International Journal of Veterinary Science

www.ijvets.com P-ISSN: 2304-3075 E-ISSN: 2305-4360 editor@ijvets.com

RESEARCH ARTICLE

Photoxicity of Rose Bengal against the Camel Tick, *Hyalomma dromedarii*

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ARTICLE INFO

Received: March 09, 2014
Revised: March 30, 2014
Accepted: April 06, 2014

Key words:
Hyalomma dromedarii
Ivermectin
Malformation
Reproductive potential
Rose Bengal

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ABSTRACT

Hyalomma dromedarii is the predominant tick species infesting camels. Consequently, the aims of the present study were to compare the photodynamic efficacy of rose bengal (RB) to that of ivermectin (IVR) against the engorged females of *H. dromedarii* through *in vitro* immersion bioassays and to test the effect of the applied materials on the reproductive potential of the survived females. RB has been tried as acaricides for the first time, to the best of our knowledge. Different concentrations of RB (0.01, 0.03, 0.13, 0.5 and 2%) and IVR (0.02, 0.08, 0.6, 2.5, and 10%) were freshly prepared in distilled water. The minimal lethal concentrations that cause 100% acaricidal effect were 2%, 8 h post treatment (PT) with RB, and 2.5%, 24 h PT with IVR. Eight hours PT with RB and IVR, the LC50 (lethal concentration, 50%) values were 0.08 and 0.35%, respectively, whereas those for LC95 were 1.45 and 30.07%, respectively. At the levels of LC50 and LC90, RB was 4 and 15 times more potent than IVR. The median lethal time, LT50, values of 2% RB and 2.5% IVR were 0.92 and 2.63 h, respectively. Treatment with the lowest concentrations of RB and IVR induced reduction in the number of survived and ovipositing females, eggs per female, ticks laid hatched eggs, and hatched eggs (48.98, 93.33, 1854.53±45, 97.5 and 93.64%) and (26.53, 86.67, 7661.27±377, 87.80 and 89.40%), respectively. The low cost of RB, together with the availability of inexpensive low-power light sources or sunshine, suggests that this approach is of great potential as an interesting alternative to chemical acaricides.

Cite This Article as: Khater HF and NI Hendawy, 2014. Photoxicity of rose bengal against the camel tick, *Hyalomma dromedarii*. Inter J Vet Sci, 3(2): 78-86. www.ijvets.com

INTRODUCTION

Dromedary camels, *Camelus dromedarius*, are very important source of meat, milk, and hide in arid and semi-arid environments. Ticks are haematophagous ectoparasites that lead to anaemia and damaged hides and predispose animals to secondary bacterial infections and infestation with myiasis-causing flies. Ticks can cause paralysis and probably transmit a greater variety of organisms linked to production losses in livestock (Mukhebi *et al*., 1999). The camel tick, *Hyalomma* (*H.*) *dromedarii* (Koch 1818) (Acari: Ixodidae), is widely distributed throughout North Africa, the northern regions of West, Central and East Africa, the Middle East, Asia Minor, and Central and South Asia (Apanaskevich *et al*., 2008) . It is suspected to play an important role in transmitting haemoprotozoan diseases and bovine tropical theileriosis, caused by *Theileria annulata* (Bhattacharyulu *et al*., 1975).

H. dromedarii is the predominant tick species infesting camels, 89% in Sudan (ELGhali and Hassan 2009) as well as 95.6% in Sinai (van Straten and Jongejan 1993) and 57.13% in Benha and Belbis, Egypt (Ramadan 1997). Other tick species are found in very low numbers, including other *Hyalomma spp.* (Ramadan 1997; ELGhali and Hassan 2009). *H. dromedarii* can behave as a three- or two host species, but the two-host life cycle is the most common (Walker *et al*., 2003). Camels are the main hosts of *H. dromedarii* adults, which also parasitize other domestic animals. Nymphs and larvae can parasitize the same hosts as adults, especially camels, but birds, rodents, and hedgehogs can also serve as hosts (Anderson, 2002). The mean tick burden is relatively high and the range of number of ticks per camel, in Sinai, Egypt, is very broad (6-173) (van Straten and Jongejan 1993). Larval–nymphal feeding periods ranged from 16 to 27 days according to the season, whereas females fed for 6-9 days (ELGhali

and Hassan 2010). Female camels harbored more ticks than males and higher infestations were recorded on camels with a grey- coat color compared to those with a brown- coat color; furthermore, ticks were found on camels throughout the year and increased in numbers during the period from March to October with a peak in September (ELGhali and Hassan 2009).

