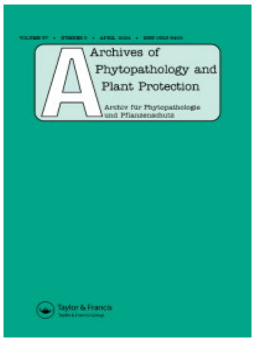

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RESEARCH ARTICLE

Casting light on the potency of photosensitizing compounds to combat the spiny bollworm, *Earias insulana* (Biosd.)

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ABSTRACT
The emergence of resistance among pests to conventional pesticides underscores the necessity for substantial efforts in identifying alternatives for managing cotton pests. So the research aimed to use the photosensitizing compounds Rose Bengal, Rhodamine B, and Methylene Blue for sustainable and eco-friendly managing of cotton pests, mainly, the second instar larvae of *Earias insulana*. Results indicated a high efficacy of Rose Bengal when scored 1.24x10^{-5}M and 1.26x10^{-4}M LC_{50} and LC_{90}, respectively followed by other compounds. Rose Bengal, Rhodamine B and Methylene Blue scored 2 h highest LT_{50} at concentrations 3x10^{-5}M. While LT_{50} values were 0.30, 0.55 and 1:45 h at 1x10^{-4}M. Rose Bengal was high effective against *E. insulana* larvae compared to Methylene Blue and Rhodamine B. Moreover, the findings revealed a discernible negative correlation between the concentrations applied and the necessary median lethal time for all tested compounds indicating their efficacy against *E. insulana* larvae.

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KEYWORDS
Spiny bollworm; *Earias insulana*; photosensitizers; median lethal time (LT_{50})

Background

Cotton is one of significant crops supporting Egyptian agricultural economy, as a source of raw materials for local industries and those closely connected. Additionally, the cotton industry supports a substantial labor force, making a notable impact on the influx of international funds (Ref.). The cotton plant is attracted by various insect pests including spiny bollworm (SBW) *Earias insulana* (Boisd.) (Sayed and Hams 2022). It has been reported to impact cotton, maize, and okra crops, not only in Egypt but also worldwide (Ref.). This persistent pest is feeding on the reproductive parts of cultivated cotton plants, resulting in a potential loss

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of one cotton boll for every one to two plants affected. *Earias* spp. can cause a qualitative deterioration of roughly 50% in cotton due to lint discoloration, coupled with an estimated 40% decrease in yield (Sayed and El-Ghobary 2019, Abd-ElAzeem et al. 2023).

Sunlight-triggered photo-pesticides are a promising approach in pest control methods, providing an eco-friendly substitute for conventional chemical pesticides, including *E. insulana* (Ben Amor and Jori 2000). When sunlight- activated photo-pesticides exposed to sunlight, these molecules initiate a photochemical reaction, releasing the active compounds against the specific pest they target. The 2nd instar larvae of *E. insulana* exhibit the behavior of entering and exiting cotton bolls makes it more prone to sunlight exposure. They come into contact with the surface of the treated cotton bolls making them vulnerable to the used photoactive compounds and subsequently to sunlight, which is crucial to activate the compounds in the insect's body. The photo pesticides would then reach the larva's midgut through ingestion. The impact on the insect's midgut would be a result of the specific mode of action of the photo pesticides, affecting processes such as enzyme activity or nutrient absorption (Katagi 2018; Abdel-Aziz and Habit 2021).

Photosensitizers are ecofriendly compounds with low environmental impact and irrelevant toxicological hazard for humans, animals, or plants, (Ben Amor and Jori 2000). Applying these compounds to cotton through spraying during sunny hours is recommended for optimal effectiveness against target pests. The short stability of these compounds minimizes their impact on non-target organisms, emphasizing their relative safety to beneficial insects and other unintended species in the cotton field offers numerous unique benefits. Also, significantly diminishes dependence on traditional chemical pesticides, thus lessening the potential risks to the environment, unintended species, and human well-being. Moreover, its reliance on natural sunlight as a catalyst enables precise scheduling of application, enhancing its efficiency and potentially cutting down overall expenses (Sonhafouo-Chiana et al. 2022). Moreover, sunlight-activated photo-pesticides are in harmony with the worldwide transition toward sustainable and environmentally friendly agricultural methods. They effectively tackle drawbacks related to pesticide resistance, pollution of the environment, and health risks associated with conventional chemical pesticides (Pathak et al. 2022).

