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The photosensitizing activity of different photosensitizers irradiated with sunlight against aquatic larvae of *Culex pipiens* L. (Diptera: Culicidae)

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ABSTRACT

The response of *Culex pipiens* early 3rd instar larvae to three photosensitizing dyes (Toluidine blue O, Methylene blue, and Rose Bengal) was studied under laboratory conditions. Despite lack of toxicity in dark and with exposure to only sunlight, *C. pipiens* third instar larvae were highly sensitive to the three tested photosensitisers with 20 minutes of sunlight irradiation. Larvae showed the most sensitivity to Rose Bengal (RB) with LC_{50} values of 1.07, 1.19, 1.35, 1.65µM, and LC_{90} values of 3.88, 3.98, 4.19, and 4.51µM. Also, less sensitivity to methylene blue (MB) was recorded with LC_{50} values of 2.70, 2.79, 2.99, 3.08µM and LC_{90} values of 4.82, 4.90, 5.04, and 5.22µM. In addition, the least sensitivity was recorded by larvae towards toluidine blue o (TBO) with LC_{50} values of 3.30, 3.39, 3.58, and 3.87µM and LC_{90} values of 5.23, 5.32, 5.57, and 5.96 µM, respectively. Results showed that photosensitizers could be an excellent replacement for the traditional insecticides avoiding their negative impact on the environment.

INTRODUCTION

The domestic mosquito *Culex pipiens* L. is one of the most dangerous vectors that carry several pathogens for humans **(Hassan *et al*., 2014; Hasaballah and El-Naggar, 2017).** It is very common in Egypt and acts as a vector of many diseases such as West Nile virus, valley fever virus, and filariasis **(El-Naggar *et al*., 2017; El-Naggar and Hasaballah, 2018).** Globally, the negative impact caused by *C. pipiens* on human health exceeds the impact of other different mosquito species **(Goddard *et al*., 2002).** So, controlling *C. pipiens* is a paramount strategy for the prohibition of the spread of diseases and epidemic outbreaks **(Elango *et al*., 2009).**

For several years, *C. pipiens* immature stages usually targeted by synthetic compounds and the public health units are often face the challenge of balancing between the hazard of infection and other hazards of these synthetic compounds on humans, the environment, as well as other living organisms **(Shapiro and Micucci, 2003).** From there, the need to use a new strategy for controlling *C. pipiens* with more effectiveness and less negative impact on the environment and human health had elevated.

Photosensitizing agents, which are activated by sunlight, attracted more attention as a new generation of insecticide that is highly efficient and environmentally safe due to its rapid photo-degradation in the visible light **(Ben Amor and Jori, 2000).** Photoinsectisides have been used in agriculture against crop pests **(Moreno *et al*., 2001).** Photodynamic therapy (PDT) is used clinically to treat a wide range of medical conditions, including psoriasis, atherosclerosis and has shown some efficacy in anti-viral treatments, including herpes. It also treats malignant cancers including head and neck, lung, bladder, and particular skin **(Saini, *et al*., 2016).** Also, this technology has been tested for the treatment of prostate cancer in a dog model and in human prostate cancer patients **(Swartling *et al*., 2010; Swartling *et al*., 2016).** In addition, PDT is recognized as a treatment strategy that is both minimally invasive and minimally toxic **(Lazaro-Carrillo *et al*., 2018).**

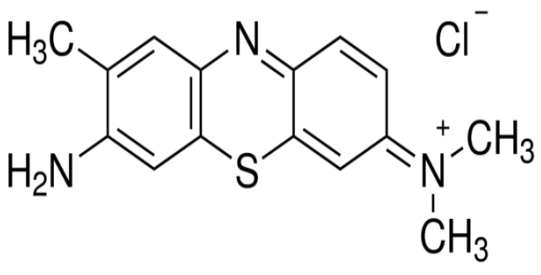
The mechanism of PDT depends on involving light source and a photosensitizing chemical substance, is used in conjunction with molecular oxygen to elicit cell death by the formation of reactive oxygen species (ROS) **(Saini *et al*., 2016).** Photodynamic therapy is a multi-stage process; first, a photosensitiser with negligible dark toxicity is administered, either systemically or topically, in the absence of light. When a sufficient amount of photosensitiser appears in diseased tissue, the photosensitiser is activated by exposure to light for a specified period. The light dose supplies sufficient energy to stimulate the photosensitiser, but not enough to damage neighbouring healthy tissue. The reactive oxygen kills only target cells **(Chen *et al*., 2002).**

The current study aimed to compare the phototoxicity of Toluidine blue O, Methylene blue, and Rose Bengal as photosensitized dyes against *C. pipiens* larvae.