The current methods used to control ticks rely mainly on conventional acaricides leading to appearance of acaricideal resistance (Nolan 1990) and environmental pollution (Bhattacarya *et al*., 2003). Accordingly, search for alternative methods for tick control is an important demand. Other strategies have been proposed including photoinsecticides, organic acids, botanicals, biological (Khater 2011, 2012, 2013) and immunization (Ramadan 1997; Habeeb *et al*., 2009) control methods. Photodynamic processes are used in plants as chemical defense weapons against attack by herbivorous insects (Wainwright 2009). The highest photoinsecticidal activity is displayed by rose bengal followed by eosin, erythrosine, and fluorescein (Ben Amor and Jori, 2000).

Phloxin B, a polyhalogenated fluorescein, has been developed for commercial use as a pesticide. The developments in this field have been reviewed by Ben Amor and Jori (2000). A photoactive compound or a photosensitizer is an organic chemical that uses light energy to "catalytically" generate toxicity. Such compound accumulates within the insect body and exposure to visible light induces lethal photochemical reactions and death of the organism (Lukðienë *et al*., 2005).

The advancement of effective acaricidal formulations that are inexpensive and less toxic to the environment should receive an urgent attention. Although rose bengal and the other photosensitizers showed insecticidal effects (Dondji *et al*., 2005; Aref 2010); their acaricidal effect has not been tested yet, according to the best of our knowledge. As a result, the aims of the present study were to compare the photodynamic effect of rose bengal to that of ivermectin, a commercially available acaricide, against the engorged females of *H. dromedarii* and their effects on the reproductive potential of the treated female ticks.

MATERIALS AND METHODS

Ticks

Fully engorged *H. dromedarii* females were collected from camels at Toukh's slaughterhouse (35 km North Cairo), Qalyubia Governorate, Egypt. The animals (5-15 years old) were brought originally for slaughter from Sudan and Saini. *H. dromedarii* were identified according to Aapanaskevich *et al*. (2008). No information on prior parasitic treatment was obtained. Considering parasitic fauna of infested animals and absence of dead ticks on the inspected camels, it is unlikely that they had received any treatment for controlling *H. dromedarii*.

Applied materials

1. Rose Bengal (RS), a xanthene dye belongs to the Fluorone group, $C_{20}H_2.Cl_4I_4Na_2O_5$ (RevectoR Microscopical Stain, c.l. 45446, Hopkin & Williams, England).
2. Ivermectin 1% (IVR), a conventional acaricide, (Ivomec®, Merk Sharp and Dohme Agvet Inc.)

The absorption spectra

The absorption spectra of RB were studied using UV-VIS spectrometer (PG instruments Limited- Model 80+).

The light source

Illumination was achieved through a spot-white-light source (power 100 W, model 212, 80 ml, spectral lamp with continuous light emission at UV-V-IR spectra). Measurements of the light irradiance for each wavelength (uw cm^{-2}- sr- nm) of the used light source was done by the spectral measurement system (SMS) 500 device at the Egyptian Organization for Standardization and Quality (EOS), Cairo, Egypt.

In vitro bioassays

In vitro immersion bioassays were carried out to determine the efficacy of RB and IVR against *H. dromedarii*, according to Khater (2014) and Khater *et al*. (2013a,b). Preliminary experiments were conducted to determine suitable experimental parameters, such as dilution factors for tested substances and the duration of their exposure to ticks. For calculation of the lethal concentration (LC) and lethal time (LT) values, diverse concentrations of RB (0.01, 0.03, 0.13, 0.5, and 2%) and IVR (0.02, 0.08, 0.6, 2.5, and 10%) were freshly prepared in distilled water. IVR treated group was used as the positive control group.

Ten engorged females of *H. dromedarii* were used per replicate in each test. Each concentration was tested in five replicates (i.e. 50 ticks were tested for each concentration). Each group of ticks was placed in a mesh cloth piece and immersed for 60 s in 100 ml solution of the drug of each tested concentration, and then the solution was continuously stirred during the process. The immersed ticks were kept in Petri dishes containing filter papers (Whatman No. 1). The negative control group was treated with distilled water. Petri dishes were kept at 26±2°C and 80±5% relative humidity (RH). RB- treated ticks were illuminated, 15 cm distance, with the previously mentioned light source for 30 min PT.

The mortality of females in all dishes was observed after different time intervals (0.5, 2, 8, 24, and 48 h) post treatment (PT). Alive and dead ticks were counted. Ticks were considered alive if they exhibited normal behavior when breathed upon or physically stimulated with wooden dowels; ticks which were incapable of movement, maintaining normal posture, leg coordination, ability to right themselves, or any signs of life were considered moribund or dead, according to Khater and Ramadan (2007) and Khater *et al*. (2013a).

Efficacy against reproduction potential

Each survived female was incubated individually, in a right position and placed in a vertical test tube covered with a cotton plug, under 27ºC and 80±5% RH. The numbers of survived and ovipositing females as well as deposited and hatched eggs were determined.