This work aimed at testing sunlight-triggered photo-pesticides against the spiny bollworm, due to their unique composition and eco-friendly benefits.

Methods

The insect rearing

The *E. insulana* strain used in the study was provided by the Cotton Bollworms Research Department at the Plant Protection Research Institute within the Agricultural Research Center in Dokki, Giza, Egypt. This pest was maintained in a laboratory, insecticide free-environment, spanning over six generations, at a constant temperature of 26 ± 1 °C and relative humidity 65–75%. The insects were fed on daily diet composed of an artificial formulation primarily derived from beans, prepared following the procedure outlined by Amer (2015).

Sunlight-responsive compounds used

A. Rose Bengal
Product Name: Rosets
Chemical Formula: C_{20} H_4 Cl_4 I_4 O_5
Molecular Weight: 973.67 g/mol
Quantum Yield: 0.76

B. Rhodamine B
Product Name: Rhodamine 610
Chemical Formula: C_{28} H_{31} Cl N_2 O_3
Molecular Weight: 479 g/mol
Quantum Yield: 0.65

C. Methylene Blue
Product Name: Urolene Blue
Chemical Formula: C_{16} H_{18} N_3 S Cl
Molecular Weight: 319.85 g/mol
Quantum Yield: 0.52

Bioassays

The three newly formulated photosensitive compounds were initially prepared through dissolving 1 g of dye in 100 mL distilled water) to make stock solutions. Subsequently, a serial of concentrations (3×10^{-5}, 5×10^{-5}, 8×10^{-5}, 1×10^{-4} M for Rose Bengal; 3×10^{-5}, 5×10^{-5}, 8×10^{-5}, 1×10^{-4}, 1×10^{-3}M for Rhodamine B and 1×10^{-5}, 1×10^{-4}, 1×10^{-3}, 8×10^{-3}M for Methylene Blue) were freshly prepared before application. An initial experiment involved in applying 1 ml of each concentration to 3 g portion of the synthetic diet. The diet was prepared as follows: Boiled water was added to ground kidney beans (250 g) and wheat (125 g). The mixture was heated for 70 min, and let to cool and dry for 20 min. About 100 ml of milk was added to the mixture and whipped in a waring blender and kept for 24 h at 4 °C. After that 49 g dry active yeast, 3 g ascorbic acid, 1.75 g sorbic acid, 1.75 g methyl parahydroxy benzoate, 8 ml of vitamin mixture and 2.5 ml formaldehyde 34–38% were added. All thoroughly blended and kept for 24 h at 4 °C before use (Amer 2015). Twenty larvae of *E. insulana* at 2nd stage were fed on each treated diet in 3 replicates. A control group was fed on an artificial diet mixed with distilled water. After one day of feeding in darkness, the treated larvae were exposed to sunlight and checked every 15 min for a duration of 2 h. The mean mortality percentages were adjusted using Abbott's formula (1925). The mortality percentages were statistically analyzed following Finney (1971). For each compound, the LC_{50} and LC_{90} values were calculated. Additionally, the toxicity index was measured according to Sun's method (1950), and potency levels were computed as follows:

$$\left(\text{Sun's Toxicity index}\right)=\frac{\text{LC50 or LC90 of the most toxic compound}}{\text{LC50 or LC90 of the tested other compounds}}$$

$$\left(\text{Potency levels}\right)=\frac{\text{LC50 or LC90 of the least toxic compound}}{\text{LC50 or LC90 of the tested other compounds}}$$

Statistical analysis

The data obtained were subjected to statistical scrutiny through analysis of variance to elucidate notable variations among treatments. A significance threshold of 5% was consistently employed in all statistical examinations. CoStat (1995) statistical software program was utilized for statistical analyses.

Results

Toxicological studies

Susceptibility of E. insulana 2nd instar larva to three photosensitizing compounds

Laboratory experiments revealed a considerable toxicity of Rose Bengal, Rhodamine B, and Methylene Blue against the tested larvae (Table 1). LC_{50} values ranged 8.521×10^{-5} M – 1.24×10^{-5} M. In terms of toxicity, Rose Bengal scored 1.24×10^{-5} M and 1.26×10^{-4} M highest LC_{50} and LC_{90} values, respectively. Rose Bengal caused 38.71% and 100% mortality at concentration 3×10^{-5} M and 1×10^{-4} M, respectively. Whereas,

Table 1. Susceptibility of 2nd instar larvae of the spiny bollworm, *E. insulana* to three photosensitizing compounds.