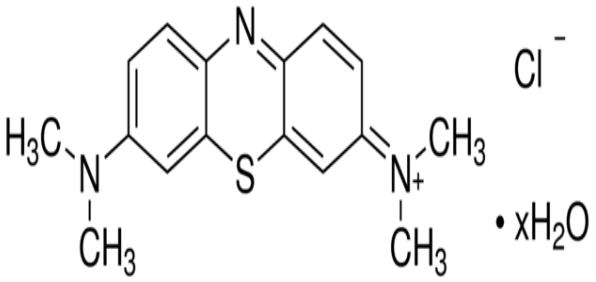
MATERIALS AND METHODS

1. Photosensitizers and Light Sources:

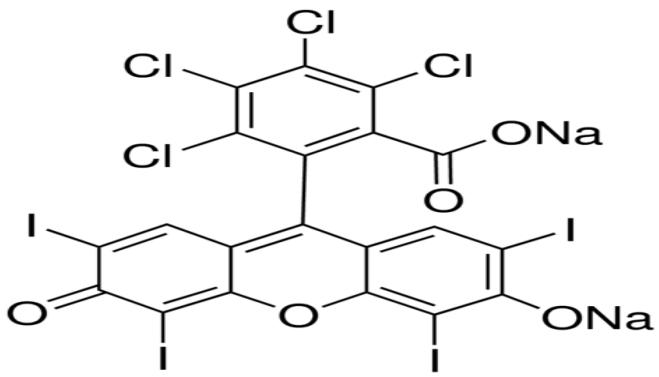
Three photosensitizers had been tested against *Culex pipiens* third larval instar, toluidine blue o (TBO), methylene blue (MB), and rose bengal (RB) with molecular weights of 305.83, 319.85, and 1017.64. All photosensitizers were purchased from Sigma-Aldrich Company, Egypt. The concentrations of 1, 2, 3, 4, 5, and 6 µM were prepared using dechlorinated tap water for larvicidal application. Sunlight was used as a light source of irradiation for activation of all three photosensitizers.



Toluidine blue O (TBO)



Methylene blue (MB)



Rose Bengal (RB)

2. Mosquito culture:

Immature stages (larvae and pupae) of *Culex pipiens* mosquito were collected from the fish farm in Saint Makarios monastery, Beni Salama, Al-Natron Valley, El-Beheira, Egypt (30º17'29.9" N, 30º28'32.3' 'E). The collected Larvae were identified according to key described by **Harbach (1985)** and reared for six generations in Mosquito insectary, Animal house, Department of Zoology, Faculty of Science, Al-Azhar University using the standard procedure described by **Hassanain *et al.* (2019)** to provide larvae needed for the assay.

3. Detection of incubation time:

In order to achieve maximum accumulation of each tested photosensitizer inside larvae before irradiation, fifty early third instar larvae were placed in a beaker containing 500 ml of each tested photosensitizer (6µM) for different incubation times. After 2, 4, 6, and 24 hrs., ten larvae from each beaker were collected, washed extensively to remove the excess of photosensitizers and transferred into 15 cm Falcon tube containing 5 ml deionized water.

Later the falcon tubes were centrifuged for ten minutes at 1500 rpm, the supernatant was collected and their spectral absorbance was measured using UV-Vis spectrophotometer [BioTek power wave XS2 Ultraviolet-visible (UV-Vis) spectroscopy] at a specific wavelength for each photosensitiser 640 nm for Toluidine blue O, 670 nm for methylene blue and 575 for Rose Bengal. It turns out that the optimum incubation time is 6 hours, in order not to compromise the larval age.

