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ORIGINAL PAPERS

Acaricide resistance and novel photosensitizing approach as alternative acaricides against the camel tick, *Hyalomma dromedarii*

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Abstract

The control of the camel tick, *Hyalomma dromedarii* is very crucial. This study evaluated the novel toxicity of photosensitizers and Phoxim insecticide against *H. dromedarii* males using the adult immersion tests. Ticks were subjected to sunlight for 10 min post-treatment (PT). The optical characters of the applied materials were determined by UV–Vis spectroscopy (250–900 nm wavelengths). The intensity of spectra decreased as dye concentration decreased. The optical bandgap energies of the dyes at different concentrations were not changed as the concentration changed and decreased as the absorption peak of individual dyes red-shifted. The mortalities 72 h PT reached 42.2%, 44.4%, 51.1%, 71.1%, 46.7%, 48.9%, 44.4%, and 55.6% for chlorophyllin, echinochrome, field stain, methylene blue, phthalocyanine, rhodamine 6G, riboflavin, and safranin, respectively. Methylene blue recorded the highest median lethal concentration (LC_{50} = 127 ppm) followed by safranin, field stain, rhodamine 6G, phthalocyanine, echinochrome riboflavin, and chlorophyllin (LC_{50} = 209, 251, 271, 303, 324, 332, and 362 ppm, respectively, 72 h PT). Their median lethal time, LT_{50}, values PT with 240 ppm were 45, 87, 96, 72, 129, 115, 131, and 137 h, respectively. The relative toxicities of the LC_{50} values 72 h PT showed that chlorophyllin, echinochrome, field stain, methylene blue, phthalocyanine, rhodamine 6G, riboflavin, and safranin were 3.2, 3.6, 4.6, 9.1, 3.8, 4.3, 3.5, and 5.6 times, respectively, more effective than Phoxim. Methylene blue, safranin, and field stain showed a broad absorbance area indicating a large photoactivity and better phototoxicity and could be used as alternative agents to synthetic acaricides.

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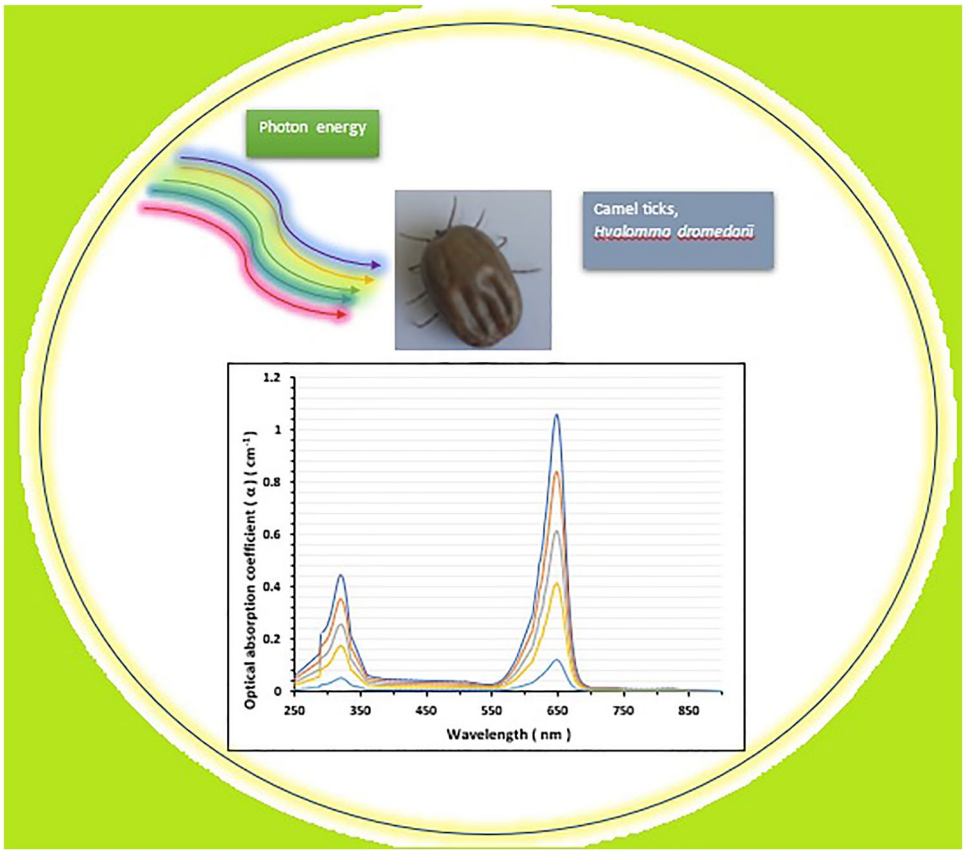
Graphical Abstract



Keywords Photosensitizers · Optical parameters · *Hyalomma dromedarii* · Acaricidal efficacy · Optical bandgap energy

1 Introduction

Camel, *Camelus dromedaries*, is raised for milk and meat production, tourism, and transportation and has a significant role in the economy, especially in the Arabian cultures. Ticks are major vectors of diseases of economic importance as they transmit a variety of pathogens affecting humans, livestock, and domestic animals [1, 2]. Ticks also cause discomfort through annoying bites, anorexia, skin spoilage, blood loss, and growth reduction [3]. The camel tick, *Hyalomma (H.) dromedarii* (Ixodidae), is spread throughout the northern regions of West, Central and East Africa, the Middle East, Asia Minor, and Central and South Asia [4]. It is the dominant species infesting camel in Egypt [5, 6]. *H. dromedarii* is the vector of *Babesia* spp. (*B. caballi*, *B. ovis*, and *B. bigemina*), *Theileria* spp. (*Theileria ovis* and *Th. annulata)*, and *Anaplasma* spp. [7–10].

The arbitrary expenditure of organic chemical pesticides led to the development of tick resistance and environmental pollution; there are great challenges to using affordable alternatives to reduce the risks to humans, non-target organisms and the environment [11–15]. Plant-based pesticides [16] and photosensitizer are efficient substitutes to the ordinary conventional pesticides and might be environmentally sound [17, 18]. Photoactive materials are not toxic in absence of light, but they are activated in light and transform into a reactive semi-stable triplet-excited state [19]. This semi-stable triplet-excited state can accommodate and support photochemical reactions; as the oxygen ($^3O_2$) reactive singlet oxygen is gene substantial challenges rated ($^1O_2$) and induced a high toxic effect [20].

$$^3P + {}^3O_2 \rightarrow P + {}^1O_2.$$

The optical parameters implement the effect of light energy at a broad range of wavelengths and find out the peak absorption and optical bandgap energy. The absorption UV–Vis spectroscopy is an appropriate method for investigating the efficacy of light on the dyes [21–23]. Therefore, using photosensitizers for tick management

protocols against *H. dromedarii* could start a new generation of acaricides.