Photosensitizing compounds	Conc. (M)	% Mortality	Slope	LC_{50} (M)	LC_{90} (M)
Rose Bengal	1×10^{-4}	100.00	1.319	1.24×10^{-5}	1.26×10^{-4}
	8×10^{-5}	53.33			
	5×10^{-5}	62.50			
	3×10^{-5}	38.71			
Rhodamine B	1×10^{-3}	73.33	0.748	8.52×10^{-5}	4.38×10^{-3}
	1×10^{-4}	62.07			
	8×10^{-5}	57.07			
	5×10^{-5}	53.33			
	3×10^{-5}	19.23			
Methylene Blue	8×10^{-3}	100	1.284	1.95×10^{-5}	2.002×10^{-4}
	1×10^{-3}	100			
	1×10^{-4}	53.33			
	1×10^{-5}	46.67			

% Mortality was determined after two hours from exposure to sun light.

Table 2. Toxicity index, slope values, $LC_{90}/LC_{50,}$ and potency levels of the 2nd instar larvae of *E. insulana* treated with three tested compounds.

Photosensitizing compounds	Toxicity index based on		Slope	LC_{90}/LC_{50}	Potency levels	
	LC_{50}	LC_{90}			LC_{50}	LC_{90}
Rose Bengal	100	100	1.319	10.16	6.87	34.76
Rhodamine B	14.55	2.87	0.748	51.41	1.00	1.00
Methylene Blue	63.59	62.93	1.284	10.26	4.37	21.88

mortality rates resulted from Methylene Blue and Rhodamine B treatments were 46.67, 100% and 19.23, 73.33% at concentrations 1×10^{-5} M, and 3×10^{-5} M and 1×10^{-3} M, respectively.

Toxicity index and potency levels

Rose Bengal the highest toxicity index measured at LC_{50} and LC_{90} concentrations, followed by Rhodamine B and Methylene Blue (Table 2). The toxicity index values were 14.55 and 63.59%, and 2.87 and 62.93%, respectively, against the 2nd instar larvae of the pest following a two-hour exposure to sunlight.

T Rose Bengal treatment slope value was 1.319 compared to Rhodamine B and Methylene Blue treatments scoring 0.748., and 1.284 slope values.

Similarly, the potency at LC_{50} and LC_{90} levels showed the two photosensitizing compounds, Rose Bengal and Methylene Blue, against the 2nd instar larvae of the tested pest, were 6.87,34.76 and 4.37,21.88 times more toxic, respectively, compared to the toxicity of Rhodamine B.

Photodynamic effect of tested compounds

The mortality rate of 2nd instar larvae treated with Rose Bengal concentration 3×10^{-5}M and exposed to 30 min of sunlight scored 18.45%. The mortality increased, with no significant differences scored the maximum 37.88% 2:00h of sunlight exposure (Table 3 and Figure 1) The highest Rose Bengal concentration (1×10^{-4} M), scored maximum 90.00% larval mortality following h the longest 2 h exposure periods under sunlight.

In contrast, during the initial 0:30hr of sunlight exposure, Rhodamine B did not cause any mortality among treated larvae across all tested concentrations. However, mortality increased when sunlight exposure was extended up to 2 h scoring 19.23%, at the lowest 3×10^{-5}M concentration. Similarly, larvae treated with higher concentrations scored maximum mortality up to 72.46% 2:00 h of light exposure. (Table 4 and Figure 2).

Finally, Methylene Blue lowest 1×10^{-5} M concentration, induced a 6.67% mortality rate after 0:30 hr of sunlight exposure. Larval mortality increased when a longer sunlight exposure time was applied, reaching a peak of 46.67% after one and two h of exposure. Similarly, at higher

Table 3. Photodynamic effect of different concentrations of Rose Bengal on the 2nd instar larva of *E. insulana* at different periods of sunlight exposure.