Data analysis

For bioassay tests, probit analysis was done on the mortality data using the computer program Biostat (2009)

to calculate the lethal concentration (LC) as well as the lethal time (LT) value. The biological data were subjected to one-way analysis of variance (ANOVA) by Duncan's multiple range test (Duncan 1955) using the computer program PASW Statistics 2009 (SPSS version 18).
Reduction (R) % was calculated according to the following formula: R% = (Control - Treated) / Control x100

RESULTS

The absorbed energy (λ_{max}) from the light source by RB exhibited two peaks of absorption. The lowest peak occurred at 302 nm, corresponding to the UV radiation (UVR), but the highest peak occurred at 536 nm, equivalent to the green spectrum (Fig. 1). In contrast to UVR, the green light is strongly absorbed by RB. The highest irradiance occurred at the end of the visible and infra-red regions (Fig. 2).

The minimal lethal concentrations (MLC) that cause 100% acaricidal effects were 2 and 2.5% PT with RB and IVR, for 8 and 24 h, respectively (Table 1). The LC50 (lethal concentration, 50%) values 8 h PT with RB and IVR were 0.08 and 0.35%, respectively, whereas those 24 h PT were 0.05 and 0.15%, respectively. The LC95 values, 24 h PT, were 0.99 and 1.76%, respectively (Table 2). Two hours PT, RB was 12 and 47 times more potent than IVR at the levels of LC50 and LC90, respectively (Table 3). The lethal time values, LT50, LT90, and LT95 were 0.92, 2.13, and 2.71 h, respectively, PT with 2% RB and 3.95, 38.63, and 73.75 h, respectively, PT with 10% IVR (Table 4).

After treatment with the lowest concentrations of RB and IVR, there is a reduction of the number of survived and ovipositing females, eggs laid per female, ticks laid hatched eggs, and hatched eggs (48.98%, 93.33%, 1854.53±45, 97.5% and 93.64%) and (26.53%, 86.67%, 7661.27±377, 87.80% and 89.40%), respectively (Table 5).

DISCUSSION

H. dromedarii is the major tick species infesting camels (ELGhali and Hassan 2009); subsequently, its control is an essential demand. Macrocyclic lactones (MCL) such as ivermectin and doramectin act as effective pesticides (Khater *et al*. 2013b; Seddiek *et al*. 2013; Khater 2014). Our results indicate that 100% acaricidal effect was reached PT with 2.5% IVR for 24 h and the LC50 values 8 and 24 h PT were 0.35 and 0.15%, respectively, whereas those for LC95 were 30.07 and 1.76%, respectively. LT50 and LT95 were 3.95 and 73.75% h, respectively, PT with 10% IVR. In contrast to our result, IVR was not effective against *Hyalomma* tick spp. infesting camels (van Straten and Jongejan 1993) and the other tick species (George *et al*., 2004**;** Klafke *et al*., 2006). Cypermethrin was more effective than IVR (as pour-ons) against *H. anatolicum (a.) anatolicum* in bovines (Sajid *et al*., 2009).

Unfortunately, the use of MCL erased some safety and ecological concerns (Lumaret and Errouissi 2002; El-Nahas and El-Ashmawy 2008; Seddiek *et al*., 2013). IVR induces several side effects including adverse effects on male fertility in cattle (Avery and Schmidt 1995), goats (Tanyildizi and Bozkurt 2002) and rats (El-Nahas and El-Ashmawy 2008). The toxic effects of IVR on liver (Seddiek *et al*., 2013) and kidney (Seddiek *et al*., 2013) functions were temporary and rabbits required not less than 3 months after injection with IVR to resume normalcy of liver and kidney functions (Eman and Abdella 2000).

More critically, residues of IVR were found in muscle, liver, and milk (Galarini *et al*., 2013). Ill-advisedly, cooking cannot be taken into account as protection against ingestion of residues of systemic pesticides of veterinary drugs (Cooper *et al*., 2011). Besides, residues of IVR were stable in milk after one year of freezing at -20°C and they had reduced by approximately one quarter after two years of freezing (Cerkvenik *et al*., 2001). As a result, it is not permitted to use IVR during lactation (Imperiale *et al*., 2004). Ecologically, most MCL have been shown to be highly toxic for the dung beetles, *Onthophagus taurus*, a non-target (beneficial) organism (Wardhaugh *et al*., 2001; Lumaret and Errouissi 2002). Consequently, healthcare providers now face a serious lack of new alternative

Table 1: The efficacy of rose bengal and ivermectin on engorged females of *Hyalomma dromedarii*