Phoxim is a potential organophosphorus acaricide [24] licensed for use in the presence of animals in most European countries [25, 26]. This study aimed to evaluate the optical properties and the novel acaricidal efficacy of eight photoactive compounds and Phoxim and to evaluate their lethal concentration and time values against male *H. dromedarii*, as well as their toxicity indices and relative toxicities.

2 Materials and methods

2.1 Ticks collection

Hyalomma dromedarii (males) were collected from places around camels (5–15 years) at Toukh city, Qalyubiya Governorate, Egypt (30° 21′ 11.6″ N and 31° 11′ 31.5″ E). Ticks were morphologically identified.

2.2 Photoactive compounds

Seven photosensitizers were purchased from Alfa Aesar, (Kandel, Germany): chlorophyllin, Natural green (3, E141-$C_{34}H_{31}CuN_4Na_3O_6$); field Stain (Methylene blue–potassium phosphate–disodium hydrogen phosphate–fresh distilled water); methylene blue ($C_{16}H_{18}ClN_3S$); phthalocyanine $(C_8H_4N_2)_4H_2$); rhodamine 6G, Basic Red ($C_{28}H_{31}N_2O_3Cl$); riboflavin (Vit B_2, $C_{17}H_{20}N_4O_6$), and safranin [a fluorescent dye ($C_{20}H_{19}ClN_4$)].

Phoxim (50%, an analogous dimethyl ester, $C_{12}H_{15}N_2O_3PS$), is a commercial acaricide purchased from CURE VET (Pharmaceutical Company, Alexandria Governorate, Egypt).

2.3 Chemical extraction of sea urchin pigments

The Red Sea urchin, *Strongylocentrotus franciscanus* pigments (echinochrome extract) was freshly prepared according to a previously described protocol [27] with little modification. Urchins were collected by divers from the Mediterranean shoreline of Alexandria (Egypt) and shipped in iceboxes and kept at − 20 °C until used. After thawing the ice, testes were cut, and the internal organs were evacuated. The spines and shells were washed with water and left to dry in the dark at a temperature > 10 °C for 24 h, then spines and shells are ground to powder, vacuum-packed in plastic bags, and kept at − 20 °C. The final extract included polyhydroxylated naphthoquinone pigments and stored in the darkness at − 30 °C as a stock solution until used and we would refer to it as echinochrome.

2.4 Optical properties

The absorption spectra as a function of wavelengths (250–900 nm) using a double beam Jasco spectrophotometer (Model V-670, Japan) were measured for the applied materials. The absorption spectra were useful in estimating transmittance, reflectance, absorption coefficient, and optical bandgap energy. The optical absorption coefficient (α) as a function of wavelength was determined from the absorption spectra according to the following formula [28]:

$$\alpha = \left(\frac{2.303 \times A}{d}\right)$$

where A is a function of I and I_0 (the intensity of the transmitted and incident beam, respectively) $A = \log\,(I/I_0)$, d is the film thickness.

To measure the energy absorbed or accomplished, during dye exposure time (to sunlight), the optical indirect bandgap energy was determined according to Makuła work [29]. The indirect optical bandgap $(\alpha h\upsilon)^{1/2}$ can be acquired from the relation between the incident photon energy and the absorption coefficient in different electronic transitions [29]:

$$\alpha h\upsilon = \beta\left(h\upsilon - E_g\right)^{\gamma}$$

where E_g is the optical bandgap energy, β is a constant and γ determines the type of electronic transition which is equal to 1/2 or 2 for the direct and indirect transition bandgaps, respectively. h is the Planck constant, υ is the photon's frequency.

2.5 Adult immersion test

The efficacy of the applied materials against *H. dromedarii* was evaluated through an adult immersion test, according to a previously described protocol**,** with the exception that ticks were subjected to direct sunlight for 10 min instead of a light source. Five concentrations of photosensitizers (240, 180, 120, 60, and 30 ppm) were freshly prepared in distilled water.

Each treatment group containing three replicates, 15 males/each (45 ticks/concentration) was placed in a piece of mesh and immersed for 60 s in 100 mL solution of each concentration, and then the solution was constantly whiskered during the procedure. The immersed ticks were added to a Petri dish containing filter paper. The negative control group was immersed in distilled water and the positive control group was treated with Phoxim (700, 500, 300, 50, and 25 ppm).

Petri dishes containing ticks were exposed to direct sunlight, between 12.00 and 2.00 PM for 10 min Petri dishes were left at 27 ± 2 °C and 80 ± 5% relative humidity. Tick

mortalities (MOs) were recorded 0.5, 1, 24, 48, and 72 h post-treatment (PT).

2.6 Statistical analysis

The mortality data were evaluated using Probit analysis through SPSS V23 (IBM, USA) to calculate lethal concentration (LC) and lethal time (LT) values. The relative toxicity and toxicity indices were determined [30] for a comparison of the tested photosensitizers, where the most toxic photosensitizer has given 100 units on the toxicity index scale.

Toxicity index = LC_{50} of the most toxic photosensitizer × 100/LC_{50} of each tested photosensitizer.

Relative toxicity = LC_{50} (or LC_{90}) of the least toxic photosensitizer/LC_{50} (or LC_{90}) of each tested photosensitizer.

Times potency = LT_{50} of the least toxic photosensitizer/LT_{50} of each tested photosensitizer.

3 Results

3.1 Optical properties

The absorption peaks of dyes were determined as follows; chlorophyllin (408 and 638 nm), echinochrome (634 nm), field stain (302 and 666 nm), methylene blue (292, and 666 nm), phthalocyanine (652 and 322 nm), rhodamine 6G (530 nm), riboflavin (450, 378 and 288 nm), and safranin (522 nm).

The tested photosensitizers exhibited various absorption spectra in near ultraviolet, visible, and near infrared regions (250–700 nm). The absorption spectrum of methylene blue, phthalocyanine, and echinochrome appeared in the visible to near infra-red regions (550–680 nm). Safranin and rhodamine 6G showed absorption in the middle visible light region, whereas field stain, chlorophyllin, and riboflavin showed absorption in the near ultraviolet region (250–440 nm) (Fig. 1).