Sunlight exposure periods (hrs.)	% Mortality at indicated concentrations of Rose Bengal expressed as mole			
	3×10^{-5}	5×10^{-5}	8×10^{-5}	1×10^{-4}
0:30	18.49	37.88 ab	36.67 a	62.76 b
1:00	28.18	53.33 a	43.33 a	75.93 ab
1:30	34.85	59.39 a	50.00 a	79.93 ab
2:00	37.88	62.42 a	53.33 a	90.00 a
Control	0.0	0.0 b	0.0 b	0.0 c
F test	1.79	4.04	13.83	21.29
$LSD_{0.05}$	35.98	40.20	18.19	24.49
P	ns	0.0335*	0.0004***	0.0001***

The same letter in the same column means not significant at *P*0.05.
LSD The least significant difference.
*slightly significant, ***high Significant.

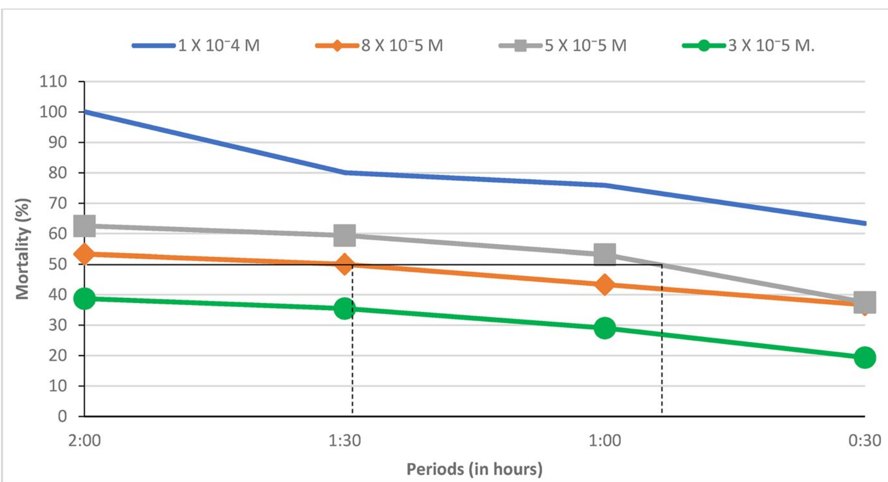

Figure 1. Effect of Rose Bengal on the mortality percentages of E. *insulana* 2nd instar larva exposed to sunlight for different time intervals.

Table 4. Photodynamic effect of different concentrations of Rhodamine B on the 2nd instar larva of *E. insulana* at different periods of sunlight exposure.

Sunlight exposure periods (hrs.)	% Mortality at indicated concentrations of Rhodamine B expressed as mole				
	3 X 10^{-5}	5 X 10^{-5}	8 X 10^{-5}	1 X 10^{-4}	1 X 10^{-3}
0:30	0.00	0.00 c	0.00	0.00 b	0.00 b
1:00	15.38	36.67 b	31.67	58.89 a	62.76 a
1:30	19.23	43.33 ab	31.67	62.22 a	69.13 a
2:00	19.23	53.33 a	55.00	62.22 a	72.46 a
Control	0.00	0.00 c	0.00	0.00 b	0.00 b
F test	0.79	47.08	2.62	33.66	45.06
$LSD_{0.05}$	30.44	11.51	45.96	18.19	17.59
P	ns	0.0000 ***	ns	0.0000 ***	0.0000 ***

The same letter in the same column means not significant at *P*0.05.
LSD The least significant difference.
***high Significant.

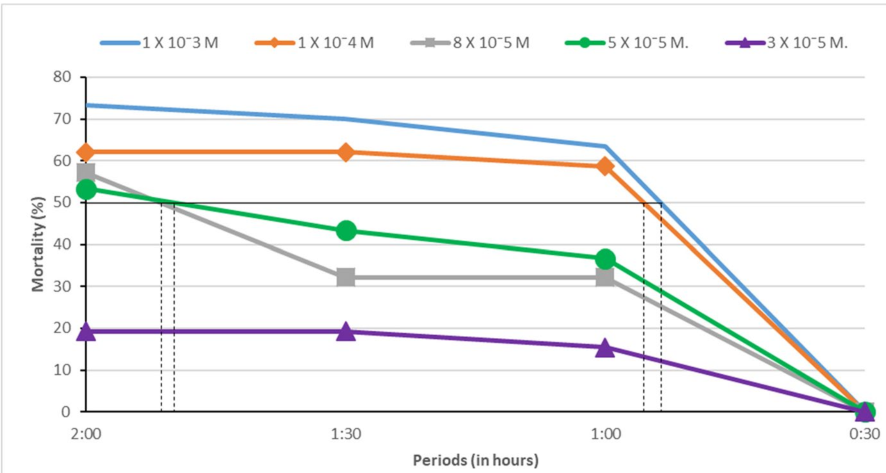

Figure 2. Effect of Rhodamine B on the mortality percentages of E. *insulana* 2nd instar larva exposed to sunlight for different time intervals.

concentrations, larval mortality increased up to 100% after 2:00 h of sunlight exposure, (Table 5 and Figure 3).

