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Evaluating the efficiency of photoactive compounds against armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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Abstract

The present investigation aimed to evaluate the efficacy of three photosensitizing compounds, namely rose bengal, rhodamin B and methylene blue against 4th instar larvae of the fall armyworm, *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) under laboratory conditions. The obtained results indicated that rose bengal was the most effective photosensitizer compound. The corresponding LC_{50} and LC_{90} values against the 4th instar larvae were $0.018x10^{-7}$M and $0.260x10^{-7}$M; followed by *rhodamine* B, where the LC_{50} and LC_{90} levels recorded $0.040x10^{-7}$M & $0.320x10^{-7}$M; on the other hand, exhibited least efficient against the 4th instar larvae of the tested pest, the corresponding LC_{50} and LC_{90} values being $0.020X10^{-5}$M &$0.340x10^{-5}$M; respectively. Photosensitization associated with the treated 4th instar larvae revealed that the LT_{50} value of the lowest concentration $0.005x10^{-7}$ M of rose bengal is more than 1:15 hours and at higher concentrations 0.05 $x10^{-7}$ M, $0.01x10^{-5}$M and $0.3x10^{-5}$M were 1:10, 0:55 and 0:38 hours; respectively. In the case of the photosensitizing *rhodamine* B, the LT_{50} values of the lowest concentration 0.002 $x10^{-7}$ M and 0.18 $x10^{-5}$ M were > 1:15 hrs.; whereas at the three higher concentrations 0.02 $x10^{-7}$ M and 0.20 $x10^{-5}$ M and 2.00 $x10^{-5}$ M, the LT_{50} values were 0:40, 0:30 and 0:25 hour; respectively. Through the light of methylene blue, the LT_{50} values at the lowest concentrations $0.001x10^{-5}$ M exhibited > 1:15 hours. On the other hand, at the higher concentrations $0.02x10^{-5}$M, the LT_{50} value was recorded 1:00 hour. Concerning the highest two concentrations, $0.60x10^{-5}$M and $6.00x10^{-5}$M of the product, the corresponding LT_{50} value attained 0:40 and 0:30 hour; respectively.

Introduction

Fall armyworm *Spodoptera frugiperda* (Smith) (FAW) (Lepidoptera: Noctuidae) invasion has exacerbated maize (*Zea mays* L.) crop yield losses in sub-Saharan Africa (SSA) (Matova *et al.*, 2020) and recently in Egypt, already threatened by other stresses, especially those that are climate-change induced. The FAW is difficult to control, manage, or eradicate, because it is polyphagous and trans-boundary, multiplies fast, has a short life cycle and migrates easily, and lacks the diapause growth phase. Fall armyworm is currently the most damaging crop pest affecting maize (Kumela *et al.*, 2019). It is a polyphagous (Feeds on several hosts) and migratory (Can spread to other countries) pest that survives on at least 80 plant species (Harrison *et al*., 2019). FAW has some distinct features that can help separate it from its close relatives (Prasanna *et al.*, 2018). These include: (a) a white colored inverted "Y" mark on the front of the dark head and (b) a brown head with dark honey-combed markings (Figure 1a) and, (c) four dark spots displayed in a square on top of the eighth abdominal segment (Prasanna *et al*., 2018), as shown in Figure (1b). Typical early FAW infestation signs and symptoms include small "pin holes" and "window panes" (Figure 2a), resulting from feeding of the small worms on leaves (Figure 2b). Figure (2c) observed advanced FAW damage, showing dead heart on the growing point and Figure (2d) also observed large FAW larvae protected.

Therefore, it is becoming clear that alternative pest management tools are needed, which will be less hazardous to humans, non-target organisms and the environment; in the same time these alternative tools must be used in the field application with minimum cost. In this context, sunlight-activated photo-pesticides represent a possible alternative to traditional 4chemical compounds. The use of photochemical processes as a tool to control the population of several types of insects has been repeatedly examined in both laboratory experiments and field studies on corn rootworm, *Diabrotica* spp. (Schroder *et al*., 1998) and house fly, *Musca domestic*a (Attia, 2016).

