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Potency of some Photosensitizing Compounds against the Cotton Leaf Worm, *Spodoptera littoralis* (Boisduval) in Relation to some Biochemical Aspects

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ABSTRACT

The present study aimed to evaluate the toxicological as well as biochemical effects of the three photosensitizer compounds; rose bengal, eosin yellow lactone and methylene blue on the fourth larval instar of the cotton leaf worm, *Spodoptera littoralis* (Boisd.). Results of insecticidal activity against the fourth larval instar of *S. littoralis* revealed that rose bengal is the most toxic photosensitizer compound followed by eosin yellow lactone, then methylene blue. The corresponding LC_{50} values were $37X10^{-5}$M, $81X10^{-5}$M and $136X10^{-5}$M; respectively. Concerning, the photodynamic process of the thee photosensitizer compounds to control the 4th instar larvae of *S. littoralis* , the LT_{50} values of the lowest concentrations of rose bengal, eosin yellow lactone and methylene blue were >4hrs for each. On the other hand, the LT_{50} values of the highest concentrations were 2.2, 3.25 and 2.50 hrs; respectively. This indicates that rose bengal was the most active compound. On the light of the median lethal effects of the three tested photosensitizer compounds on the total protein ,total lipids and total carbohydrates content in the tested fourth larval instar of the cotton leaf worm, the results proved that the three photosensitizer compounds decreased these biochemical contents except methylene blue slighty increased total protein content relative to control.

INTRODUCTION

The cotton leaf worm *Spodoptera littoralis* (Boisduval) is one of the most destructive pests of cotton in Egypt. It caused damages a wide range of cultivated crops specially cotton crop causing important economic losses by feeding on vegetations as well as reproductive structures in these crops (Abdel-Aziz and El-Gohary, 2013). The efficient control of the cotton leaf worm *Spodoptera littoralis* has been the goal of investigators in the field of Agricultural entomology. Chemical treatments create problems by leaving undesirable residues in food. Due to the random use of insecticides, the pests can develop levels of resistance to most available insecticides. Therefore, it is becoming clear that alternative pest management tools are needed, which will be less hazardous to human, 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 chemical 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), Mexican fruit fly, *Anastrepha ludens* (Moreno and Mangan, 1995) 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 of 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 an carbohydrates(Attia, 2016).

The objective of this work is to determine the toxicity and lethal time of some photosensitizing compounds against the fourth larval instar of the pest. To study the effect of the median lethal concentration of the tested compounds on some biochemical contents in the treated larvae.

MATERIALS AND METHODS

1.Rearing technique of insect culture:

The laboratory strain of the cotton leaf worm *Spodoptera littoralis* (Boisd.) was obtained from the Cotton Leaf Worm 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 ten generations at27+_ ^{o}C and 65-75% R.H. The standard tested insect used in the bioassay for all experiments were fed daily on fresh on castor bean leaves, *Ricinus communis* (L.) in Bollworms Research Department, Sakha Station, Kafrel-Sheikh according to the method described by El-Defrawi (1964).

2. Photoactive compounds used:

Common name: Rose Bengal
Group: Xanthene
Class: Fluorone
Trade name: Rosets
IUPAC name:4,5,6,7-Tetrachloro-3,6-dihydroxy- 2,4,5,7-tetraiodo-3H- spiro[isobenzofuran-1,9 xanthen]-3-one.
Chemical formula: C_{20} H_4 Cl_4 I_4 O_5
Molar mass: 973.67 g/mole
Common name: Eosin yellow lactone
Group: Xanthene
Class: Fluorone
Trade name: Eosin Yellowish
IUPACname:2-(2,4,5,7-tetrabromo-6-oxido-3-ozo-3H-xanthene-9-yl)benzoate
Chemical formula: C_{20} H_6 Br_4 Na_2 O_5
Molar mass: 691.85g/mole
Common name: Methylene blue
Group: Quinone-imine
Class: Thiazin
Trade name: Urolene blue
IUPAC name: 3,7-bis(Dimethyl amino)-phenothiazin-5-ium chloride.
Chemical formula: C16H18N3SCl
Molar mass: 319.85g/mole

3. Bioassay:

The three new photosensitizing compounds were diluted with water to prepare stock solutions, and then

serial concentrations around the LC values based on ppm of the tested products were prepared freshly before treatments. Preliminary bioassay was carried out by dipping castor bean leaves in the serial concentration solutions for 30 seconds, then the treated leaves were left to dry at room temperature. The fourth larval instar were confined with the treated castor bean leaves in glass jars covered with muslin for 24 hours. The tested larvae were tested in four replicates containing 25 larvae per each. Bioassay included untreated check in which leaves was dipped in water only.

Inspection every 15 min. were recorded till four hours after treatments. 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.

4.Biochemical studies:

Quantitative analysis of total protein, total lipid and total carbohydrates was carried out in The Pest Physiology Department, Plant Protection Research Institute, Agricultural Research Center. The analysis was used for the fourth larval instar treated with LC_{50} values of the tested Photosensitizing compounds previously calculated by bioassays. The tested larvae were fed on the treated as well as untreated leaves for four hours.

5. Preparation of samples for biochemical analysis:

Batches of the treated larvae were homogenized in phosphate buffer saline. The homogenate was centrifuged at 6000 r.p.m. for 10 minutes. The deposited were discarded and supernatants transferred to eppendrof tubes and kept in deep freezer at – 20 ^{o}C till use for measuring total protein, total carbohydrates and total lipids.