The median lethal time (LT_{50}) of Rose Bengal value passed over 2h of sunlight exposure period at the lowest concentration (3×10^{-5} M). This duration decreased to minimum 0:30 h of sunlight exposure at the higher concentrations (Table 6 and Figure 1).

Similarly, the LT_{50} value at the lowest Rhodamine B concentration exceeded 2h of sunlight exposure, while decreased at higher concentrations (Table 6 and Figure 2).

Discussion

The toxicity mechanisms induced by these photosensitizers include damaging the midgut membrane, altering potassium levels in the hemolymph, affecting membrane permeability, and causing physiological and

Table 5. Photodynamic effect of different concentrations of Methylene Blue on the 2nd larval instar of *E. insulana* at different periods of sunlight exposure.

Sunlight exposure periods (hrs.)	% Mortality at indicated concentrations of Methylene Blue expressed as mole			
	1×10^{-5}	1×10^{-4}	1×10^{-3}	8×10^{-3}
0:30	6.67 b	16.67 b	46.03 b	63.36 b00
1:00	42.20 a	46.67 a	79.89 a	100 a
1:30	42.20 a	46.67 a	100 a	100 a
2:00	46.67 a	53.33 a	100 a	100 a
Control	0.00 b	0.00 b	0.00 c	0.00 c
F test	29.65	7.25	45.06	432.80
$LSD_{0.05}$	12.91	27.09	21.25	8.42
P	0.0000***	0.0052**	0.0000***	0.0000***

The same letter in the same column means not significant at *P*0.05.
LSD The least significant difference.
**moderate significant, ***high Significant.

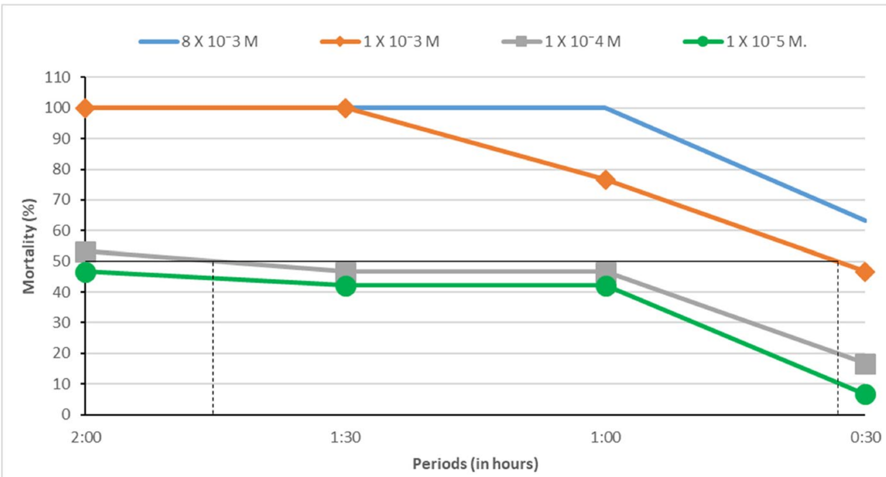

Figure 3. Effect of Methylene Blue on the mortality percentages of E. *insulana* 2nd instar larva exposed to sunlight for different time intervals.

morphological malformations at various developmental stages, ultimately impacting arthropod development and fecundity (Ben Amor and Jori 2000; Baptista and Wainwright 2011).

The photochemical process initiated by sunlight liberates active compounds that prove fatal to the targeted pest, ensuring a precise and effective mechanism (Abdel-Aziz and Habit 2021).

The intriguing aspect lies in recognizing the pivotal role conventional pesticides play in cotton pest control programs. Nonetheless, with the emergence of pest resistance to diverse pesticides, it becomes apparent that considerable endeavors are necessary to pinpoint effective alternative control strategies (Siddiqui et al. 2023). As indicated by numerous studies, compounds functioning as photosensitizers have the potential to serve as substitutes for traditional chemical pesticides in the future (Pieterse et al. 2023).