The mechanism for photodynamic activity has been described by Heitz (1995). Toxicity occurs at the cellular level with the dye as a catalyst for generation of the singlet oxygen molecules. The photoactive compound accumulates within the insect and, following exposure to visible light, induces damage to its cuticle, midgut wall, followed by feeding inhibition and eventual death (Amor *et al.*, 1998). Moreover, these products affect biochemical contents such as proteins, lipids and carbohydrates (Attia, 2016 and El-Ghobary *et al*., 2018).

The objective of this work is to determine the toxicity and lethal time of some photosensitizing compounds against the 3rd instar larvae of the pest in relation to some biochemical contents in the treated larvae.

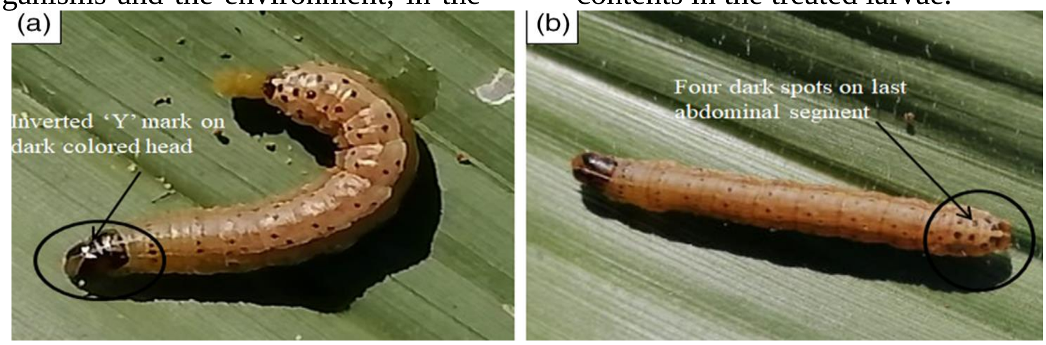

Figure (1): Physical appearance of the fall armyworm (FAW) larvae highlighting the most distinguishing features of the worm (a) a white colored inverted "Y" mark on the front of the dark head. (b) four dark spots displayed in a square on top of the eighth abdominal segment.

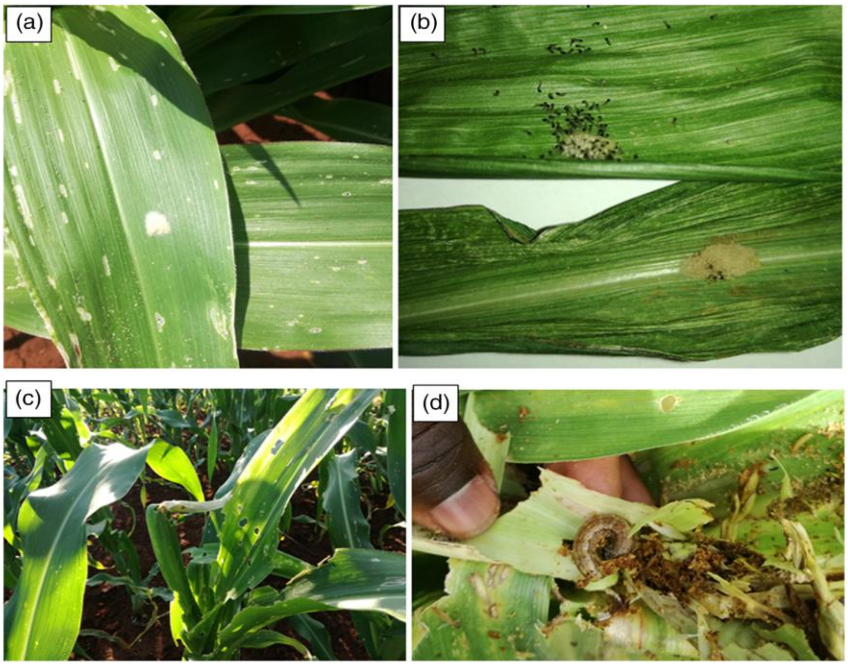

Figure (2): (a) Fall armyworm (FAW) egg masses and first signs of FAW infestation on leaves, (b)Young FAW larvae (black heads) emerging from egg masses on window pane damaged leaves, (c) Advanced FAW damage, showing dead heart on the growing point, (d) Large FAW larvae protected.