The UV–Vis absorption spectra of the dyes as a function of various photosensitizer concentrations (30, 60, 120, 180, and 240 ppm) were measured. The optical absorbance of tested dyes follows Beer's Law, where the absorbance intensity decreased with decreasing dye concentration (Fig. 2). The experimental data show smooth linear fitting, which means that as the concentration decreases, the value of photon energy was decreased linearly. The solution of each dye was diluted to be readable within the spectrophotometer manufacture limit and the factor of dilution for each dye as relative absorption intensity was presented (Fig. 3).

3.2 Optical bandgap energy

The optical bandgap energy of Phthalocyanine at the two peak values was very small compared to the rest of the dyes. Most dyes showed more than one bandgap energy except safranin and filed stain. Methylene blue, field stain, and phthalocyanine showed low optical bandgap energies (1.75, 1.70, and 0.95 eV, respectively). Echinochrome showed two optical bandgap energies (1.72 and 3.40 eV). Chlorophyllin, rhodamine 6G, and riboflavin optical bandgap energies were 2.60, 2.20, and 2.40 eV, respectively (Table 1). Safranin showed moderate optical bandgap energy (2.09 eV) (Fig. 4).

3.3 Adult immersion test

All dyes in this study showed moderate toxic effects against camel tick *H. dromedarii* after 72 h PT, where the mortality percent (MO%) reached 42.2%, 44.4%, 51.1%, 71.1%, 46.7%, 48.9%, 44.4%, and 55.6% for chlorophyllin, echinochrome, field stain, methylene blue, phthalocyanine, rhodamine 6G, riboflavin, and safranin, respectively (Fig. 5). The acaricidal efficacy represented by the LC_{50} values of chlorophyllin, echinochrome, field stain, methylene blue, phthalocyanine, rhodamine 6G, riboflavin, and safranin 24 h PT were 648, 704, 521, 340, 589, 614, 631, and 520 ppm, respectively. On the other hand, the corresponding values 72 h PT were 362, 324, 251, 127, 303, 271, 332, and 209 ppm, respectively (Table 2). The data of the positive control group showed that all tested materials were more effective than Phoxim and its LC_{50} values 24, 48, and 72 h PT were 924, 1690, and 1161 ppm, respectively (Table 2).

The relative toxicities 24 h PT indicated that chlorophyllin, field stain, methylene blue, phthalocyanine, rhodamine 6G, riboflavin, and safranin were 1.1, 1.4, 2.1, 1.2, 1.2, 1.1, and 1.4 folds, respectively, as toxic as echinochrome. The relative toxicities 48 h PT for chlorophyllin, echinochrome, field stain, methylene blue, rhodamine 6G, riboflavin, and safranin were 1.1, 1.1, 1.3, 2.4, 1.2, 1.1, and 1.5-folds, respectively, as potent as phthalocyanine. While the relative toxicity 72 h PT with echinochrome, field stain, methylene blue, phthalocyanine, rhodamine 6G, riboflavin, and safranin were 1.1, 1.4, 2.9, 1.2, 1.3, 1.1, and 1.7 folds, respectively, more toxic than chlorophyllin (Table 3).

The relative toxicities 24 h PT with methylene blue showed a high toxic effect, followed by safranin, field stain, and phthalocyanine (toxicity indices = 100, 65.4%, 65.3%, and 57.7%, respectively). Meanwhile, the toxicity indices of chlorophyllin, echinochrome, rhodamine 6G, and riboflavin were 52.5%, 48.3%, 55.4%, and 53.9%, respectively (Table 3).

The toxicity indices 48 h and 72 h PT pointed out that methylene blue was the most effective photosensitizer with 100% toxicity index followed by safranin (62% and 60%,