Conc. %	Time / h														
	0.5			2			8			24			48		
	D	L	MO%	D	L	MO%	D	L	MO%	D	L	MO%	D	L	MO%
Rose bengal															
0	0	50	0^{d}	0	50	0^{e}	0	50	0^{f}	1	49	2^{f}	1	49	2^{d}
0.01	0	50	0^{d}	0	50	0^{e}	5	40	10^{e}	9	41	18^{e}	25	25	50^{c}
0.03	0	50	0^{d}	17	33	34^{d}	19	31	38^{d}	24	26	48^{d}	30	20	60^{b}
0.13	6	44	12^{c}	21	29	42^{c}	26	24	52^{c}	30	20	60^{c}	50	0	100^{a}
0.5	8	42	16^{b}	30	20	60^{b}	41	9	82^{b}	45	5	90^{b}	50	0	100^{a}
2	9	41	18^{a}	44	6	88^{a}	50	0	100^{a}	50	0	100^{a}	50	0	100^{a}
Ivermectin															
0	0	50	0^{c}	0	50	0^{f}	0	50	0^{f}	1	49	2^{e}	1	49	2^{d}
0.02	0	50	0^{c}	5	45	10^{e}	9	41	18^{e}	8	42	16^{d}	14	36	28^{c}
0.08	0	50	0^{c}	9	41	18^{d}	13	37	26^{d}	11	39	22^{c}	19	31	38^{b}
0.6	5	45	10^{b}	12	38	24^{c}	26	24	52^{c}	40	10	80^{b}	50	0	100^{a}
2.5	5	45	10^{b}	20	30	40^{b}	40	10	80^{b}	50	0	100^{a}	50	0	100^{a}
10	7	43	14^{a}	40	10	80^{a}	45	5	90^{a}	50	0	100^{a}	50	0	100^{a}

Conc. %: Concentration %; D.: Number of dead ticks; L.: Number of life ticks; MO%: mortality%; In vitro immersion assays have been performed. Concentrations were freshly prepared in distilled water; Values within a column followed by different lowercase letters were significantly different (P≤0.05), while values within a column followed by the same lowercase letters were not significantly different (P≤0.05).

Table 2: Lethal concentration (LC) values (%) of rose bengal and ivermectin after treatment of *Hyalomma dromedarii*

LC	Time post treatment/ h			
	2	8	24	48
Rose bengal				
50	0.19±0.13	0.08±0.01	0.05±0.01	0.01±0.01
60	0.33±0.27	0.13±0.02	0.08±0.01	0.02±0.01
70	0.60±0.66	0.20±0.04	0.13±0.02	0.03±0.01
75	0.83±1.09	0.26±0.06	0.17±0.04	0.03±0.02
80	1.19±1.91	0.35±0.08	0.23±0.05	0.04±0.03
84	1.65±3.18	0.46±0.12	0.30±0.08	0.05±0.05
90	3.07±8.24	0.77± 0.23	0.51±0.15	0.07±0.11
95	6.72±27.33	1.45±0.52	0.99±0.37	0.11±0.30
99	29.21±257.49	4.82±2.36	3.44±1.78	0.27±1.85
Ivermectin				
50	2.34±2.85	0.35±0.09	0.15±0.05	0.07±0.04
60	5.29±10.04	0.70±0.18	0.22±0.08	0.09±0.06
70	12.65±39.83	1.46±0.43	0.33±0.13	0.13±0.11
80	35.16±201.81	3.44±1.25	0.53±0.24	0.20±0.23
90	145.12±1936.08	11.28±5.50	1.03±0.60	0.36±0.67
95	467.73±12613.96	30.07±18.30	1.76±1.26	0.58±1.60
99	4198.08±428427.28	189.21±164.47	4.87±4.91	1.42±8.00

Table 3: The relative efficacy of rose bengal against *Hyalomma dromedarii* according that of IVR

Time post treatment/ h							
2		8		24		48	
LC50	LC90	LC50	LC90	LC50	LC90	LC50	LC90
12	47	4	15	3	2	5	5

LC: Lethal concentration value

Table 4: Lethal time (LT) values (per hour) of rose bengal and ivermectin after treatment of *Hyalomma dromedarii*

Rose	Concentrations (%)		
bengal	0.13	0.5	2
50	10.65±4.56	1.95±1.35	0.92±0.10
60	26.84±15.74	3.21±2.75	1.08±0.12
70	72.28±60.05	5.46±1.80	1.29±0.15
80	230.65±276.40	10.20±15.12	1.60±0.21
90	1152.95±2173.00	24.24±115.87	2.13±0.35
95	4352.92±11615.34	49.52±554.76	2.71±0.53
99	52561.92±262149.44	189.08±9972.87	4.25±1.09
Ivermectin	0.6	2.5	10
50	7.89±1.80	2.63±0.39	3.95±1.80
60	13.43±3.48	3.59±0.55	6.19±2.72
70	23.77±7.45	5.01±0.84	10.03±3.86
80	46.39±18.23	7.39±1.46	17.65±4.31
90	117.23±61.14	12.68±3.18	38.63±13.60
95	252.03±161.28	19.79±5.94	73.75±44.04
99	1058.67±942.06	45.64±18.37	247.98±259.11

acaricides and searching for safe alternatives to the currently available acaricides is very crucial, for use when resistance appears or to be used instead of existing pesticides to delay the appearance of resistance and to avoid environmental pollution (Khater 2011, 2012, 2013a) and drug residues (Cooper *et al*., 2011).