Materials and methods

1. Rearing technique of insect culture:

The laboratory strain of the fall armyworm *S. frugiperda* was obtained from the Cotton Bollworm Department, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt. The pest was reared under laboratory conditions without contamination with insecticides for more than six generations at 27+-ºC and 65-75% R.H. The standard tested insect used in the bioassay for all experiments was fed daily on fresh castor bean leaves, *Ricinus communis* (L.) in Bollworms Research Department, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt according to the method described by El-Defrawi *et al.* (1964).

2. Photoactive compounds used:

2.1. Common name: Rose bengal

Trade name: Rosets

Chemical formula: C20 H4 Cl4 I4 O5

Molar mass: 973.67 g/mole

Quantal yield :076

2.2. Common name: *Rhodamine* B

Trade name: *Rhodamine* 610

Chemical formula: $C_{28}H_{31}ClN_2O_3$

Molar mass: 479 g/mole

Quantal yield: 0.65

2.3. Common name: Methylene blue

Trade name: Urolene blue

Chemical formula: $C_{16}H_{18}N_3SCl$

Molar mass: 319.85 g/mole

Quantal yield: 0.52

3. Bioassays:

The three new photosensitizing compounds were diluted with water to prepare stock solutions, and then serial concentrations were prepared freshly before treatments. A preliminary bioassay was carried out by dipping castor bean leaves in the serial concentration solutions for 20 seconds, then the treated leaves were left to dry at room temperature. The 4th instar larvae were confined with the treated castor bean leaves in glass jars (15 cm in diameter X 30 cm in high) covered with muslin. The larvae were tested in three replicates containing 20 3rd larvae per each. Bioassays included untreated check in which leaves were dipped in water only. After feeding one day in dark, the glass jars were taken out doors where exposure to sunlight. Inspection every 15 min. was recorded till one and half hours during sunlight exposure. Average of mortality percentages were corrected using Abbott's formula (1925). The corrected mortality percentages were statistically computed according to Finney (1971). The slope, LC_{50} and LC_{90} values were estimated for each compound. The toxicity index (Sun, 1950) and potency levels were also calculated.

$$\text{Sun's Toxicity index} = \frac{LC_{50} \text{ or } LC_{90} \text{ of the most toxic compound}}{LC_{50} \text{ or } LC_{90} \text{ of the tested other compounds}}$$

$$\text{Potency levels} = \frac{LC_{50} \text{ or } LC_{90} \text{ of the least toxic compound}}{LC_{50} \text{ or } LC_{90} \text{ of the tested other compounds}}$$

4. Statistical analysis:

The results were statistically evaluated by analysis of variance to explain the significant differences between treatments. The 5 % level of probability was used in all statistical tests. The statistical software program CoStat (1995) was used for all analysis.

Results and discussion

1. Toxicological studies:

Laboratory trials were implemented to investigate the toxic efficacy of three photosensitizing compounds, namely rose bengal, *rhodamine* B and methylene blue against the 4th instar larvae of the armyworm *S. frugiperda* as follows:

1.1. Susceptibility in the 4th instar larvae of the armyworm, *Spodoptera frugiperda* to photosensitizing compounds:

The efficiency of different concentrations of the photoactive compounds; rose bengal, *rhodamine* B and methylene blue against the 4th instar larvae of *S. frugiperda* after 1:15 hrs. with exposure to sunlight was investigated. The results are represented in Table (1) and depicted graphically in Figure (3), Figure (4) and Figure (5). The obtained results revealed that the selected compounds had promising toxic effects on the 4th instar larvae of *S. frugiperda* where the LC_{50} values ranged between $0.018X10^{-7}$ M and $0.02X10^{-5}$ M (Table 1). The order of toxicity according to the LC_{50} values could be discerningly arranged as follows: rose bengal, *rhodamine* B and methylene blue. The corresponding LC_{50} values were $0.018X10^{-7}$ M, $0.040X10^{-7}$ M and $0.02X10^{-5}$ M; respectively. On the other hand, the corresponding LC_{90} values were $0.26X10^{-7}$ M;$0.32X10^{-7}$ M and $0.34X10^{-5}$M; respectively. The maximum toxic effect was observed in the feeding of the 4th instar larvae with rose bengal compound. It caused 25% mortality in the treated larvae at concentration $0.005X10^{-7}$ M. then increased to reach 100% at the highest concentration $0.3X10^{-5}$ M. On the other hand, *rhodamine* B at concentrations $0.002X10^{-7}$ M and $2X10^{-5}$ M caused 20 and 95% mortality; respectively. Concerning methylene blue, it caused 24 and 84% mortality at concentrations $0.001X10^{-5}$ M and $6X10^{-5}$ M.