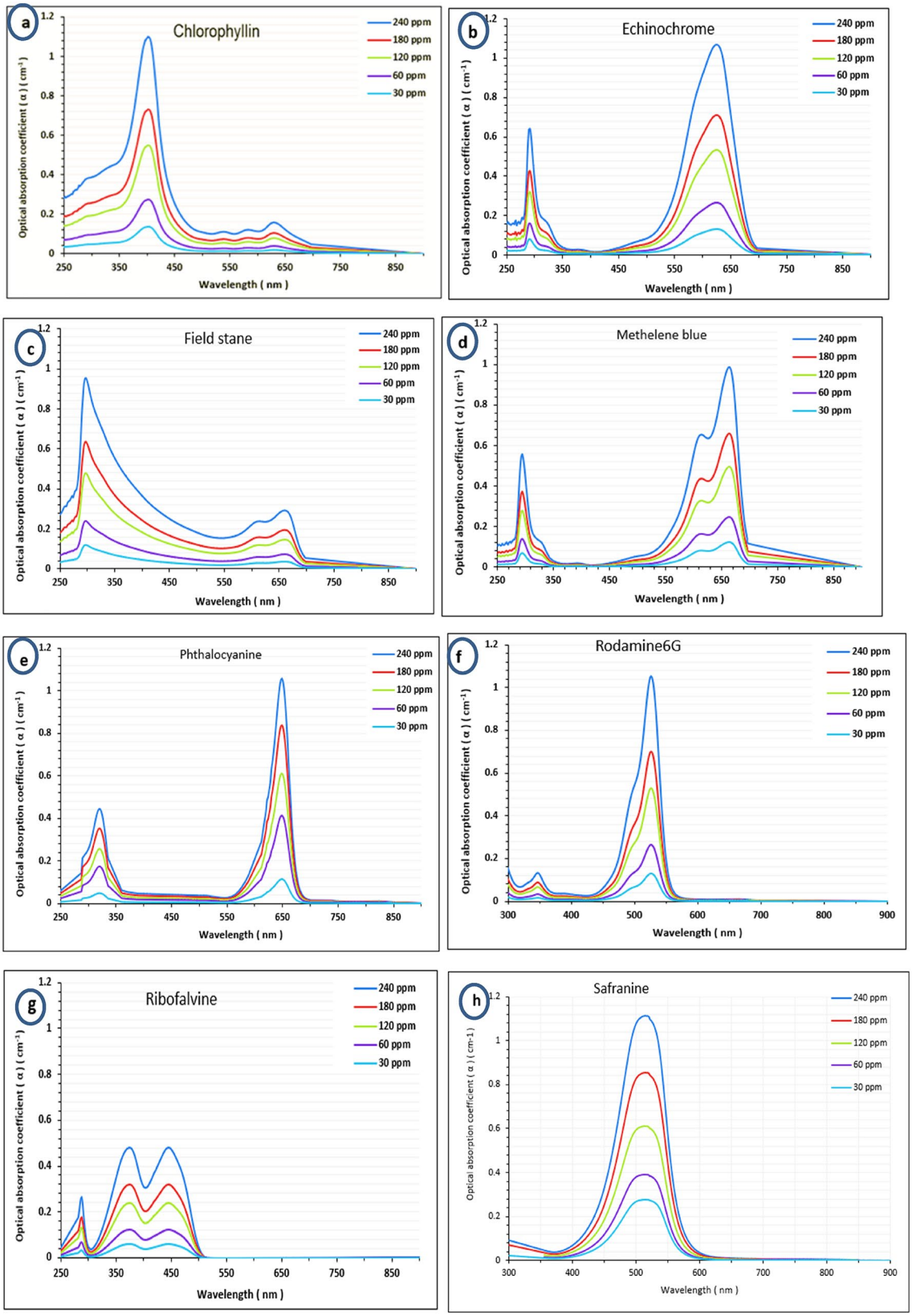


Fig. 1 UV–Vis optical absorption coefficient spectra of chlorophyllin (**a**), echinochrome (**b**), field stain (**c**), methylene blue (**d**), phthalocyanine (**e**), rhodamine6g (**f**), riboflavin (**g**), and safranine (**h**)

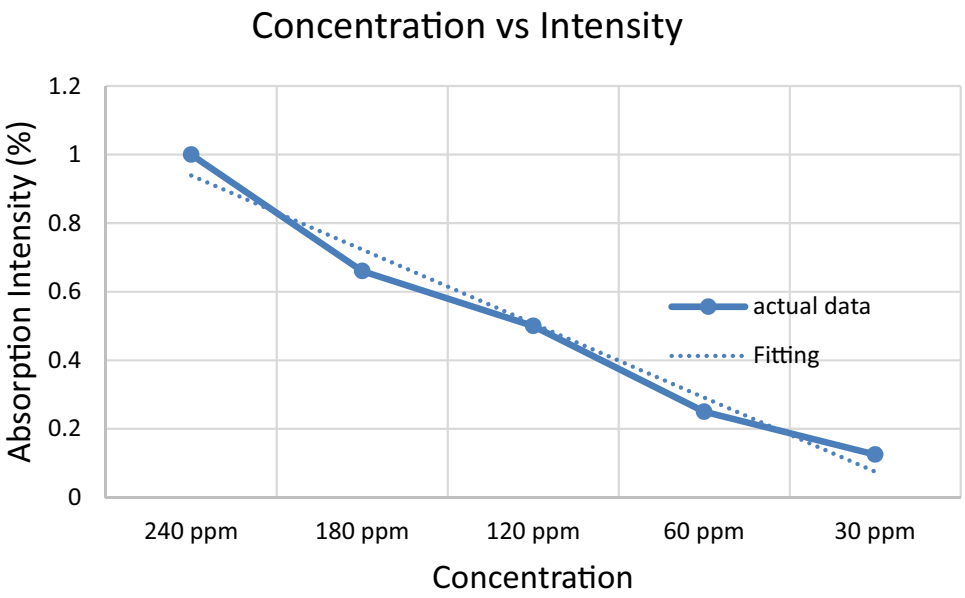


Fig. 2 Absorption intensity as a function concentration of individual photosensitizer

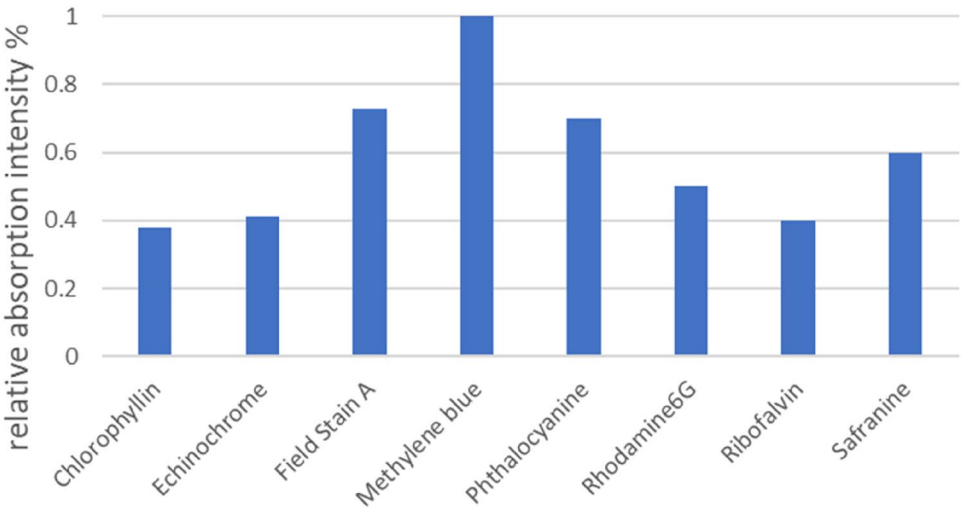


Fig. 3 Relative absorption intensity for each tested photosensitizer

respectively), then field stain (52.6% and 50.6%, respectively). Such values were also recorded for chlorophyllin (44.3% and 35.1%) echinochrome (47.5% and 39.2%), phthalocyanine (41.8% and 74.3%), rhodamine 6G (48.5% and 46.8%), and riboflavin (42.9% and 38.3%) (Table 3).

Regarding the relative toxicities of the LC_{50} values 72 h PT, chlorophyllin, echinochrome, field stain, methylene blue, phthalocyanine, rhodamine 6G, riboflavin, and safranin were 3.2, 3.6, 4.6, 9.1, 3.8, 4.3, 3.5, and 5.6, respectively, times more toxic than Phoxim. On the other hand, 24 h PT, relative toxicities were 1.4, 1.3, 1.8, 2.7, 1.6, 1.5, 1.5, and 1.8-folds, respectively (Table 4).

According to their speed of killing ticks, the LT_{50} values PT with 240 ppm of chlorophyllin, echinochrome, field stain, methylene blue, phthalocyanine, rhodamine 6G, riboflavin, and safranin were 137, 131, 87, 45, 115, 96, 129, and 72 h, respectively (Table 5). The matching values PT with 180 ppm, chlorophyllin, echinochrome, stain, methylene blue, phthalocyanine, rhodamine 6G, riboflavin, and safranin were 150, 188, 117, 52, 166, 129, 194, and 100 h, respectively. While LT_{50} values PT with 120 ppm were 264, 434, 227, 103, 212, 182, 205, and 203 h, respectively (Table 5). It worth to mention that LT_{50} values PT with 300, 500, and 700 ppm were 246, 190, and 81 h, respectively (Table 6).

