**The Potential Role of Photosensitizers in Fight against Mosquitoes: Phototoxicity of Rose Bengal Against *Culex Pipiens* Larvae**

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**Abstract**

Thecommon house mosquito, *Culex pipiens,* is abundant in Egypt and act as a vector of pathogens of medical and veterinary importance. The present study aimed to compare the toxic effect of rose bengal exposed to sunlight from 10 am to 4 pm a photosensitizer to that of chlorpyrifos, a commercially available larvicide, against the early 3rd larval instar of *Cx. pipiens*. Treatments revealed dose-dependent mortality, reaching 100% after treatment with rose bengal for 6 hrs and 90.6% for chlorpyrifos for 24 hrs. Six hours post-treatments, the LC50 of rose bengal and chlorpyrifos were 4.9x10-6 and 4.9x10-4, respectively; while the LC95 were 2.0x10-3 and 4.0x0-3, respectively. Based on the LC50 values of chlorpyrifos as a reference substance, rose bengal was found 100 times more potent than chlorpyrifos. The LT50 of rose bengal ranged from 34.8 to 1.1 hrs post-treatment with1×10-6 M and 1×10-2, respectively. The LT50 values of chlorpyrifos ranged from 3065.9 to 6.1 hrs after subjecting to 1×10-4 M and 1×10-3, respectively. It could be concluded that rose bengal could be used to prevent mosquito bites and their associated diseases as an alternative to traditional insecticides and an eco-friendly larvicide.

**Keywords**: photodynamic treatment, sunlight, larvicides. Mosquitoes, Egypt

1. **Introduction**

Dipterous insects cause serious public health problems for both humans and animals **(Edman,2009; Linthicum, 2012)**. *Culex pipiens,* thecommon house mosquitos (Diptera: Culicidae) is found in Egypt **(Ammar et al., 2012; Abdel-Shafie et al., 2016)** and applying synthetic pesticides **(Emtithal and Thanaa, 2012; El- Zayyat et al., 2017)** is mainly used for its control*. Cx. pipiens* acts as a vector of West Nile virus, Rift valley fever virus **(Conley et al., 2014),** Japanese encephalitis virus **(Campbell et al., 2011),** andfilariasis **(Joseph et al., 2011; Dyab et al., 2015; El-Naggar et al., 2017)**.

Botanical insecticides have long been used as the main weapons against insect pests before using of synthetic insecticides, developed in the mid- 1930s to 1950s **(Khater, 2012a, 2017).** Applying mosquito repellent is used for prevention form mosquito bites and its associated diseases (**Khater et al. 2019**). Synthetic insecticides are efficient, speedy, easily used, and inexpensive. Therefore, they replaced many natural control strategies like using botanicals, predators, and parasitoids. Repeated and inappropriate use of insecticides induced environmental contamination, toxicity to non-target organisms, development of pest resistance, and negative impact on animal and human health **(Khater, 2012a,b; Killeen et al., 2017; Khater et al. 2019).**

*Cx. pipiens* acquired resistance against insecticides in Egypt **(Zayed et al., 2006).** Consequently, there is an urgent need to explore and utilize safe alternatives for its control **(Shalaby and Khater 2005; Khater and Shalaby 2008).**

The biorational insecticides for mosquito control are preferable for environmental protection and public concerns because they have limited or no adverse effects on the environment, non- target organisms (**Murugan et al., 2015; Govindarajan et al., 2016a)** including humans. They include biochemicals insecticides (botanicals, insect growth regulators, insect pheromones, photoinsecticides, and inorganics); biological insecticides using of natural enemies as predators, nematodes, and pathogens (virus, bacteria, fungi, or protozoa); nanoinsecticides, and transgenic insecticides **(Khater, 2012b;** [**Roni**](http://www.sciencedirect.com/science/article/pii/S0147651315300129) **et al., 2015; Govindarajan** **et al., 2016b).** Most of them are effectively controlled the Egyptian strain of *Cx. pipiens* **(Khater 2003).**

Photodynamic processes are used in plants as chemical defense weapons against the attack by herbivorous insects, and the same strategy is used by parasitic fungi to help break plant cell walls **(Wainwright, 2009).** Such photodynamic action is an emerging strategy for control of multidrug-resistant microorganisms by producing singlet oxygen and/or reactive oxygen species (ROS) released from the interaction between photoactive compounds (photosensitizers) and light in the presence of molecular oxygen. Although photodynamic control (PDC) of mosquitoes was first explored in 1928 **(Barbieri, 1928),** PDC has started to regain its importance because of growing concerns about pesticide-resistant mosquitoes **(Ben Amor and Jori, 2000; Azizullah et al., 2014; Lucantoni et al., 2015; Souza et al., 2017).**

A photosensitizer accumulates within the pest body, and exposure to visible light induces lethal photochemical reactions and death of the organism **(Ben Amor et al., 1998a; Lukðienë et al., 2005; Khater and Hendawy, 2014; Khater et al., 2016).**

Halogenated xanthenes as rose bengal have proven to be effective photo-insecticides against several insect species (**Ben Amor and Jori, 2000).** The aims of the present study were comparing the toxic effect of rose bengal as a photosensitizer insecticide to that of chlorpyrifos, a commercially available conventional larvicide, against the early 3rd larval instars of *Cx. pipiens,* determination of lethal concentration (LC) and time (LT) values, and determination of the relative efficacy of rose bengal over chlorpyrifos.