Table (1): Susceptibility status of the 4th instar larvae of *Spodoptera frugiperda* to three photosensitizing compounds.

Photosensitizing compounds	Conc. (M)	% Mortality	Slope	LC_{50}(M)	LC_{90}(M)
Rose Bengal	0.005×10^{-7}	25.00	1.102	0.018×10^{-7}	0.260×10^{-7}
	0.05×10^{-7}	70.00			
	0.01×10^{-5}	95.00			
	0.3×10^{-5}	100.00			
***Rhodamine* B**	0.002×10^{-7}	20.00	2.265	0.040×10^{-7}	0.32×10^{-7}
	0.02×10^{-7}	75.00			
	0.20×10^{-5}	80.00			
	2.00×10^{-5}	95.00			
Methylene blue	0.001×10^{-5}	24.00	0.999	0.02×10^{-5}	$.0.34 \times 10^{-5}$
	0.06×10^{-5}	42.00			
	0.6×10^{-5}	75.00			
	6.00×10^{-5}	84.00			

% mortality was determined after1:15 hours from exposure to sunlight.

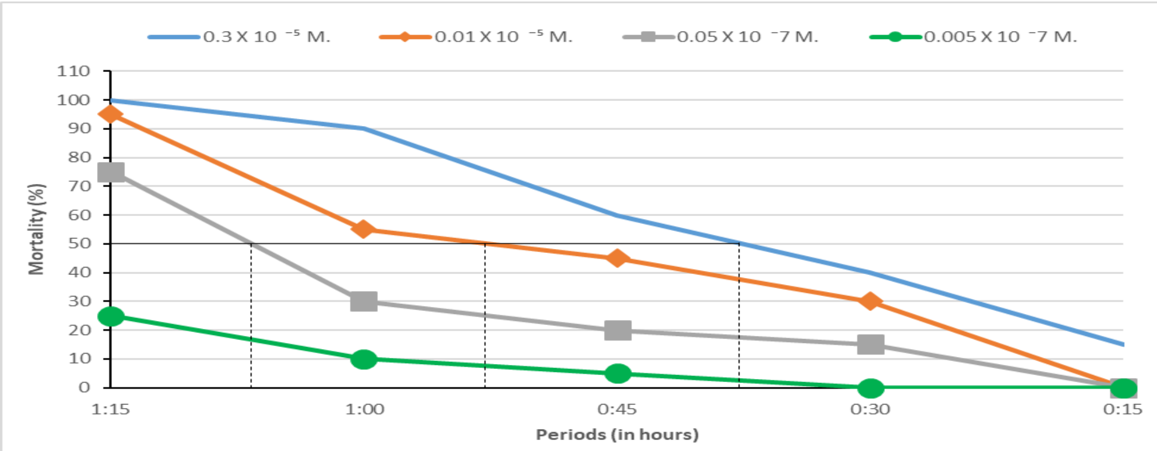

Figure (3): Effect of rose bengal on the mortality percentages of *Spodoptera frugiperda* 4th instar Larvae exposed to sunlight for different time interval