According to time potency, PT with 240 ppm, echinochrome, field stain, methylene blue, phthalocyanine, rhodamine 6G, riboflavin, and safranin were 1.04, 1.6, 3, 1.2, 1.4, 1.1, and 1.9 times, respectively, faster than chlorophyllin. On other hand and PT with 180 ppm, the time potency of chlorophyllin, echinochrome, field stain, methylene blue, phthalocyanine, rhodamine 6G, riboflavin, and

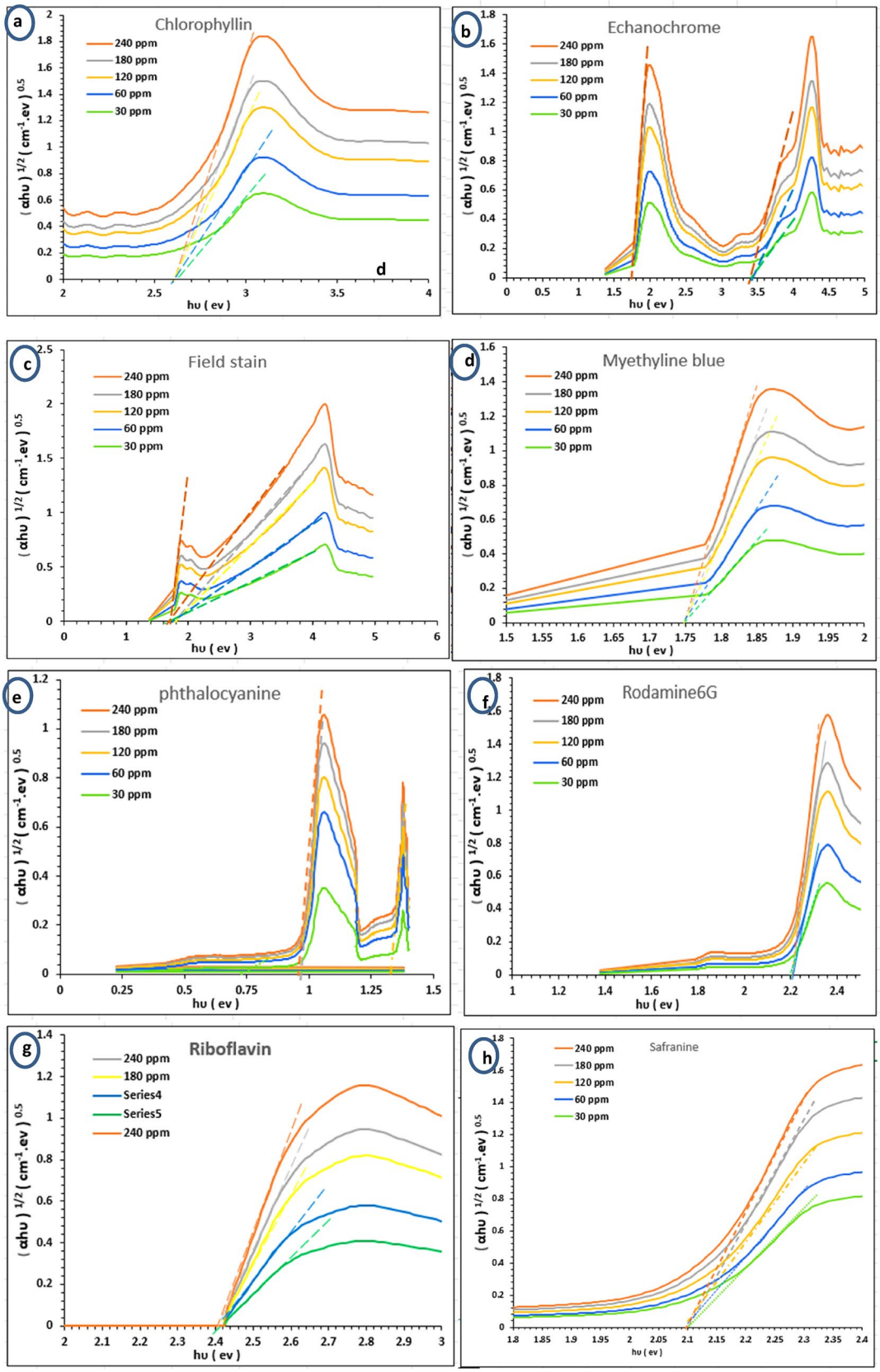


Fig. 4 Optical bandgap energy plots of chlorophyllin (**a**), echinochrome (**b**), field stain (**c**), methylene blue (**d**), phthalocyanine (**e**), rhodamine6g (**f**), riboflavin (**g**), and safranine (**h**)

Table 1 Optical bandgap energy (E_g) [measured in electron-volt (eV)] of tested photosensitizer against male *Hyalomma dromedarii*

Photosensitizer	Chlorophyllin	Echinochrome	Field stain	Methylene blue
The optical bandgap energy E_g (eV)	2.60	1.72, 3.40	1.70	1.75
Photosensitizer	Phthalocyanine	Rhodamine 6G	Riboflavin	Safranine
The optical bandgap energy E_g (eV)	0.95, 1.35	2.20	2.40	2.09

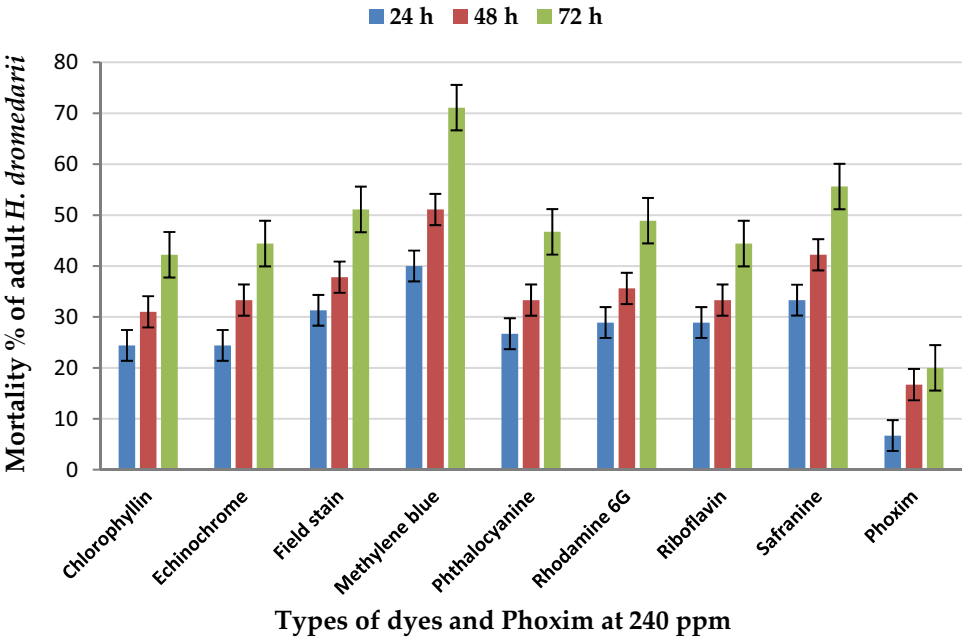


Fig. 5 Mortality of the tested dyes compared to phoxim at a concentration of 240 ppm