1. **Materials and methods**

**2.1.Insect**

*Cx. pipiens* mosquitoes were obtained from the Research and Training Center on Vectors of Diseases, Faculty of Science, Ain Shams University, Cairo, Egypt, and reared in laboratory according to that of **Kasap and Demirhan (1992)** and modified by **Umaru and Akogun (2015)**.

**2.2.Materials**

Chlorpyrifos (Dursban) is a commercially available insecticide, obtained from AGRINE SERVE-Agricultural products, Giza, Egypt.

Rose bengal , Rosets, C20H4Cl4I4O5, is water-soluble pink dye, (4, 5, 6, 7-Tetrachloro-3', 6'-dihydroxy-2',4',5',7'-tetraiodo-3H-spiro[isobenzofuran-1,9'-xanthene]-3-one), obtained from LOBA Chemie, Mumbai, India.

**2.3.The light source and absorption spectra**

Sunlight was used as a source of illumination following the recommendation of **Khater and Hendawy (2014**) and **Khater et al., (2016)** during the period from 10 am to 4pm.

The absorption spectra of rose bengal were studied using UVVIS spectrometer (PG instruments Limited- Model 80+). The absorption occurred at wavelengths ranged from 450 to 600 nm corresponding to the visible region (Vis). The maximum absorption occurred at 536 nm which, equivalent to the green spectrum; whereas the weakest absorption occurred at the 302 nm wavelength, corresponding to the Ultraviolet (UV) region (Fig.1).

**2.4.Bioassys**

A preliminary study was performed to define the range of concentrations. Bioassy was done according tothat of the **WORLD HEALTH ORGANIZATION (1981).** Early 3rd larval instars of *Cx. pipiens* were used in this study. Four molar concentrations of each material were solved in dechlorinated water. Each concentration of the tested materials together with control groups were replicated three times, 25 larvae were used per replicate. Larvae were divided into four groups (Grs) as follows:

Gr. 1: Larvae were treated with rose bengal in different concentrations, 1x10-2, 1x103, 1x10-4, and 1x10-6, and exposed to sunlight from 10 am to 4 pm.

Gr. 2: Larvae were treated with chlorpyrifos in different concentrations, 1×10-4, 3×10-4, 6×10-4, and 1×10-3, and exposed to sunlight as in group (1).

Gr. 3: Larvae were treated with rose bengal at concentrations applied in Group but kept in dark.

Gr. 4: Larvae were not treated and exposed to sunlight as Gr. 1.

Larval mortalities were recorded after 2, 4, 6, and 24 hours post-treatments.

**3.Data analysis**

Z test of two proportions was used to assess the significance among different concentrations at each time using Microstat software. Multiple comparisons were done (P=0.0083) according to that of **Turner and Thayer (2001).**

Mortality data were subjected to Probit analysis **(Finney, 1952)** using the computer program Biostat (2009) for calculation of the lethal concentration (LC) values as well as of the lethal time (LT) values. The relative potency of rose bengal and chlorpyrifos were calculated according to the following formula and that of **Zidan and Abdel-Mageed (988)**

*Relative potency= LC value of chlorpyrifos /LC value of rose bengal*

**4.Result**

Rose bengal absorbs sunlight at three regions including the Ultraviolet, Visible, and Infrared. The riches of sunlight occur in the visible region and the absorption spectra of rose bengal involved it. The intensity of the spectra from the sunlight is increasing during the period from 10 am to 4 pm.

Treatment of 3rd larval instars of *Cx. pipiens* with rose Bengal and chlorpyrifos revealed dose-dependent mortality, reaching 100% after treatment with rose bengal for 6 hrs and 90.6% for chlorpyrifos for 24hrs. The present study indicated that there were no mortalities among larvae treated with different rose bengal concentrations and kept in the dark (Gr.3). Exposure of larvae to sunlight without treatment in the control light group (Gr.4) induced no mortalities among larvae (Table1).

Six hours post-treatments, the LC50 (lethal concentration, 50%) values of rose bengal and chlorpyrifos were 4.9x10-6 and 4.9x10-4, respectively; while the LC95 (lethal concentration, 95%) were 2.0x10-3 and 4.0x0-3, respectively. Based on LC50 values of chlorpyrifos as a reference substance, rose bengal was found 100 times more potent than chlorpyrifos (Table 2).

The LT50 (the times required to kill 50%) values of rose bengal ranged from 34.8 to 1.1 hrs post-treatment with1×10-6 M and 1×10-2, respectively. The LT50 values of chlorpyrifos ranged from 3065.9 to 6.1 hrs after subjecting to 1×10-4 M and 1×10-3, respectively (Table 3).

