that with increasing concentrations of the solutions the mortality rate and subsequent metamorphosis of the larvae to a new stage of development decreased accordingly. The 1000µl concentration had a 100% observed mortality of the larvae just after 72 hours exposure to sunlight, with an LC_{50} of 1.51µl/ml gotten from the probit and log concentration analysis, the P-value of 0.003 which is less than 0.05 level of significance indicated that the concentrations of eosin were significantly different. The effect of the different concentrations of eosin solution on *Anopheles* mosquito larvae, agrees with the work by Alessandra *et al.*,[22], while studying the evaluation of eosin as a photosensitizer for larval control of *Aedes aegypti*, and concluded that eosin was phototoxic to *Aedes aegypti* larvae even at low concentration and presented a fast penetration time into the larvae and was able to induce a quick and efficient larval mortality using sunlight or irradiation from a white light source, but was non-toxic to the larvae in the dark. The Eosin was the second most effective solution on the *Anopheles* mosquito in the various concentrations, 100% mortality was also observed in the 1000µl concentration. Eosin had an LC_{50} of 2.39µl/ml which resulted in the mortality of more than 50% within 200µl to the 1000µl concentrations in 72 hours, which means that a dilution of 239µl in a 100ml of water can yield 50% mortality within 24 to 72hrs. In the research work by Hussein *et al.*, 2015 on the effect of some photosensitizing compounds on house fly (*Musca domestica*), the LC_{50} value in the insects treated with eosin measured 7×10^{-4}M showing that it had effect on the larvae, and his conclusion supports this study that observed eosin as the most effective dye on the *Anopheles* mosquito larvae. Each of the eosin concentrations differed significantly on the effect on *Anopheles* mosquito larvae exposed to sunlight at a P-value of 0.017 less than 0.05 level of significance.

Mosquito biochemical and physiological processes can be influenced by various factors such as ambient environmental temperature [23], pH, salinity, dissolved oxygen in the water body, total

dissolved solids, and conductivity [24]. Mosquitoes make use of stagnant water for breeding which can be conditioned by environmental factors. In these settings, the prevailing physicochemical factors play a crucial influence on mosquito survival and growth. The amount of dissolved oxygen available for the larvae's survival is determined by the ambient (environmental) temperature, which in turn is determined by the temperature fluctuation in their habitat.

The estimation of dissolved oxygen is important because it supports the survival of aquatic life and serves as a marker in the ecosystem dissolved oxygen which was found to be considerably reduced below the World Health Organization reference of 6-8.5mg/L after the days of exposure to sunlight.

Changes in the physicochemical parameter was observed in the dissolved oxygen values between the day one as seen in table 2a in comparison to day six in table 2b showing dissolved oxygen (DO) at 6.5mg/l in day one in the 1000µl concentration to 3.9mg/l in rapid reduction at day six in the same solution and concentrations as compared to the control values and the World Health Organization references which was observed in all the solutions of different concentration. The eosin increased the intensity as a photosensitizer by penetrating the larval internal organelles, hence it lead to a higher possibility of sunlight to trigger the singlet oxygen excited state causing an alteration of the morphological structure of the mosquito larvae hence causing rapid internalization of the dye leading to the altering of the physiological function of larva [22].These would have being a major cause in the quick reduction in the values of measured dissolved oxygen after exposure to sunlight. The efficacy of photoactive compounds as pesticides certainly depends also on feeding intensity and ingestion of the dye by a target insect [25]. According to Silberbush *et al*.,[26] they state that reduced levels of dissolved oxygen (DO) caused by changes in physicochemical determinants in the water resulted in reduced larval survival and prolonged development time. Also based on another view of excited state generation of molecules by Hussein *et al*., [25] they inferred that the photosensitive dyes with the greater number of the halogen atom substituents yield greater toxicity. Therefore, the effect is higher in dyes with a higher number of halogen atom substituents. As a result, the reactions are amplified by the halogen atoms. The amplification is caused by increased intersystem crossover from the dye's first excited singlet state to its first excited triplet state, allowing for more efficient interaction with oxygen molecules, eosin contains 4 bromine atoms as supported in the study by Fondren and Heitz, [27] , eosin Y contains only 4 bromine atoms [28].The interaction of the molecules causes a dysfunction of the cellular activities in the internal organelles of the *Anopheles* mosquito species larvae, hence mortality was recorded.

The study showed a decrease in salinity and conductivity at day six in the test solutions. According to a previous study by Embidi *et al*., [29], it was revealed that high salinity and conductivity were important factors for *Anopheles* larvae survival. *Anopheles* mosquitos have been shown to breed in clear, pH-balanced water. After six days, the pH in the test liquids had risen to a slightly alkaline level, according to the study. A study by Manilla and Frank [30],

reported that larvae of *Anopheles* mosquitos were more suited to slightly acidic pH. The increased pH may have contributed to the mortality rate of the mosquito larvae in association with the other physicochemical parameters such as the dissolved oxygen which was found to be considerably reduced below the World Health Organization reference of 6-8.5mg/L after the day six exposure to sunlight.

Although the temperature in both control and test solution were lower than the World Health Organization reference value of 33^{o}C, there was a more slightly relative temperature levels in the test solution. From the study, it is possible that the temperature was not a factor in the mortality of the mosquito larvae as it goes contrary to previous studies by Christiansen-Jucht *et al*. [32], Bayoh and Lindsay [33] reported that increasing environmental temperature (from 23°C to 37°C, 27°C to 39°C, and 32°C to 38°C) during the larval stages contributed to decreased larval survival because of marked changes in temperature of above 35^{O}C.

CONCLUSION

This research work has shown that photosensitizers like eosin an organic molecule that is a brilliant red dye may be used for the control of mosquito larvae, since there are different authorities that have put up the argument that mosquito control should include the application of larvicides as a technique, and this has been accepted into the management of disease transmission by vectors. The use of photosensitizers in the control of larvae of mosquitoes has become necessary since their products are environmentally friendly. Photosensitizers undergo different reaction pathways in reaching their final product stage, by either donating an electron to the substrate or abstracting a hydrogen atom from the substrate, as the photosensitizers triggers a physicochemical shift in a nearby molecule [33]. The photosensitizer gradually returns to its ground state, where it stays chemically unchanged until more light is absorbed. Singlet oxygen ($^{1}O_{2}$) can be generated by photosensitizer molecules that absorb ultraviolet (UV) and visible liht through photochemical reactions, and $^{1}O_{2}$ is an oxidizing agent that can cause oxidative damage to biomolecules when it reacts with them.

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