Table 2 Acaricidal efficacy of photosensitizer against *Hyalomma dromedarii*

Treatment	Time post-treatment hour								
	24 h		48 h		72 h		X^2	Equation	R^2
	LC_{50} (lower–upper)[a]	LC_{90} (lower–upper)	LC_{50} (lower–upper)	LC_{90} (lower–upper)	LC_{50} (lower–upper)	LC_{90} (lower–upper)	72 h	72 h	72 h
Chlorophyllin	648 (354–4276)	4326 (1254–2.5×10^5)	508 (295–2286)	4332 (1280–1.6×10^5)	362 (236–972)	2849 (1035–3.9×10^5)	0.13	$y=-3.60+1.40*x$	0.99
Echinochrome	704 (362–6322)	5719 (1437–7.1×10^5)	474 (281–1911)	4058 (1239–1.3×10^5	324 (218–767)	2477 (955–2.6×10^5)	0.23	$y=-3.63+1.44*x$	1.00
Field stain	521 (300–2420)	4407 (1293–1.7×10^6)	428 (253–1864)	3628 (1358–2.7×10^5)	251 (177–498)	2064 (846–1.7×10^5)	0.02	$y=-3.31+1.38*x$	0.99
Methylene blue	340 (222–907)	2950 (1041–4.6×10^5)	225 (156–468)	2439 (893–3338)	127 (100–167)	699 (422–1806)	0.07	$y=-3.61+1.72*x$	0.99
Phthalocyanine	589 (332–3180)	4133 (1238–1.8×10^6)	538 (295–3239)	4729 (1102–2.0×10^5)	303 (205–694)	1655 (713–1.3×10^5)	0.74	$y=-3.42+1.38*x$	0.99
Rhodamine 6G	614 (332–4717)	5902 (1515–6.8×10^5)	464 (269–2116)	4966 (1359–2.6×10^5)	271 (190–558)	2128 (868–1.8×10^5)	0.11	$y=-3.46+1.42*x$	1.00
Riboflavin	631 (326–4384)	6053 (1499–6.3×10^5)	525 (287–3331)	6446 (1533–7.6×10^5)	332 (215–926)	3264 (1089–6409)	0.15	$y=-3.24+1.29*x$	1.00
Safranin	520 (290–2868)	4500 (1072–1.6×10^5)	363 (223–1280)	2365 (1255–1.7×10^5)	209 (153–365)	1031 (742–1.1×10^5)	0.03	$y=-3.23+1.39*x$	0.99
Phoxim 50%	924 (691–3666)	2303 (1226–7.6×10^5)	1690 (810–1.2×10^5)	25,614 (5290–3.4×10^6)	1161 (648–4360)	13,379 (3788–3.9×10^5)	0.76	$y=-3.23+1.39*x$	0.89

[a]Dose = ppm

Table 3 Relative toxicities and toxicity indices of the tested photosensitizer against *Hyalomma dromedarii*

Photosensitizer	Treatment					
	Time post-treatment hour					
	24 h		48 h		27 h	
	Relative toxicities	Toxicity indices	Relative toxicities	Toxicity indices	Relative toxicities	Toxicity indices
Chlorophyllin	1.1	52.5	1.1	44.3	1.0	35.1
Echinochrome	1.0	48.3	1.14	47.5	1.1	39.2
Field stain	1.4	65.3	1.3	52.6	1.4	50.6
Methylene blue	2.1	100.0	2.4	100.0	2.9	100.0
Phthalocyanine	1.2	57.7	1.0	41.8	1.2	74.3
Rhodamine 6G	1.2	55.4	1.2	48.5	1.3	46.8
Riboflavin	1.12	53.9	1.1	42.9	1.1	38.3
Safranin	1.4	65.4	1.5	62.0	1.7	60.0
Reference	Echinochrome	Methylene blue	Phthalocyanine	Methylene blue	Chlorophyllin	Methylene blue

Toxicity index% = LC_{50} of the most toxic photosensitizers × 100/LC_{50} of the tested photosensitizer

Relative toxicity (folds) = LC_{50} of the least toxic photosensitizers/LC_{50} of the tested photosensitizer

Table 4 Relative toxicities of tested photosensitizer over Phoxim insecticide against the camel tick, *Hyalomma dromedarii*

Photosensitizer	Treatment		
	Time post-treatment hour		
	24 h	48 h	72 h
	Relative toxicities	Relative toxicities	Relative toxicities
Chlorophyllin	1.4	3.3	3.2
Echinochrome	1.3	3.6	3.6
Field stain	1.8	3.9	4.6
Methylene blue	2.7	7.5	9.1
Phthalocyanine	1.6	3.1	3.8
Rhodamine 6G	1.5	3.6	4.3
Riboflavin	1.5	3.2	3.5
Safranin	1.8	4.7	5.6
Phoxim	1	1	1

Relative toxicity (folds) = LC_{50} of the least toxic photosensitizers/LC_{50} of the tested photosensitizer

safranin were 1.3, 1.03, 1.7, 3.7, 1.2, 1.5, and 1.9 times, respectively, faster than riboflavin (Table 5).

4 Discussion

The camel tick, *H. dromedarii*, is infesting camels worldwide [5, 6, 31] and considerable efforts are needed to evaluate new ecofriendly acaricides [32, 33]. Photosensitizer accumulates in the pest body and stimulates lethal photochemical reactions [17, 18]. Photosensitizers use light (natural or artificial) at specific absorption wavelengths to be fully functional and enhance their capability as pesticides [19, 34].

In the current study, the toxicity of eight photosensitizers against *H. dromedarii* male as alternative tick control methods was evaluated for the first time, according to our knowledge, except for safranin which was applied in our previous study against another stage, engorged females, of *H. dromedarii* [32, 35], but this study evaluated its effect against *H. dromedarii* males.