Table 6. Median lethal time of photosensitizing compounds against the 2nd larval instar of *E. insulana* exposed to sunlight for different time intervals.

Photosensitizing compounds	Conc. (M)	LT_{50} in hours
Rose Bengal	1×10^{-4}	< 0:30
	8×10^{-5}	1:30
	5×10^{-5}	0:50
	3×10^{-5}	>2
Rhodamine B	1×10^{-3}	0:50
	1×10^{-4}	0:55
	8×10^{-5}	1:50
	5×10^{-5}	1:55
	3×10^{-5}	>2
Methylene Blue	8×10^{-3}	< 0:30
	1×10^{-3}	0:35
	1×10^{-4}	1:45
	1×10^{-5}	>2

Several studies have indicated the insecticidal mechanism of photosensitizing compounds as they accumulate within the insect body induces lethal photochemical reactions after exposure to visible light, causing destruction and finally death of cells (Lukšiene et al. 2007). Photosensitizers, typically chemical compounds, absorb light energy and then transfer it to molecular oxygen, initiating the production of reactive oxygen species (ROS) within the insect's cells. These generated ROS, including singlet oxygen and free radicals, induce oxidative stress, causing damage to essential cellular components such as proteins, lipids, and DNA. The cumulative impact of this oxidative damage disrupts vital cellular functions, interferes with metabolic pathways and compromises cellular membranes. Ultimately, the collective disturbances lead to the demise of the insect. This process aligns with the mechanism of photodynamic activity. The thrilled 1Sens state of the photosensitizer transforms into the lively 3Sens state by navigating a crossing system, aided by the absorption of light photons. Because of the prolonged duration of the excited triplet state, it significantly aids in elevating molecular oxygen's triplet ground state ($3\,O_2$) to the exceedingly harmful $1\,O_2$ state when exposed to light. The extended lifespan of the triplet state plays a vital role in initiating the excitation of molecular oxygen into a potent and cytotoxic singlet state, offering versatile applications, notably in insect control. This mechanism underscores the role of photosensitizers in initiating a cascade of reactions that result in oxidative stress and, consequently, the demise of the targeted insect (Vilensky and Feitelson 1999, Attia 2016, El-Ghobary et al. 2018, Lima et al. 2018). Mangan and Moreno (2001) mentioned that the efficiency of the photosensitizers used as pesticides depends on the feeding intensity, sunlight exposure and ingestion of the target insect species.

The three photosensitizing compounds were highly toxic against 2nd instar larvae of *E. insulana* when exposed to varying concentrations and different durations of sunlight. The outcomes of this experiment revealed distinct susceptibilities among the treated larvae. The LC_{50} values ranged from 1×10^{-5} M to 8×10^{-3} M. The results consistently indicated that Rose Bengal exhibited the highest efficacy, followed by Methylene Blue. This observation aligns with prior studies conducted by various researchers on different insects in both laboratory and field settings. For instance, studies by Fairbrother et al. (1981) on *Musca domestica*, Aref (2010) on *Hylemyia antiqa*, Abdel-Aziz et al. (2013) on *Spodoptera littoralis* also, Abdel-Aziz and Habit (2021) on *Agrotis ipsilon* all reported similar trends.

This concurs with the findings of Younis et al. (2020), who emphasized the crucial role of sunlight in activating the Rose Bengal photosensitizer compound, highlighting its notable effectiveness at low concentrations and short exposure times. The collective evidence underscores the potential of these photosensitizing compounds as promising agents for insect control, substantiated by both current and previous research outcomes.

The chemical structure of Rose Bengal, comprises the highest number of halogen atoms, including featuring four iodine (I) and four chlorine (Cl) atoms t, compared to Rhodamine B and Methylene Blue. Halogen composition may be involved in their pesticidal and photosensitizer activities and the time of exposure to sunlight, as well (Attia 2016, El-Ghobary et al. 2018; Sułek et al. 2020).

Conclusion

Our investigation demonstrated the high activity of Rose Bengal, Rhodamine B, and Methylene Blue against the 2nd instar larvae of SBW *E. insulana* under laboratory conditions. Based on LC values, Rose Bengal emerged as the most potent photosensitizer, followed by Methylene Blue and Rhodamine B. These eco-friendly alternatives can be potential for integration into SBW pest management programs. Further in-depth investigations are recommended to assess the feasibility for comprehensive pest control strategies.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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