4. Larvicidal bioassay:

Bioassay was carried out according to the procedure of the World Health Organization **(World Health Organization, 1996)** with minor modifications. Twenty larvae of *C. pipiens* early third instar were deprived of food for 6 hrs and then placed in 300 ml beakers containing 200 ml of different concentrations from each photosensitizer. Then, the larvae were allowed to feed on a small piece of bread for six hours in dark. After that, larvae were washed extensively to remove the excess photosensitisers and transferred into 200 ml of clean dechlorinated tap water for the irradiation process.

Control larvae represented in different concentrations from all tested photosensitizers in dark.

5. Irradiation process:

The irradiation process was carried out using sunlight for 20 min. from 12.00 to 12.20 am along with control groups (photosensitizers' free) to assess the impact of sunlight alone on larvae. Survived larvae were recorded subsequently right after radiation and after 24, 48, and 72 h. This process was done 3 replicate times and all values were calculated as Mean±SD (N=3).

6. Statistical analysis:

Probity analysis was applied to average survived larvae values for calculating LC_{50} and LC_{90} at 95% lower and upper confidence limits. All the statistical analyses were carried out using Statistical Package Social Science (SPSS) software version 11.5 (SPSS, 2007). Results represented as Mean±SD.

RESULTS

1. Detection of incubation time:

As shown from figure 1, the maximum accumulation of photosensitizers inside larvae recorded at 24 hrs however 24 hrs is too long time to incubate that would jeopardize the larval age, so the incubation time of choice was the second-best (6 hrs).

2. Larvicidal activity of photosensitisers:

The third larval instar of Culex pipiens showed high sensitivity to the tested three photosensitizers, toluidine blue o (TBO), methylene blue (MB), and rose bengal (RB) after 20 minutes of sunlight irradiation. Also, no toxicity was observed either in dark incubation with photosensitizers or with only sunlight exposure (Figure 2).

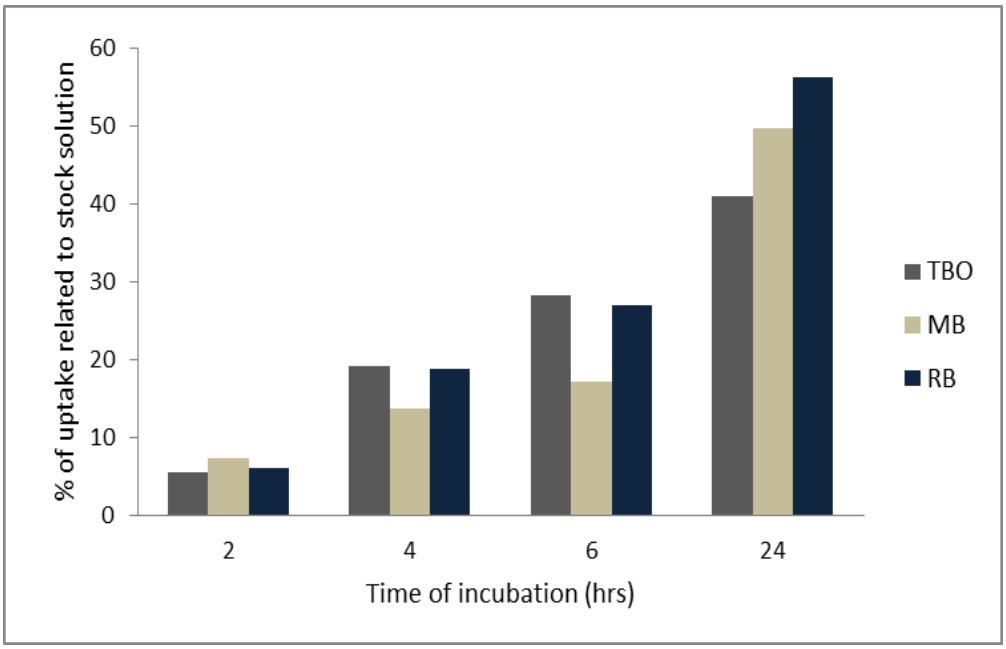


Fig. 1. Diagram shows different incubation times of toluidine blue o (TBO), methylene blue (MB), and rose bengal (RB).