This study showed that ticks were highly susceptible to all tested photosensitizers, as mortalities indicated a time and dose-dependent relationship. The susceptibility of Phoxim as a synthetic acaricide was made to compare its efficacy against the applied photosensitizer. The data showed that LC_{50} of Phoxim was very high, 1161 ppm, 72 h PT and its LC_{90} values 48 and 72 h PT were very high 25,614 and 13,379 ppm, respectively. As the recommended dose of Phoxim is 1 mL/L (1000 ppm), *H. dromedarii* has acquired resistance as it needs doses of Phoxim, 5 and 98 times, respectively, more than the recommended dose is to be effective.

This study indicated that the toxicity indices of tested photosensitizers 72 h PT with chlorophyllin, echinochrome, field stain, methylene blue, phthalocyanine, rhodamine 6G, riboflavin, and safranin were 3.2, 3.6, 4.6, 9.1, 3.8, 4.3, 3.5, and 5.6, respectively, more toxic than Phoxim.

Methylene blue was the most effective photosensitizer followed by safranin and field stain (LC_{50} = 127, 209, and 251 ppm, respectively); their toxicity indices were 100%,

Table 5 Lethal time values (h) and time potency of tested photosensitizer against male *Hyalomma dromedarii*

Photosensitizer	Concentrations								
	Photosensitizer conc. (ppm)								
	120			180			240		
	Lethal time		Times potency	Lethal time		Times potency	Lethal time		Times potency
	LT_{50} (Lower–upper)	LT_{90} (Lower–upper)		LT_{50} (Lower–upper)	LT_{90} (Lower–upper)		LT_{50} (Lower–upper)	LT_{90} (Lower–upper)	
Chlorophyllin	264 (116–9320)	3477 (583–1.5×10^6)	1.6	150 (84–833)	3498 (427–1.6×10^6)	1.3	137 (72–447)	1662 (856–7.4×10^5)	1.0
Echinochrome	434 (155–7516)	15,350 (1791–9.5×10^7)	1.0	188 (94–868)	4220 (869–1.8×10^6)	1.03	131 (68–427)	4026 (980–9.0×10^5)	1.04
Field stain	227 (105–1338)	5671 (1062–4.2×10^6)	1.9	117 (67–327)	2942 (588–3.2×10^5)	1.7	87 (48–224)	2034 (789–3.7×10^5)	1.6
Methylene blue	103 (45–259)	3728 (931–6.2×10^5)	4.2	52 (32–99)	1111 (462–4664)	3.7	45 (23–90)	241 (93–1514)	3.0
Phthalocyanine	212 (103–3360)	2530 (505–2.2×10^6)	2.0	166 (86–660)	3453 (791–1.1×10^5)	1.2	115 (62–339)	3389 (866–5.8×10^6)	1.2
Rhodamine 6G	182 (95–1608)	2152 (482–5.6×10^6)	2.4	129 (72–395)	2571 (630–4.4×10^5)	1.5	96 (54–248)	2299 (720–3.2×10^5)	1.4
Riboflavin	205 (101–1763)	2972 (609–6.4×10^6)	2.1	194 (91–906)	6616 (1252–3.0×10^6)	1.0	129 (63–446)	5625 (1177–1.4×10^6)	1.1
Safranin	203 (94–995)	6864 (1275–3.4×10^5)	2.14	100 (57–253)	2162 (637–2.6×10^5)	1.9	72 (37–169)	1247 (788–3.7×10^5)	1.9
Reference	Echinochrome			Riboflavin			Chlorophyllin		

Times potency = LT_{50} of the least toxic compound/LT_{50} of the tested compound

Table 6 Lethal time values (h) of Phoxim against male *Hyalomma dromedarii*

300 ppm		500 ppm		700 ppm	
Lethal time		Lethal time		Lethal time	
LT_{50} (lower–upper)	LT_{90} (lower–upper)	LT_{50} (lower–upper)	LT_{90} (lower–upper)	LT_{50} (lower–upper)	LT_{90} (lower–upper)
246 (209–299)	510 (422–422)	190 (159–22)	417 (354–524)	81 (51–105)	246 (210–304)

60%, and 50.6%, respectively; their relative toxicities were 2.9, 1.7, and 1.4 times, respectively, more effective than chlorophyllin.; and their LT_{50} values were 45, 72 and 87 h, respectively. Analogous to our results, methylene blue was more toxic than hmatomporphyrin (a photosensitizer) against the cotton leafworm, *Spodoptera littoralis*, as MO% PT with 10^{-2}, 10^{-3}, and 10^{-4} mg/L were 64%, 34%, and 18%, respectively, which decreased to 33.7%, 21%, and 5.7%, respectively, 10 days PT [36].

Our results are in harmony with former studies that displayed that the toxicity indices of methylene blue and eosin yellow lactone were 35.78% and 45.68%, respectively, as potent as rose Bengal against the cotton leafworm [37]. Methylene blue could be attached easily to the biological membrane [37]; therefore, it is considered a good pesticide in the presence of light.

This study indicated that safranin is a highly potent acaricide against male *H. dromedarii* (LC_{50}, 24 h PT = 520 ppm, respectively). Similar to our finding, safranin is a highly effective acaricide against the engorged females of *H. dromedarii* in Egypt (MO% = 100 PT with 4% for 8 h), and its LC_{50} after 24 h was 0.78% (7800 ppm). At the LC_{50} and LC_{90} levels 24 h PT, safranin was six and 73 times more effective than tetramethrin. LT_{50} of safranin was 0.80 h PT with 4% [32]. Male *H. dromedarii* in this study is highly susceptible to safranin than the engorged females of the same species [32].

Alike our results, safranin and methylene blue were effective acaricides against the fourth larval instars of the black cutworm, *Agrotis ipsilon*, as poison baits and induced stomach and contact toxicities (LC_{50} = 0.107 and 0.125%, respectively, 72 h PT). Sublethal concentrations of both photosensitizers adversely affected the developmental stages of *A. ipsilon* [37]. Sublethal concentrations of safranin [32] and rose Bengal [35] adversely affected the reproductive potential of treated engorged females of *H. dromedarii* by reducing the number of ovipositing females, fecundity, and egg hatchability.

Some other dyes (photosensitizers) act as effective pesticides; rose Bengal was 100 times more toxic than chlorpyrifos against the common house mosquito, *Culex pipiens* [38].

As far as we know, there is a patent related to the superior toxic effects of some photosensitizers as safranin O, auramine O, eosine Y; erythrosine B, D, and C; orange 5; and thioflavine T against arthropods and reported that they exhibit far toxic efficacy compared to phloxine B, once used with an optimal adjuvant. Without the specific adjuvant, such dyes have little or no lethal effect [39]. Unluckily, this patent did not specify which arthropods or the adjuvant was used.