Eight hours PT with RB, our data revealed that the MLC that provoke 100% acaricidal effect was 2% and the LC50 and LC95 values were 0.08 and 1.45%, respectively. RB was 4 and 15 times more intoxicating than IVR at the levels of LC50 and LC90, respectively. The LT95 value was 2.71 h PT with 2% RB. Because we used photosensitizer against ticks for the first time, according to our knowledge, we faced a shortage of literature to discuss our results with. Consequently, we discussed our data with eco friendly materials applied to *Hyalomma spp.* and other tick species as well as sensitizers applied to other arthropods.

Consistent with our results, several botanicals induce acaricidal effects against *Hyalomma spp*., purified cardiac glycosides from *Digitalis purpurea* (digitoxin) and *Calotropis procera* induce acaricidal effect against *H. dromedarii* (Al-Rajhy *et al*., 2003). Tick population densities of *Hyalomma truncatum* on animals treated with neem seed extract, *Azadirachta indica*, were lower than those found on untreated animals (Weeb and David 2002). The toxic effects of the extracts of garlic, *Allium sativum,* effectively controlled adults of *Hyalomma marginatum rufipes* and *Rhipicephalus pulchellus* (Nchu *et al*., 2005). The commercial product, Neem Azal F, containing extract of neem seed oil effectively controlled *Hyalomma excavatum* as 100% mortality of unfed larvae and adults was reached at concentrations of 1.6-3.2%, seven days PT (Abdel-Shafy and Zayed 2002). Furthermore, oral administration of *Jatropha curcas* seed meal could be used in the treatment of *Hyalomma marginatum marginatum* at levels of less than 10% in the diet of rabbits without any serious effects on liver and kidney functions for 8 weeks PT (Abdel-Shafy *et al*., 2011). Furthermore, microbial control of *H. dromedarii* includes the potential activity of three varieties of *Bacillus thuringiensis*. Dipel 2x (*B. thuringiensis var. kurstaki*) is the most potent, followed by Vectobac (*B. thuringiensis var. israeliensis*), then HD 703 (*B. thuringiensis var. thuringiensis*) (Hassanain *et al*., 1997).

Our data indicate that the mean number of eggs per female of the negative control group ranged from 8529.06±84 to 12680.31±541. Similar observation was recorded for *H. dromedarii* (8076.0±989) (Alahemd and Kheir 2003). Moreover, we observed that sublethal concentrations of IVR (0.02 and 0.08%) adversely affected the reproduction potential of *H. dromedarii.* Likewise, flumethrin1%, in pour-on formulation and 87 µg active ingredient, adversely affected the fertility of engorged females of *H. dromedarii,* PT with contact method through reducing the percentage of ovipositing females, egg mass weight, number of eggs, percentage of females laying eggs that hatch, and the conversion efficiency of female weight to egg mass weight (El-Azazy and Lucas 1996). Furthermore, the number of ovipositing females, deposited eggs, and hatched larvae of *Argas persicus* decreased markedly as the dose of IVR increased (Marzouk *et al*., 2004). Our records indicate that the hatchability percentage of the untreated control groups ranged from 93.56 to 94.48%. Consistent results were also reported for *H. dromedarii,* 94.49% (85.80 ~ 99.70%) (El Hakim *et al*. 2011); 82-94% (ELGhali and Hassan 2010); and 99% (Alahmed and Kheir 2003).

Photodynamic acaricides, in the present study, induced not only acute toxic effects, but also manifested subacute or chronic effects by impairing the reproduction of ticks, through reducing the numbers of survived and ovipositing females as well as the numbers of laid and hatched eggs. Similar reproduction failures were reported for botanicals. The essential oil of *Citrus sinensis* var. *balady* has strong toxic effect on eggs of *H. dromedarii* especially in the earlier embryonic development (Habeeb *et al*. 2007). Egg hatchability of *H. excavatum* is seriously impaired PT with Neem Azal F (Abdel-Shafy and Zayed

Table 5: The efficacy of rose bengal and ivermectin on the reproductive potential of *Hyalomma dromedarii*