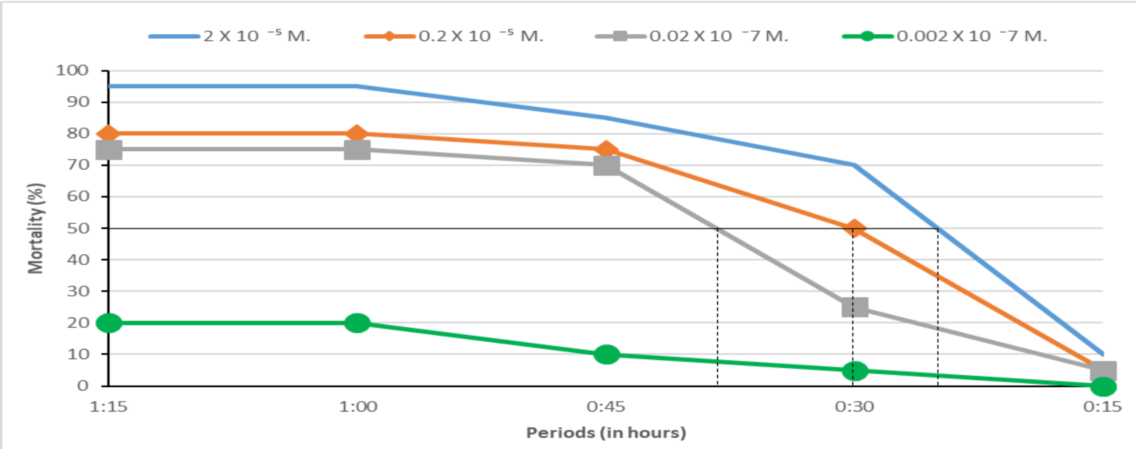

Figure (4): Effect of rhodamine B on the mortality percentages of *Spodoptera frugiperda* 4th instar larvae exposed to sunlight for different time intervals.

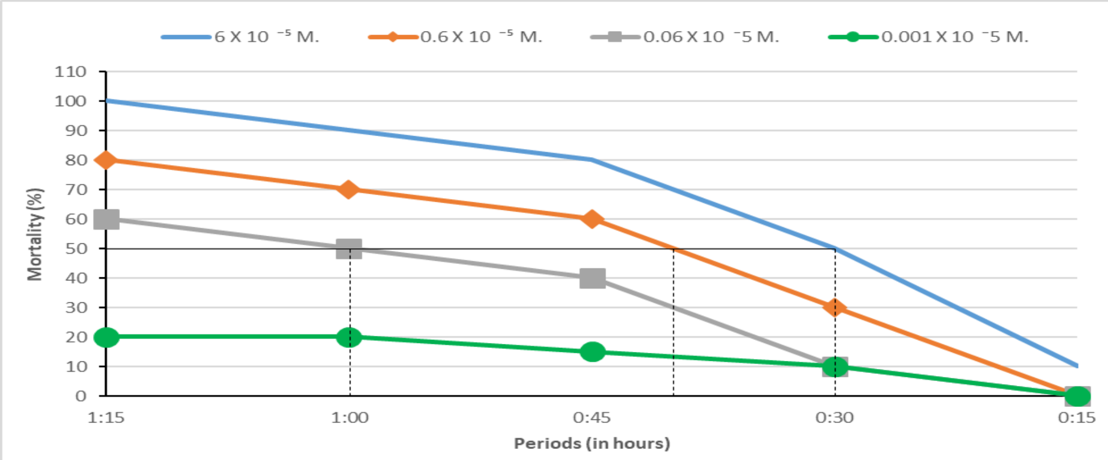

Figure (5): Effect of methylene blue on the mortality percentages of *Spodoptera frugiperda* 4th instar larvae exposed to sunlight for different time intervals.

1.2. Comparison on basis of toxicity index, slope values, LC_{90}/LC_{50} ratio and potency levels:

Concerning the efficiency of the tested compounds against the 4th instar larvae of *S. frugiperda*, the toxicity index method of **Sun (1950)** is used to determine the degree of toxicity of different insecticides by comparing the with a standard compound exhibited by the most toxic one. In this study, rose bengal which showed the highest toxicity against the treated larvae was chosen as the standard compound and given arbitrary 100 units. Results represented in Table (2) showed general similarity trend of the toxicity index of the tested photosensitizing products at both LC_{50} and LC_{90} levels.

Table (2): Toxicity index, slope values, LC90/LC50 and potency levels of the 4th instar larvae of S. frugiperda treated with three tested compounds.

Photosensitizers	Toxicity index based on		Slope	LC_{90}/LC_{50}	Potency levels	
	LC50	LC90			LC50	LC90
Rose bengal	100	100	1.102	14.14	270.270	130.77
***Rhodamine* B**	47.37	45	2.265	8.00	50.00	106.25
Methylene blue	0.90	0.76	0.999	17.00	1.00	1.00

On the ground of the toxicity index at LC_{50} and LC_{90} levels, the toxicity index values of the two photosensitizing compounds, *rhodamine* B and methylene blue were 47.37 and 45,00 and 0.90 and .0.76 % as toxic as toxicity of the photosensitizing product, rose bengal; respectively against the 4th instar larvae of *S. frugiperda* at both LC_{50} and LC_{90} levels, respectively.