**5. Discussion**

In Egypt, resistance strains of *Cx. pipiens* larvae to insecticides were reported against Organophosphates **(Zayed et al., 2006)** and a bacterial agent **(Saleh et al., 2003),** hence it is very important to use alternative control strategies against it as photosensitizers. rose bengal showed a highly toxic effect against larvae of *Cx. pipiens*. 100 % larvicidal effects were reached 6 hrs post-treatment at concentration 1x 10-2 M, chlorpyrifos was found to be less toxic than rose bengal.

In agreement with the present results, rose bengal was the highly effective dye against instar larvae of *Cx. pipiens* (**Carpenter et al., 1984; El-Shourbagy et al., 2018);** *Aedes triseriatus* **(Carpenter et al., 1984),** *Aedes* *aegypti*, *Anopheles stephensi*, and *Culex* *quinquefasciatus* **(Dondji et al., 2005).** Xanthene derivatives (a mixture of Rose bengal and Erythrosin) applied under artificial irradiation were the most efficient agents for *Anopheles* and *Aedes* larval control **(Barbieri, 1928).** Xanthene, chlorin, and porphyrin derivatives also exhibited larvicidal activity *on Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* after photoactivation **(Dondji et al., 2005).** In the same trend, a variety of photosensitizers are efficient mosquito larvicides (**Amor and Jori, 2000; Azizullah et al., 2014; Lucantoni et al., 2015; Souza et al., 2017).**

Rose bengal is also effectively controlled the other pests as the adult house fly, *Musca domestica* **(Fondren et al., 1979; Tawfik et al., 2015);** engorged females of *Hyalomma dromedarii* **(Khater and Hendawy, 2014);** andthe 4th larval instars of *Spodoptera* *littoralis* (**El-Ghobary****el al., 2018)**. After the addition of a specific hydrocarbon, rose bengal effectively controlled different stages of the onion fly, *Hylemyia antiqua* **(Aref, 2010).**

Our data indicated that rose bengal quickly killed larvae in the present study as indicated by the LT values. It excepted similar results against ticks **(Khater and Hendawy 2014).** Rose bengal was 100 times more effectual than that of chlorpyrifos, 6 hrs post-treatment. Similar relative potency over pesticides was reported **(Ahmed et al., 1985; Khater and Hendawy 2014; Khater et al. 2016).**

The results in the present work revealed that exposure to sunlight alone (Gr. 4) and exposure to Rose bengal in the dark (Gr. 3) are not lethal to mosquito larvae. These results come along with that recommendation by **Khater and Hendawy (2014) and Khater et al**. (2016) of using sunlight instead of a light source. photo**t**oxicity occurs at the cellular level with the dye acting as a catalyst for the generation of singlet oxygen molecules leading to initiation of oxidation reactions that destroy several target molecules in the cell. To induce larval death byoxidative stress, a good photosensitizer (PS) should have efficient singlet oxygen or strong ROSgeneration **(Downum and Wen, 1995; Hamblin, 2016).**

The toxicity mechanisms triggered lethal energy stress by a photodynamic sensitizer against arthropods were summarized by **Ben Amor and Jori (2000)** as follows:damaging the membranes of the midgut wall, alteration of the potassium levels in the hemolymph indicating changes in the membrane permeability, and physiological and morphological abnormalities at the larval, pupal, and adult stages affecting development and fecundity. More importantly, pests do not acquire resistance against photoactive compounds **(Lukðienë et al., 2005; Almeida et al. 2009).**

Concerning the safety of photosensitizers, they are inactive in the dark and do not accumulate because they are degraded by light. Sunlight-activated compounds are characterized by a low environmental impact and insignificant toxicological risk for humans, animals, or plants, **(Ben Amor et al., 1998a).**

Besides their pesticidal effect, photosensitizers have been shown to act as very efficient photodynamic agents against a broad number of microbial pathogens, including bacteria, fungi, and protozoa **(Decraene et al., 2006; Baptista and Wainwright, 2011)**. This property has promising applications as water and blood disinfectants besides mosquito control **(Baptista and Wainwright, 2011).** Photodynamic processes are used as food additives or therapeutic agents **(Ben Amor et al., 1998a)** andaddress environmental problems such as the decontamination of wastewaters, the disinfection of fish-farming tanks, and protection of nontarget aquatic creatures as amphibians and reptiles form pathogens **(Jori et al., 2011).**

**6. Conclusion**

It could be concluded that rose bengal is highly effective when used at lower concentrations and short exposure times when compared to those of chlorpyrifos. Sunlight is an essential factor for the activation of rose Bengal; consequently, it is recommended to subject photosensitizers to sunlight at the regions from 300 nm to 600 nm for future work. Rose bengal could be applied to prevent mosquito bites and their associated diseases as an alternative to traditional insecticides and an eco-friendly larvicide.

**7. Acknowledgments**

Our sincere gratitude is given to Prof. Dr. Nabil Hendawy, Physics Department, Faculty of Science, Benha University, Egypt, for his fruitful assistance with the physics part of this study.

**N.B:** This work is a part of the PhD thesis submitted by Heba M Aboelela, Department of Parasitology, Faculty of Medicine, Benha University, Benha, Egypt

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