	Survived females		Ovipositing Females			Eggs/Female	Ticks laid hatched eggs			Hatched eggs		
Conc. %	NO.	R%	NO.	%	R%	Mean ± SE	NO	%	R%	No.	%	R%
Rose bengal												
0	49[a]	0	45[a]	91.84	0	8529.06±84[a]	40[a]	88.89	0.00	7980.00±361[a]	93.56	6.44
0.01	25[b]	48.98	3[b]	12.00	93.33	1854.53±45[b]	1[b]	33.33	97.5	542.40±7[b]	29.25	93.64
0.03	20[c]	59.18	1[b]	5.00	97.78	1376.40±43[c]	0[b]	0.00	100	0[c]	-	100.00
0.13	0[d]	100.00	0[c]	-	100.00	0[d]	0[b]	-	100	0[c]	-	100.00
0.5	0[d]	100.00	0[c]	-	100.00	0[d]	0[b]	-	100	0[c]	-	100.00
2	0[d]	100.00	0[c]	-	100.00	0[d]	0[b]	-	100	0[c]	-	100.00
Ivermectin												
0	49[a]	0.00	45[a]	91.84	0.00	12680.31±541[a]	41[a]	91.11	0	11980.70±600[a]	94.48	5.52
0.02	36[b]	26.53	6[b]	16.67	86.67	7661.27±377[b]	5[b]	83.33	87.80	1345.00±52[b]	17.56	89.40
0.08	31[c]	36.73	5[b]	16.13	88.89	1393.72±112[c]	0[c]	0.00	100	0	-	100.00
0.6	0[d]	100.00	0[c]	-	100.00	0[d]	0[c]	-	100	0	-	100.00
2.5	0[d]	100.00	0[c]	-	100.00	0[d]	0[c]	-	100	0	-	100.00
10	0[d]	100	0[c]	-	100.00	0[d]	0[c]	-	100	0	-	100.00

NO. Number; R%: reduction %; Mean ± SE: Mean ± standard error; Values within a column followed by different lowercase letters were significantly different (P≤0.05), while values within a column followed by the same lowercase letters were not significantly different (P≤0.05).

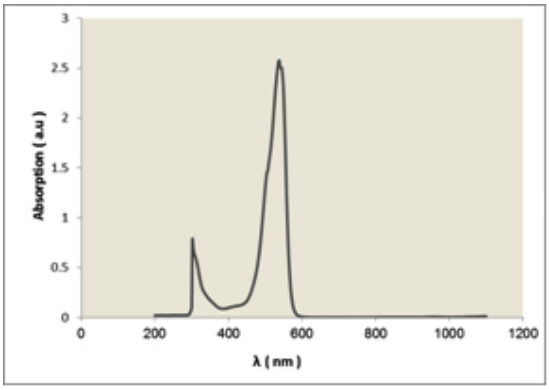

Fig. 1: The absorption spectra of rose bengal

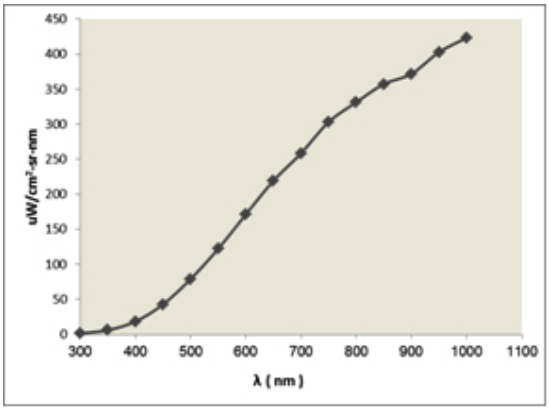

Fig. 2: Emission spectra of used white- light source

2002). *Artemisia absinthium* has acaricidal and ovicidal effect against the dog tick, *Rhipicephalus sanguineus* (Godara *et al*., 2014). Eggs of *H. dromedarii* are mostly affected 25 days PT with varieties of *Bacillus thuringiensis* (Hassanain *et al*., 1997). Moreover, the detrimental effect of peracetic acid, 0.25%, against the cattle tick, *Boophilus annulatus*, extended beyond the adult stage, lead to significant decrease in the mean number of the laid eggs (Khater and Ramadan 2007). Besides, peracetic acid effectively controlled larvae of *A. persicus in vitro* (Khater and Ramadan 2007, Khater *et al*., 2013a) and in vivo and inhibited its molting (Khater *et al*., 2013a).

Analogues to the light-induced killing observed in the present study, RB was used as effective insecticide against mosquito larvae, *Culex pipiens* and *Aedes triseriatus* (Carpenter *et al*. 1984). RB effectively controlled the 4th instar larvae of three species of mosquito larvae, *Aedes aegypti* (L.), *Anopheles stephensi* (Liston), and *Culex quinquefasciatus* (Say), grown in the laboratory (Dondji *et al*. 2005). Compared with other photosensitizers, RB seemed to be more efficient at even lower concentration than chlorin (e6) and chlorophyllin on *Ae. aegypti* larvae. Moreover, RB effectively controlled C*x. quinquefasciatus* in field test in Bobo-Dioulasso, Burkina Faso and the mortality induced by RB varied from 80 to 96% obtained with unfiltered cesspit water to 0.4 to 6.7% in cesspits with a heavy load of organic materials (Dondji *et al*. 2005). The greatest photosensitizing activity is displayed by tetraiodo xanthene derivatives, such as RB and erythrosin B. Both dyes are significantly more efficient than their tetra-bromo analogue (eosin yellow) in killing *Musca domestica* (Fondren *et al*. 1978, 1979). After the addition of a specific hydrocarbon, RB effectively controlled different stages of the onion fly, *Hylemyia antiqua* (eggs, larvae, pupae, and adults) within 15 sec to 15 min PT with different concentrations (0.01270, 0.00145, 0.00127, and 0.000029 µg/L) and different light exposure times (Aref 2010).