The slope values and LC_{90}/LC_{50} ratios of the tested compounds against the 3rd instar larvae of the tested pest after 1:15 hrs. from exposure to sunlight were calculated. The potency levels expressed as a number of folds were obtained by dividing the LC_{50} or LC_{90} for the least effective compound (Methylene blue) by the corresponding figure for each product. The obtained results are presented in Table (2). Data showed that the steepest toxicity line was noticed in the case of treatment with rhodamine B. The corresponding slope of toxicity line was 2.265, whereas the flattest one was observed in the case of treatment with methylene blue, where the slope of the toxicity line was recorded 0.999. The slope value of the toxicity line of the other photosensitizing compound rose bengal occupied the meddle situation among the two products mentioned previously, where it recorded 1.102.

The above mentioned conclusion is correct whether it is the slope values or the LC_{90}/LC_{50} ratios, since the later method simply expressed the steepness of the LC-P lines in a reversal way to the slope values. Therefore, an increase in the slope value or a decrease in the LC_{90}/LC_{50} ratio indicates an increase in the toxicity line. Data represented in Table (2) showed similarity in the order of the potency levels at both LC_{50} and LC_{90} levels. The potency levels of the two photosensitizing compounds rose bengal and *rhodamine* B at both LC_{50} and LC_{90} values were 270.27 and 130.77 and 50 and 106.25 times as toxic as the toxicity of methylene blue; respectively against the 4th instar larvae of the tested pest.

2. Photodynamic effect of the three photosensitizing compounds:

The mortality percentages of the 4thinstar larvae of *S. frugiperda* treated with different concentrations of the three photosensitizing compounds were recorded after exposure to sunlight for different periods.

2.1. Photodynamic effect of rose bengal:

Data obtained in Table (3) and illustrated in Figure (3), revealed that the first observation of the mortality occurred among 4th instar larvae fed on treated castor bean leaves with the lowest concentration of rose bengal ($0.005X10^{-7}$M) and exposed to sunlight for 45 minutes, where the mean mortality percentage was 5.00%, and this value was increased gradually to reach 10.00 and 25.00% mortality at 1:00 and 1:15 hrs. of exposure to sunlight; respectively. At concentration $0.05X10^{-7}$ M, larval mortality was 15.00% after 30 minutes of exposure to sunlight. After 45 minutes of sunlight exposure, larval mortality increased to reach 20.00%. Moreover, larval mortality was increased to reach 55.00 and 95.00% after 1:00 and 1:15 hrs. of sunlight exposure, respectively. At the highest concentration $0.01X10^{-5}$ M of rose bengal, larval mortality exhibited 15.00% after 15 minutes of sunlight exposure, the increased significantly to reach 40.00, 60.00, 90.00 and 100 % after 0:30, 0:45, 1:00 and 1:15 hrs. of exposure to sunlight; respectively.

Table (3): The photodynamic effect of different concentrations of rose bengal on the 4th instar larvae of *Spodoptera frugiperda* at different periods of sunlight exposure.

Sunlight exposure periods (hrs.)	% Mortality at indicated concentrations expressed as mole			
	$0.005 X 10^{-7}$	$0.05 X 10^{-7}$	$0.01 X 10^{-5}$	$0.3 X 10^{-5}$
0:15	0.00d	0.00d	0.00e	15.00e
0:30	0.00d	15.00c	30.00d	40.00d
0:45	5.00c	20.00c	45.00c	60.00c
1:00	10.00b	30.00b	55.00b	90.00b
1:15	25a	75.00a	95.00a	100a
Control	0.0	0.0	0.0	0.0

Numbers with the same litters are not significantly different in the same column

2.2. Photodynamic effect of rhodamine B:

The mortality rates of the 4thinstar larvae of *S. frugiperda* treated with different concentrations of *rhodamine* B were recorded after exposure to sunlight for different periods. Results represented in Table (4) and Figure (4) showed that the lowest concentration 0.002X10^{-7} M showed slight mortality which recorded 5.00% after 0:30 hour of sunlight exposure. After 0:45, 1:00 and 1:15 hours of sun exposure, the larval mortality increased slightly to reach 10:00, 20:00 and 20:00%; respectively.

