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

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Abstract:

Background: The common house mosquito, *Culex pipiens*, is abundant in Egypt and act as a vector of pathogens of medical and veterinary importance. **Aim:** The present study aimed to compare the toxic effect of rose bengal a photosensitizer to that of chlorpyrifos, a commercially available larvicide against the early 3rd larval instar of *Cx. pipiens*. **Methods:** We compare the toxic effect of rose Bengal exposed to sunlight from 10 am to 4 pm to that of chlorpyrifos, against the early 3rd larval instar of *Cx. pipiens*. **Results:** 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. **Conclusion:** 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

Introduction

Dipterous insects cause serious public health problems for both humans and animals. *Culex pipiens,* the common house mosquitos (Diptera: Culicidae) is found in Egypt and applying synthetic pesticides is mainly used for its control. *Cx. pipiens* acts as a vector of West Nile virus, Rift valley fever virus**,** Japanese encephalitis virus**,** and filariasis (1).

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**.** Applying mosquito repellent is used for prevention form mosquito bites and its associated diseases. 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 (2)**.**

Cx. pipiens acquired resistance against insecticides in Egypt**.** Consequently, there is an urgent need to explore and utilize safe alternatives for its control (3).

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 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. Most of them are effectively controlled the Egyptian strain of *Cx. pipiens* (4).

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. 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**,** PDC has started to regain its importance because of growing concerns about pesticide-resistant mosquitoes (5)**.**

A photosensitizer accumulates within the pest body, and exposure to visible light induces lethal photochemical reactions and death of the organism (6).

Halogenated xanthenes as rose bengal have proven to be effective photo-insecticides against several insect species (7)**.** 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.

Materials and methods

This clinical trial was done during the period from January 2019 to September 2019, after approval of the ethical committee of Benha Faculty of medicine.

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** (8) and modified by **Umaru and Akogunma** (9)**.**

Materials

Chlorpyrifos (Dursban) is a commercially available insecticide, obtained from AGRINE SERVE-Agricultural products, Giza, Egypt. Rose bengal , Rosets, $C_{20}H_4Cl_4I_4O_5$, 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.

The light source and absorption spectra

Sunlight was used as a source of illumination following the recommendation of **Khater et al.,** (6) 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).

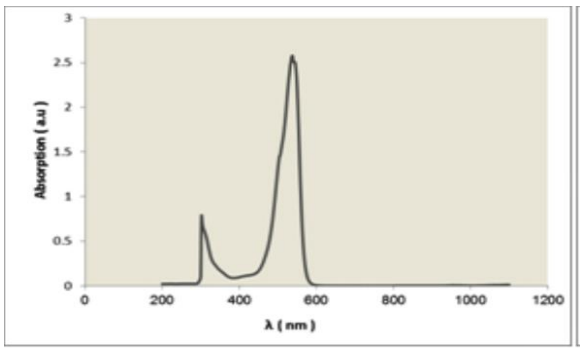

Fig.1: The absorption spectra of rose Bengal.

Bioassys

A preliminary study was performed to define the range of concentrations. Bioassy was done according to that of the **WORLD HEALTH ORGANIZATION** (10)**.** 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, $1x10^{-2}$, $1x10^{3}$, $1x10^{-4}$, 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.

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** (11)**.**

Mortality data were subjected to Probit analysis (12) 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** (13).

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

Results

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).

Table (1): The efficacy of tested compounds against early 3rd instar larvae of *Cx. pipiens.*

Groups (Grs) and Tested Compounds	Concs. (M)	Mortality (%)			
		2	**4**	**6**	**24**
	$1\ X10^{-6}$	10.7	21.3	37.3	38.7
Gr. (1): Rose bengal	$1\ X10^{-4}$	37.3c	49.3c	70.7c	74.7c
	$1\ X10-^{3}$	62.7ce	73.3ce	94.7ce	98.7ce
	$1\ X10-^{2}$	81.3ce	97.3cef	100.0ce	100ce
Group (2): Chlorpyrifos	1×10^{-4}	4	13.3	16	20
	3×10^{-4}	6.6	14.6	22.6	56e
	6×10^{-4}	25.3eg	36eg	56eg	77.3e
	1×10^{-3}	30.6eg	66.6egh	77.3eg	90.6eg
Group (3): Rose Bengal in dark.	$1\ X10^{-6}$	0	0	0	0
	$1\ X10^{-4}$	0	0	0	0
	$1\ X10-^{3}$	0	0	0	0
	$1\ X10-^{2}$	0	0	0	0
Group (4): Control	0	0	0	0	0

Concs: means concentrations

c= sig in comparison with $1X10^{-6}$, e= sig in comparison with $1X10^{-4}$, f= sig in comparison with $1X10^{-3}$, g= sig in comparison with $3X10-^{4}$, h= sig in comparison with $6X10^{-4}$, Multiple comparisons were done at adjusted P=0.0083.