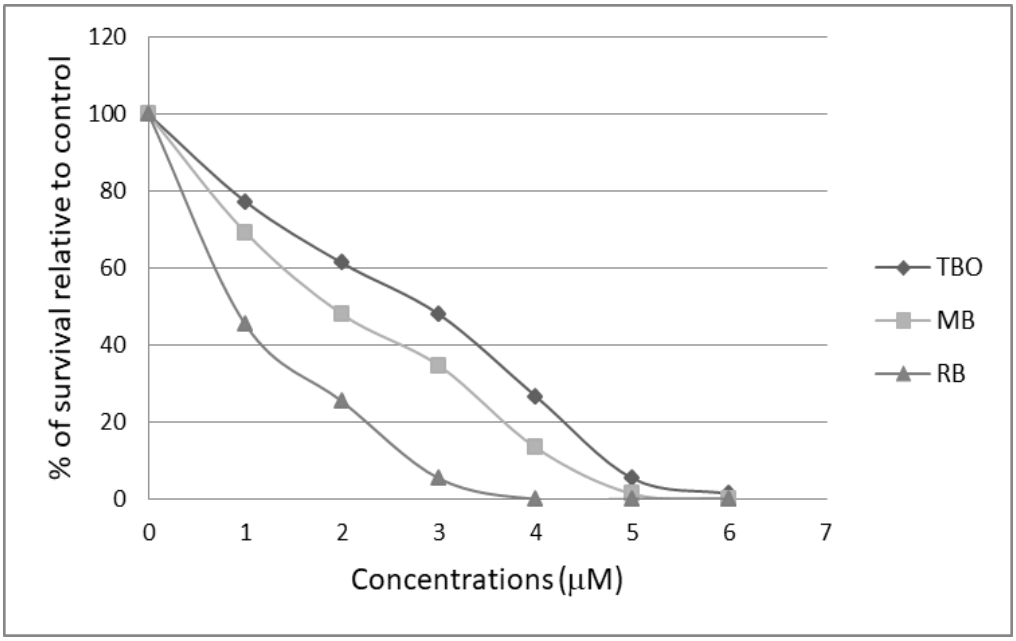


Fig. 2. Photodynamic treatment of larvae using different concentrations of Toluidine Blue O (TBO), Methylene blue (MB) and Rose Bengal (RB) after 20 min. Sunlight irradiation.

Larvae showed the most sensitivity to RB with LC_{50} values of 1.65, 1.35, 1.19, and 1.07 µM and LC_{90} values of 4.51, 4.19, 3.98, and 3.88 µM after 0, 24, 48, and 72 hrs. of radiation. Also, less sensitivity to MB was recorded by *C. pipiens* third larval instar with LC_{50} values of 3.08, 2.99, 2.79, and 2.70 µM and LC_{90} values of 5.22, 5.04, 4.90, and 4.82 µM after 0, 24, 48, and 72 hrs, respectively. In addition, larvae showed the

lowest sensitivity to TBO with LC_{50} values of 3.87, 3.58, 3.39, and 3.30 µM and LC_{90} values of 5.96, 5.57, 5.32, and 5.23 µM after 0, 24, 48, and 72 hrs, respectively (Table 1).

Table 1. Propit values (µM) of tested photosensitizers against *C. pipiens* 3th larval instar.