Treating larvae with 0.02X10^{-7} M caused 5% mortality after 0:15 hour of sunlight exposure. After 0:30, 0:45, 1:00 and 1:15 hrs. of sunlight exposure, the larval mortality increased significantly to reach 25:00, 70.00, 75.00 and 75.00%; respectively. At higher concentrations 0.20X10^{-5} M, *rhodamine* B caused 5.00% larval mortality at 0:15 hour, then increased significantly to 50.00, 75.00, 80.00 and 80.00 % at 0:30, 0:45, 1:00 and 1:15 hrs. of sunlight exposure.

Table (4): The photodynamic effect of different concentrations of rhodamine B on the 4th instar larvae of *Spodoptera frugiperda* at different periods of sunlight exposure.

Sunlight exposure periods (hrs.)	% Mortality at indicated concentrations expressed as mole			
	0.002 X 10^{-7}	0.02 X 10^{-7}	0.20 X 10^{-5}	2.00 X 10^{-5}
0:15	0.00d	5.00c	5.00c	10.00d
0:30	5.00c	25.00b	50.00b	70.00c
0:45	10.00b	70.00a	75.00a	85.00b
1:00	20.00a	75.00a	80.00a	85.00b
1:15	20.00a	75.00a	80.00a	95.00a
Control	0.00	0.00	0.00	0.00

Numbers with the same litters are not significantly different in the same column

Concerning the highest concentration 2.00X10^{-5} M, the incidence of larval mortality resulted from treating the 4th instar larvae recorded 10.00% after 0:15 hour of exposure to sunlight, then the larval mortality rates increased significantly to record 70.00, 85.00, 85.00 and 95.00% larval mortality after 0:30, 0:45, 1:00 and 1:15 hrs. of to sunlight exposure; respectively. Concerning the highest concentration**.**

2.3. Photodynamic effect of methylene blue:

The mortality rates of the 4th instar larvae of *S. frugiperda* treated with different concentrations of the photosensitizing methylene blue were determined after exposure to sunlight for different periods. Results illustrated in Table (5) and Figure (5), indicated that the lowest concentration 0.001X10^{-5} M of methylene blue caused 10.00% larval mortality after 0:30 hour of sunlight exposure. The larval mortality increased gradually as sunlight exposure periods increased to reach its maximum to 20.00% mortality after one and 1:15 hrs. of sunlight exposure. At concentration 0.06X10^{-5} M, the larval mortality was 10% after 0:30 hour of sunlight exposure. The mortality rate in the 4th instar larvae treated with methylene blue was increased significantly to 40% after 0:45 hour of sunlight exposure. The larval mortality increased to 50% after one hour of sunlight exposure. The highest mortality rate was 40.00, 50.00 and 60.00% after 0:45, 1:00 and 1:15 hrs. of sunlight exposure. It was obvious that the higher concentration 0.6X10^{-5} M, caused a remarkable increase in the 4th instar larvae of *S. frugiperda* attained which being 30.00, 60.00, 70.00 and 80.00% at 0:30, 0:45, 1:00 and 1:15 hrs. as a result of sunlight exposure; respectively. At the highest concentration 6X10^{-5} M, it is clear that promising larval mortality percentages were recorded to reach 10.00, 50.00, 80.00, 90.00 and 100% after 0:15, 0:30, 0:45, 1:00 and 1:15 hrs. of sunlight exposure.

Table (5): The photodynamic effect of different concentrations of methylene blue on the 4th instar larvae of *Spodoptera frugiperda* at different periods of sunlight exposure.

Sunlight exposur eperiods (hrs.)	% Mortality at indicated concentrations expressed as mole			
	0.001 X 10^{-5}	**0.06 X 10^{-5}**	**0.6 X 10^{-5}**	**6.00 X 10^{-5}**
0:15	0.00d	0.00e	0.00e	10e
0:30	10.00c	10.00d	30.00d	50.00d
0:45	15.00b	40.00c	60.00c	80.00c
1:00	20.00a	50.00b	70.00b	90.00b
1:15	20.00a	60.00a	80.00a	100a
Control	0.00	0.00	0.00	0.00

Numbers with the same litters are not significantly different in the same column.

As judged by a comparison based on median lethal time (LT_{50}) as presented in Table (6) and illustrated graphically in Figure (3), the LT_{50} value of the lowest concentration 0.005x10^{-7} M of rose bengal is more than 1:15 hrs. and at higher concentrations 0.05 x10^{-7} M, 0.01x10^{-5} M and 0.3x10^{-5} M were 1:10, 0:55 and 0:38 hours; respectively.