Larvae were treated and exposed to sunlight from 10am to 4pm except those in Gr. 3

Table (2): Lethal concentration (LC) values (M) of rose Bengal and chlorpyrifos against early 3rd instar larvae of *Cx. pipiens.*

Tested compounds	Time/ PT (hour)	LC_{50} (Confidence limits)	LC_{95} (confidence limits)	LC_{99} (confidence limits)	Slope
Rose bengal	**2**	$2.7X10^{-4}$ ($1.4X10^{-4}$ - $5.4X10^{-4}$)	$3.0X10^{-1}$ ($7.2X10^{-2}$ - 2.5)	5.4 (0.8 – 100)	0.541 ±0.062
	4	$4.7X10^{-5}$ ($3.9X10^{-10}$ - $2.1X10^{-3}$)	$3.3X10^{-2}$ ($1.1X10^{-3}$ - $2.3X10^{-1}$)	$4.9X10^{-1}$ ($5.7X10^{-3}$ - $7.0X10^{11}$)	0.579 ±0.061
	6	$4.9X10^{-6}$ ($7.0X10^{-11}$ - $07.0X10^{-5}$)	$2.0X10^{-3}$ (1.2X10-4 - 3.9X10-1)	$2.5X10^{-2}$ (6.7X10-4 - $7.7X10^{12}$)	0.629 ±0.070
	24	$3.7X10^{-6}$ ($5.8X10-^{19}$ - $8.0X10^{-5}$)	$9.2X10^{-4}$ ($5.0X10^{-5}$ - $9.8X10^{-1}$)	$9.1X10^{-3}$ ($2.4X10^{-4}$ - $2.0X10^{13}$)	0.686 ±0.077
Chlorpyrifos	**2**	$4X10^{-3}$ ($3.2X10^{-2}$ - $1X10^{-2}$)	$1.2X10^{-1}$ ($4X10^{-2}$ -$3X10^{-1}$)	$5X10^{-1}$ ($2X10^{-1}$ - $15X10^{-1}$)	0.541 ±0.062
	4	$1X10^{-3}$ ($1.1X10^{-6}$ -$2X10-^{3}$)	$1.2X10^{-2}$ ($6X10^{-3}$ - $2X10^{-2}$)	$3X10^{-2}$ ($1X10^{-2}$- $7X10^{-2}$)	0.579 ±0.061
	6	$4.9X10^{-4}$ ($1.X10^{-5}$ -$1X10^{-3}$)	$4X10^{-3}$ ($3X10^{-3}$- $8X10^{-3}$)	$1X10^{-2}$ ($6X10^{-3}$- $1.9X10^{-2}$)	0.629 ±0.070
	24	$2.5X10^{-4}$ ($2.1X10^{-4}$ - $3X10^{-3}$)	$1.5X10^{-3}$ ($1.1 X10^{-3}$ – $2.4X10^{-3}$)	$3.2X10^{-3}$ ($2.1X10^{-3}$ -$6.1X10^{-3}$)	0.686 ±0.077
RE [1]		**14.8**			
RE [2]		**21.3**			
RE [3]		**100**			
RE [4]		**67.5**			

PT means post treatment

[1]Ratio of chlorpyrifos LC50: ratio of rose Bengal LC50 2hrs PT.

[2]Ratio of chlorpyrifos LC50: ratio of rose Bengal LC50 4hrs PT.

[3]Ratio of chlorpyrifos LC50: ratio of rose Bengal LC50 6hrs PT.

[4]Ratio of chlorpyrifos LC50: ratio of rose Bengal LC50 24hrs PT.

Table (3): Median lethal time (LT_{50}) value, (per hour) and its 95%confidence limits of rose bengal and chlorpyrifos.

Tested compounds	Conc. (M)	LT_{50} (hours)	95% CI
Rose bengal	1×10^{-6}	34.8	10.9- 111.1
	1×10^{-4}	3.4	1.2- 9.3
	1×10^{-3}	1.7	1.0- 2.7
	1×10^{-2}	1.1	0.7- 1.8
Chlorpyrifos	1×10^{-4}	3065.9	239.1- 39317.5
	3×10^{-4}	583.6	77.5- 4392.0
	6×10^{-4}	18.2	9.7- 34.1
	1×10^{-3}	6.1	3.0- 12.4

Discussion

In Egypt, resistance strains of *Cx. pipiens* larvae to insecticides were reported against Organophosphates (14) and a bacterial agent (15)**,** 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* (16)**;** *Aedes triseriatus* (17)**,** *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* (18)**.** Xanthene derivatives (a mixture of Rose bengal and Erythrosin) applied under artificial irradiation were the most efficient agents for *Anopheles* and *Aedes* larval control (19)**.** Xanthene, chlorin, and porphyrin derivatives also exhibited larvicidal activity *on Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* after photoactivation (18)**.** In the same trend, a variety of photosensitizers are efficient mosquito larvicides (5)**.**

Rose bengal is also effectively controlled the other pests as the adult house fly, *Musca domestica* (20)**;** engorged females of *Hyalomma dromedarii* (21)**;** and the 4th larval instars of *Spodoptera littoralis* (22). After the addition of a specific hydrocarbon, rose bengal effectively controlled different stages of the onion fly, *Hylemyia antiqua* (23)**.**

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 (21)**.** Rose bengal was 100 times more effectual than that of chlorpyrifos, 6 hrs post-treatment. Similar relative potency over pesticides was reported (6).

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 et al**. (2016) of using sunlight instead of a light source. Phototoxicity 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 by oxidative stress, a good photosensitizer (PS) should have efficient singlet oxygen or strong ROS generation (24)**.**

The toxicity mechanisms triggered lethal energy stress by a photodynamic sensitizer against arthropods were summarized by **Ben Amor and Jori** (7) 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 (25)**.**

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, (7)**.**

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 (26). This property has promising applications as water and blood disinfectants besides mosquito control (26)**.** Photodynamic processes are used as food additives or therapeutic agents and address 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 (27)**.**

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.

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