Photosensitizers	Time (hrs)	LC_{50} (µM)	95% Confidence limits (LCL-UCL)	LC_{90} (µM)	95% Confidence limits (LCL-UCL)*
Toluidine Blue O (TBO)	Right after radiation	3.87	(3.38-4.37)	5.96	(5.32-6.59)
	24	3.58	(3.21-3.96)	5.57	(5.14-5.99)
	48	3.39	(3.04-3.74)	5.32	(4.95-5.69)
	72	3.30	(2.87-3.73)	5.23	(4.79-5.67)
Methylene Blue (MB)	Right after radiation	3.08	(2.67-3.49)	5.22	(5.02-5.42)
	24	2.99	(2.50-3.49)	5.04	(4.88-5.19)
	48	2.79	(2.59-2.99)	4.90	(4.80-5.0)
	72	2.70	(2.45-2.95)	4.82	(4.68-4.96)
Rose Bengal (RB)	Right after radiation	1.65	(1.12-2.17)	4.51	(4.42-4.60)
	24	1.35	(0.59-2.11)	4.19	(4.0-4.37)
	48	1.19	(0.62-1.76)	3.98	(3.69-4.28)
	72	1.07	(0.15-1.98)	3.88	(3.71-4.05)

* LCL: lower confidence limits; UCL: upper confidence limits.

DISCUSSION

Control of pathogens and vectors largely relies on effective chemical compounds. Unfortunately, these problematic creatures have developed resistance to most of synthetic chemicalsm besides the adverse effects of chemicals on the environment and health . Therefore, there is a need to devise new techniques to eliminate these problematic creatures. Recently, the main goal for scientists is to use of natural products and ecofriendly solutions for eliminate these problematic creatures **(Ibrahim *et al*., 2017; Cui *et al*., 2020; Metwally *et al*., 2020; Attia *et al*., 2021)**. One of these ecofriendly solutions is photosensitizers irradiation.

The obtained results revealed that there was no lethal impact occurred on mosquito larvae with exposure to sunlight alone or exposure to photosensitizers in the dark and this comes along with that recommendation by **Khater and Hendawy (2014)** of using sunlight instead of a light source. Also, methylene blue (MB) and toluidine blue O (TBO) can be photoactivated with light sources of range (630-700) nm while light source ranging of (380-520 nm) is used to trigger the photosensitization reaction of Rose Bengal (RB). Both light sources fit of rages are located in visible of sun light. The previous photosensitizers introduce phototoxic effects by singlet oxygen ($1O_2$) generation through

Type-II mechanism (excited triplet state photosensitiser (3Psen*) directly react with ground state triplet molecular oxygen ($3O_2$) which is chemically reactive due to the presence of unpaired valence electrons **(Chen *et al*., 2002).** These highly cytotoxic singlet oxygen molecules initiate multisite attacks against the intracellular proteins and cellular membranes in cells **(Katritzky *et al*., 1996).**

On the other hand, RB is the most effective against *Culex pipiens* larvae and this may be attributed to its big molecular weight (1017.64) which is as twice as MB (319.85) and TBO (305.83); leading to more precipitation of RB dye crystals in larvae system with the same incubation time. The activity of tested photosensitizers is coupled with previous results recorded by **Dondji *et al*. (2005),** where different photosensitizers showed the lethal effect on *Aedes aegypti*, *Anopheles stephensi*, and *C. quinquefasciatus* fourth larval instar depending on the presence of light, however, rose bengal (RB) seemed to be more efficient at even lower concentration than other photosensitizers against *Ae*. *aegypti* larvae, **Azizullah *et al* (2014)** recorded that, chlorophyll derivatives natural photosensitizers can effectively use against the dengue vector, *Ae*. *aegypti* larvae, **de Souza *et al*. (2014)** where porphyrin (Photogem) in the presence of sunlight and fluorescent lamp recorded about 100.0% mortality in *Ae*. *aegypti* second larval instar after 24 hrs and **El- Shourbagy *et al*. (2018)** where RB was the most effective dye against *C. pipiens* fourth larval instar followed by phloxine B, then rhodamine B.

CONCLUSION

As obtained from the results, tested dyes Toluidine blue O (TBO), Methylene blue (MB), and Rose Bengal (RB) have outstanding activities against *Culex pipiens* larvae superior to traditional larvicides in efficacy with low concentrations, environmentally safe, and activated using natural sources as sunlight exposure. They can be used effectively to control mosquitoes and their associated diseases. In near future, we need to initiate more studies to test more photosensitizer agents against other different mosquito species.

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