As shown in Table (6) and Figure (4), In case of the photosensitizing rhodamine B, the LT_{50} values of the lowest concentration 0.002 x10^{-7} M was> 1:15 hours; whereas at the three higher concentrations 0.02 x10^{-7} M and 0.20 x10^{-5} M and 2.00 x10^{-5} M, the LT_{50} values were 0:40, 0:30 and 0:25 hour; respectively.

As results presented in Table (6) and depicted in Fig. (5), through the light of methylene blue, the LT_{50} values at the lowest concentrations 0.001x10^{-5} M exhibited> 1:15 hours. On the other hand, at the higher concentrations 0.06x10^{-5} M, the LT_{50} value recorded 1:00 hour. Concerning the highest two concentrations, 0.60x10^{-5}M and 6.00x10^{-5} M of the product, the corresponding LT_{50} value attained 0:40 and 0:25 hour; respectively.

Table (6): Median lethal time of photosensitizing compounds against the 4th instar larvae of *Spodoptera frugiperda* exposed to sunlight for different time intervals.

Photosensitizing compounds	Conc. (M)	LT_{50} in hrs.
Rose bengal	0.005 X 10^{-7}	>1:15
	0.05 X 10^{-7}	1:10
	0.01 X 10^{-5}	0:55
	0.3 X 10^{-5}	0:38
Rhodamine B	0.002 X 10^{-7}	>1:15
	0.02 X 10^{-7}	0:40
	0.20X 10^{-5}	0:30
	2.00 X 10^{-5}	0:25
Methylene blue	0.001 X 10^{-5}	>1:15
	0.06 X 10^{-5}	1:00
	0.6 X 10^{-5}	0:40
	6.00 X 10^{-5}	0:25

It is interesting to mention that conventional insecticides play an important role in the overall cotton leaf worm suppression program. However, development of the cotton leaf worm resistance to various insecticides used indicated that great efforts should be needed to find effective alternative control methods. Several studies indicated that photosensitizer compounds represent a possible alternative to traditional chemical compounds for pest control (Attia, 2016).

According to Attia (2016), light dependent mechanism of xanthene compounds involves the production of singlet oxygen that causes toxicological as well as biochemical effects on insects. The present study is one of the trials contributing to such studies.

Concerning the toxicity of the four photosensitizing compounds used against the pest under the study, the 3rd instar larvae of *S. frugiperda* were allowed to feed on different concentrations of the tested photosensitizer compound; rose bengal, rhodamine B and methylene blue for different periods of sunlight exposure. According to aforementioned results, the treated larvae showed different susceptibilities to the tested compounds. The LC_{50} values ranged between $0.018X10^{-7}$ M and $0.02X10^{-5}$ M. This agrees with the previous findings of several authors working with different photosensitizing compounds in both laboratory and field applications (Attia, 2016). As revealed from the obtained results, rose bengal was the most promising compound, followed by rhodamine B. This result had been detected by several investigators other insects (Aref, 2010 and Attia, 2016). On the other hand, methylene blue and methyl violet exerts extremely low toxic activity against the cotton leaf worm larvae; this result is in agreement with Attia (2016) on house fly adults. 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.

According to the chemical structure of the tested compounds, rose bengal has the highest number of halogen atoms including 4 iodine and 4 chlorine atoms; whereas rhodamine B, methylene blue and methyl violet have the lowest number including one chlorine atom. The photosensitizer compounds with the greatest number of halogen atom substituents yield greater toxicity, therefore, the halogen atoms amplify the reactions (Attia, 2016). As stated from the previous results, the photosensitizer's effectiveness depends on the concentration of the tested product as well as the time of exposure to sunlight after treatments. These results are in accordance with those reported by Attia (2016) and El-Ghobary *et al.* (2018).

The mechanism for photodynamic activity has been described by Vilensky and Feitelson (1999) and Attia (2016). Upon absorption of light photons, the excited single state of photosensitizer (^{1}Sens) reaches to the excited triple state (^{3}Sens) via intercrossing system. The excited triplet stated characterized by long life time, so it can play a major role in excitation of triplet ground state of oxygen ($^3O_2$) into excited singlet state ($^1O_2$) which is donated with a high cytotoxicity.

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