In the present work, the least effective photosensitizers were echinochrome and chlorophyllin (LC_{50} = 332 and 324 ppm, respectively). In contrary to our finding, copper and magnesium chlorophyllin photosensitizers have also toxic effect against the cotton leafworm, *Spodoptera littoralis*, as they reduced the number of larvae 15 days post-spraying and in the second season, 74–90% and 65–95%, respectively [40]. Furthermore, chlorophyllin had high toxicity against *Culex* and *Chaoborus* (LD_{50} = 6.88 and 24 mg/L, respectively [41]; LD_{50} values of chlorophyllin were 2.34 and 5.88 mg/L against *Aedes* and *Anopheles* species, respectively [42] and a lethal dose of 8 mg per *Chaoborus crystallinus* larvae [43]. Chlorophyll derivatives can be effectively used against malaria, filarial, and dengue vector-borne diseases [44]. Echinochrome had high antibacterial, anti-inflammatory [45] and antiviral effects [46]. After treatment for 3 days in the present study, Rhodamine 6G, riboflavin, and phthalocyanine were the least effective photosensitizers (LC_{50} = 271, 332, and 303 ppm, respectively).

Other than the pesticidal effect, photosensitizers are efficacious photodynamic materials against many pathogens such as fungi, protozoa, and bacteria and resolve many environmental problems, such as water disinfection, sterilization, prohibition of water-borne diseases [47], purification of wastewaters, and preservation of animal species [48]. The photoactive characteristic counts on several factors, such as photostability, irradiation time, concentration, fluency rate of light delivered, and the other biological, physical and chemical features [49, 50].

Dyes are inactive in the dark and no insect mortalities were recorded in the dark [51]; therefore, sunlight is very important for dye activation. Exposure of a photosensitizer to sunlight as in this study would be more effective and viable than an artificial light source due to the fact that sunlight possesses all wavelengths from UV to visible light [32, 35]. This study revealed that methylene blue exhibited absorption peaks at wavelengths of visible to near infra-red region (560–665 nm). Similar finding was recorded [52, 53]. In addition, the absorbance of safranin and rhodamine 6G in the present study showed peaks at wavelengths of visible light at (450–550 nm: and 485–540 nm, respectively). Analogous data were reported [54–57]. Echinochrome showed absorption peaks of 294 and 550–650 nm. The present work indicated that field stain and chlorophyllin showed absorption peaks at low wavelengths, which means higher photon energy. Phthalocyanine showed a peak at 630 in this study; like finding was recorded [58, 59]. Riboflavin also shows a broad range of absorption at low values of wavelengths (342–480 nm) in this study; comparable range was reported [60].

Photosensitizer molecules absorb photon energy with the appropriate wavelength, which excites an electron into a higher energy orbital according to the Jablonski diagram [61]. The more absorption wavelengths, the more energy participates in the photosensitizer molecule. Therefore, dyes such as methylene blue, phthalocyanine, rhodamine 6G, safranin, and echinochrome are likely to acquire higher energy, because they exhibit absorption wavelengths peaks in the visible light range from 400 to 700 nm [54, 59]. Because of listed variations of the absorption ranges, these dyes are good candidates as pest control. Similar findings were recorded for safranin [32].

Optical measurements of the tested photosensitizers were recorded immediately and a few days after treatment of the ticks show high absorbance, especially for methylene blue owing to their photostability and photoliability which suggested they can be used in sunlight for a long time. Methylene blue is stable enough to be used in sunlight and not easily degraded and needs additional help for its degradation as titanate nanoparticles [62] and graphene oxide or tin oxide [63–65].

Phthalocyanine and echinochrome, as well as methylene blue, exhibit absorption peaks in the visible to near-infrared spectral region (between 640 and 700 nm) and they have a wide optical bandgap which does not provide enough energy to the photon to excite [19]. This also supports their stability in long storage and for their application in the presence of sunlight.

The optical bandgap energy determines how much energy of the light or sun is needed for the photon to excite. The behavior of the applied dyes is assumed to be of indirect bandgap (n = 1/2). It is interesting to note that the optical

bandgap was not altered with increasing dye concentration as expected because of the absorption peak was not shifted because of concentration change. This was explained by the linear relationship between photon energy and concentration.

Optical bandgap energies of phthalocyanine, chlorophyllin, echinochrome, rhodamine 6G, and Riboflavin in this investigation showed more than one optical bandgap energy indicating more activity over a broad range of photon frequencies, representing higher lethal capability. These data agree with the phototoxicity of tested photosensitizers [66–68].

Although safranin and phthalocyanine in this work have one bandgap energy and energies were still low, indicating that they could be applied as a potent pesticide. Low values of optical bandgap, as well as the broadening of the absorption spectra, were pointing out good photoactive dye [69].

Photosensitizers' toxicity mechanisms against arthropods pest were summed up [70]. The membranes of the midgut wall seem to be the first susceptible sites to be photo damaged. The symptoms appeared in membrane permeability as well as alteration of potassium levels in the hemolymph. Photosensitizers affect water level, weight, and protein mass, leading to fatal energy compression in the treated pests. A reduction in the fertility rate has been exhibited as a secondary effect. Photosensitizers also induce morphological and physiological mutations. They also affected the immature stages and the reproductive outcome of the treated pests [32, 35–37].

5 Conclusions

This study revealed that *H. dromedarii* had acquired resistance against phoxim and evaluated a novel approach of using photosensitizers against ticks as an alternative control strategy [31]. Methylene blue was the most efficient photosensitizer followed by safranin and field stain. They exhibit rather much broader absorption spectra, higher absorption intensity compared to the other dyes. Whereas the phthalocyanine, rhodamine 6G, and riboflavin exhibited almost equal toxic effects with almost comparable optical properties. While echinochrome and chlorophyllin have the least toxic effect, which was supported by their lowest relative absorption intensity. The optical parameters together with results of the toxicity measurements suggested that the phototoxicity of the tested photosensitizer dyes was strongly related to their photoactivity and can be correlated. Methylene blue, safranin, and field stain showed better stability and a broad absorption range. Therefore, it is recommended to use methylene blue followed by safranin and field stain for field application (on hosts and the environment) after revealing their ecotoxicological profile to avoid pest resistance and reduce the reliance on chemical acaricides.

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Declarations

Conflict of interest The authors declare that there is no conflict of interest.

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