Alike RB, used in the present study, porphyrins are prone to exhibit an efficient photoexcitation (Ben Amor and Jori 2000) against insects (Rebeiz *et al*., 1990). Different porphyrins and substituted porphyrins were found to be toxic against adults of several dipteran species such as *Stomoxys calcitrans* (Ben Amor *et al*. 1998a,b); *Ceratitis capitata* (Pujol-Lereis *et al*., 2010); *Bactrocera oleae*, *Liriomyza bryoniae* (Lukðienë *et al*. 2005; Buda *et al*., 2006; Luksiene *et al*., 2007); *Drosophila melanogaster* (Smijs *et al*., 2004); and *Eretmapodites quinquevittatus* (Helleck and Hartberg, 1999). Porphyrins are also highly phototoxic against the aquatic larvae of mosquitoes, like *C. pipiens* (Salama *et al*., 2002), *Ae. aegypti* (Dondji *et al*., 2005; Lucantoni *et al*., 2011), *C. quinquefasciatus* and *A. stephensi* (Dondji *et al*., 2005).

Salama *et al*. (2002) demonstrated that hematoporphyrin IX (HP IX) produced important histopathological effects on the midgut, epidermis, fat body, and muscles of *C. pipiens* aquatic larvae.

Besides their pesticidal effect, photosensitiesers 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 at a clinical level for treatment of infectious diseases, as well as for providing alternatives for blood disinfection and vector control (Baptista and Wainwright, 2011). Photodynamic processes are used also to address environmental problems of high significance, such as the decontamination of wastewaters, the disinfection of fish-farming tanks, protection of animal species (e.g., amphibians and reptiles) that are endangered by pathogens whose life cycle takes place largely in aqueous media, and control of populations of noxious insects (Jori *et al*., 2011).

A photodynamic effect occurs when photosensitiser molecules absorb light and dissipate the absorbed energy by transferring it to biological acceptors (usually oxygen), generating an excess of reactive species, redox imbalance in the cells, that are able to force cells into apoptotic pathways (Baptista and Wainwright, 2011). A photoactive compound accumulates within the insect following its exposure to visible light and induces damage of its cuticle, midgut wall (Salama *et al.,* 2002; Ben Amor *et al*. 1998b), body fat and muscles (Salama *et al*., 2002), and malpighian tubules followed by feeding inhibition and eventual death (Ben Amor *et al*., 1998b). Consequently, most photosensitizers are able to induce apoptotic cell death (Lukšienë 2003; Eggen *et al*., 2005).

Although the energy absorbed from UVR by RB is greater than their counterparts in the green spectrum, according to our measurements (Fig 1), the latter is the most influential due to severe absorbed dye proposed (RB). Therefore, we suggest that a white light spectral lamp with green filter can be used.

RB is an anionic water-soluble xanthene photosensitizer. It is an efficient generator of cytotoxic singlet oxygen (Φ= 0.79) upon photoactivation (Miller 2005) which is capable of photocatalytic conversion of oxygen molecules (O_2) to yield singlet oxygen ($^1O_2$) upon irradiation with green light. Hence, it has been considered a promising sensitizer in photodynamic therapy (PDT) of tumors, with minimal side effects (Wachter *et al*., 2003).

Analogous to our results, the optical properties of porphyrins indicated that they are interesting photoinsecticides. The intense absorption band for RB occurred at the green and UV spectral region, whereas those for porphyrins occurred at the blue spectral region. These spectral regions represent the maximum emission spectrum in the sunlight at midday, and also in the red region predominant at dawn and sunset, wavelengths >600 nm (Ben Amor and Jori, 2000). This is due to the scattering by small particles in the atmosphere and according to Rayleigh law (Jenkins and White, 1979) which indicates that the intensity of light scattering (I_s) is inversely proportional to the wavelength to the power four ($1/\lambda^4$).

Regarding safety of photosensitizers, they are non-toxic in the dark and less prone to accumulate, because they are usually bleached and further degraded by light. Sunlight-activated compounds are usually characterized by a low environmental impact and negligible toxicological risk for humans, plants, or animals and photosensitizers are registered as food additives or phototherapeutic agents (Ben Amor *et al*., 1998b). More importantly, pests do not acquire resistance against photoactive compounds (Lukðienë *et al*., 2005).

The efficiency of photoactivity depends on several parameters such as concentration, physical, chemical, and biological features, fluence rate of light delivered and irradiation time, as well as its phototability (Ben Amor and Jori 2000; Hasan *et al*., 2003, Tonnesen 2004). In addition, the property typical of photosensitizers to absorb essentially all of the wavelengths in the sun's emission spectrum allows the promotion of processes largely based on natural resources (sun light) with significant energy savings and low impact on ecosystems.

Regarding comparison between the used light source (LS) and sunlight (Su), when the light is falling on RB, the total optical energy (E) falling on it is given by $E = I\,t$ (equation 1), according to Dougls (1979), where (I) is the intensity of the light per unit area and (t) is the exposure time. Consequently, the total optical energy of the light source is $E_{LS} = I_{LS}\,t_{LS}$ (equation 2) and for sunlight is $E_{Su.} = I_{Su.}\,t_{Su.}$(equation 3). By dividing equation 1 by equation 2, we got the following equation: $E_{LS} / E_{Su.} = I_{LS}\,t_{LS} / I_{Su.}\,t_{Su.}$(equation 4).

At $E_{LS} = E_{Su}$, we get on the ratio between intensity per unit area of the sunlight ($I_{Su)}$ at the top of Earth's atmosphere and the intensity per unit area of the light source (I_{LS}), as a result, $I_{Su.}/ I_{LS} = t_{LS} / t_{Su.}$(equation 5). The intensity of the sunlight (I_{Su}) at the top of Earth's atmosphere is about $14x10^{-2}$ W/cm^2-sr-nm (Hecht 2004), whereas the intensity of the light source (I_{LS}) is corresponding to the absorbed spectrum ($\lambda_{max.}$= 536nm) by RB is 100μW/cm^2-sr-nm (Fig 2).

When we compare the exposure times of light source (t_{LS}) and sunlight (t_{Su}), we find that $t_{LS} / t_{Su} = 14x10^{-2}$ W/cm^2-sr-nm / 100μW/cm^2-sr-nm = $14x10^2$ (equation 6). The previous equation indicates that the exposure time of RB to sunlight is much less than those of the light source ($t_{Su} << t_{LS}$) at the same energy. This means that the exposure of RB to sunlight will be more efficient than its exposure to the light source, which is a very practical point. To get the same energy form the sun light, the irradiance of used light source should be 14×10^{-2} W/cm^2, at the same exposure time.

Conclusion

RB is highly effective when used at lower doses and for short exposure time. The faster acaricidal effect is very important (Khater and Ramadan 2007; Khater *et al*., 2013a) for avoidance of the hazard ensued by pathogen transmission in the course of delayed mortality caused by the currently used acaricides (Uspensky and Uspensky 2006). The photodynamic effect of RB is considered promising when compared to the effects of IVR, the commonly used acaricides. Caution should also be exercised over any use of broad-spectrum acaricides as such applications will inevitably hasten the selection for

resistance in gastrointestinal nematodes (Wall 2012). It is not advised to use IVR as systemic acaricide especially on dairy animals (Imperiale *et al*., 2004). Photosensitizers could be incorporated into programs of integrated pest management, which do not rely on biological control agents (Martin *et al*. 1998), to overcome tick populations as they are safe and do not cause environmental pollution, which is the most dangerous drawback of chemical acaricides.

Although we got good results using a weak light source in the visible region, the sun light is more powerful than the used lamb (1400 times). This could be explained as the following, if we expose ticks to RB for 1400 second illuminated by the used light source, we would get the same effect after exposure to sun light for 1 second. This is a very practical point as camels live in the desert where sun light is available all the year round.

Exposure of RB to sunlight will be more efficient and applicable than its exposure to an artificial light source. As green light, the extreme component of the sunlight during the day, is absorbed well by RB, a white- light-spectral lamp with a green filter could be used for enhancing the efficacy of RB in case of *in vitro* treatments or in situations and countries where there is limited sunshine, such as any country which is near to either the north or south pole will have a significantly reduced amount of daylight hours. Phototreatment protocols could be tailored to specific insects and environmental conditions under actual field situation and their photostability could be enhancing through carriers. For example, loading RB in multivesicular liposomes is a promising approach to improve the photodynamic efficacy of RB, by enhancing its photostability and delivery into cells (Fadel and Kassab 2011). Perhaps phtotoactive compounds will open a new avenue for the development of new generation of pesticides, which would be human-safe, environmentally friendly, low-cost, and not mutagenic for pests.

Acknowledgments

The authors thank Prof. Dr. Azza A. Moustafa, Research Institute of Medical Entomology, Egypt, for her valuable help and support and Prof. Dr. Mohamed Y. Ramadan for the identification of the parasites and his valuable advices. Our sincere gratitude is also given to Dr. Tarek Y. Elrasasi, Physics Department, Faculty of Science, Benha University, for his fruitful assistance.

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