Determination of total protein:

Total protein content of whole body was determined according to the method described by Bradford (1976).

Determination of total lipids content:

Total lipids content were estimated by the method of Knight *et al*. (1972).

Determination of total carbohydrates:

Total carbohydrates content were estimated in acid extract of the treated samples by phenol-sulphuric reaction of Dubois *et al.* (1956). Total carbohydrates content were extracted and prepared for assay according the method described by Crompton and Birt (1967).

6.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 experiments were carried out to evaluate the toxic effects of three photosensitizing compounds, namely rose Bengal, eosin yellow lactone and methylene blue on the fourth larval instar of the cotton leaf worm, *Spodoptera littoralis.*

Comparison on basis of mortality percentage, LC_{50} and LC_{90} levels:

The efficacy of different concentrations of rose Bengal, eosin yellow lactone and methylene blue against the fourth larval instar of *S. littoralis* after 4hours of exposure to sunlight. The selected compounds had toxic effects on the fourth larval instar of *S. littoralis*, where the LC_{50} values ranged between $37X10^{-5}$ M and $136X10^{-5}$ M. The order of toxicity according to the LC_{50} values could be descendingly arranged as follows: rose Bengal, eosin yellow lactone and methylene blue. The corresponding LC_{50} values were $37X10^{-5}$ M, $81X10^{-5}$ M and $136X10^{-5}$ M; respectively. The maximum toxic effect was observed in the feeding of the 4th instar larvae with rose Bengal compound. It caused 37% mortality in the treated larvae at concentration $26X10^{-5}$ M. It increased to reach 92.50% at concentration $128X10^{-5}$ M. On the other hand, eosin yellow lactone at concentrations $36X10^{-5}$ M and $181X10^{-5}$ M caused 24.00 and 79.00% mortality. Concerning methylene blue it caused 29.50 and 89.50% mortality at concentrations $78X10^{-5}$ M and $391X10^{-5}$ M; respectively.

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 fourth larval instar of *S. littoralis*, the toxicity index method of Sun (1971) is used to determine the degree of toxicity of different insecticides by comparing the compounds with a standard compound exhibited the most toxic one. In this study, rose bengal that showed the highest toxicity against the treated larvae was chosen the standard compound and given arbitrary 100 units. Results represented in Table (2) showed general similarity trend of the toxicity index of the tested products at both LC_{50} and LC_{90} levels.

On the ground of the toxicity index at LC_{50} and LC_{90} levels, the toxicity index values of the two photosensitizing compound, eosin yellow lactone and methylene blue were 45.68&35.78 and 27.21&26.29% as toxic as the toxicity of the photosensitizing product, rose bengal; respectively against the fourth larval instar of *S. littoralis*.

Table 1. Mortality percentage, LC_{50} and LC_{90} levels of the 4th instar larvae of *S. littoralis* to three photosensitizing compounds.

Photosensitizing compounds	Conc. (M)	Mortality %	Slope	LC_{50}	LC_{90}
Rose Bengal	$26 X 10^{-5}$	37.00	2.578	$37 X 10^{-5}$	$117 X 10^{-5}$
	$52 X 10^{-5}$	58.50			
	$77 X 10^{-5}$	79.50			
	$103 X 10^{-5}$	87.00			
	$128 X 10^{-5}$	92.50			
Eosin yellow lactone	$36 X 10^{-5}$	24.00	2.125	$81 X 10^{-5}$	$324 X 10^{-5}$
	$72 X 10^{-5}$	44.50			
	$108 X 10^{-5}$	59.50			
	$145 X 10^{-5}$	68.50			
	$181 X 10^{-5}$	79.00			
Methylene blue	$78 X 10^{-5}$	29.50	2.489	$136 X 10^{-5}$	$445 X 10^{-5}$
	$156 X 10^{-5}$	53.00			
	$234 X 10^{-5}$	68.50			
	$313 X 10^{-5}$	81.00			
	$391 X 10^{-5}$	89.50			

Mortality percentage was determined after four hours from exposure to sunlight.

Table 2. Toxicity index, slope values, LC_{90}/LC_{50} and potency levels of the Fourth larval instar larvae of *S. littoralis* treated with three tested compounds.

Photo	Toxicity index based on LC50	Toxicity index based on LC90	Slope	LC_{90}/LC_{50}	Potency levels LC50	Potency levels LC90
Rose bengal	100	100	2.578	3.162	3.68	3.80
Eosin yellow lactone	45.68	35.78	2.125	4.00	1.68	1.37
Methylene blue	27.21	26.29	2.489	3.272	1.00	1.00

The slope values and LC_{90}/LC_{50} ratio of the tested compounds against the fourth larval instar of the tested pest after 4 hours from the treatment were calculated. The potency levels expressed as number of fold 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 case of treatment with rose bengal. The corresponding slope of toxicity line was 2.578, whereas the flattest one was observed in case of treatment with eosin yellow lactone, where the slope of the toxicity line recorded 2.125. The slope of the toxicity line of methylene blue occupied the meddle situation among the products that mentioned previously, where it recorded 2.489.

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 of the toxicity line.

Data represented in Table (2) showed similarity in the order of the potency levels at both LC_{50} and LC_{90} values. The potency levels of the two photosensitizing compounds rose bengal and eosin yellow lactone at both LC_{50} and LC_{90} values were 3.68, 3.80 and 1.68, 1.37 times as toxic as the toxicity of methylene blue; respectively against the instar larvae of the tested pest.

2.Photodynamic effect of the three photosensitizing compounds:

Mortality percentage of the fourth larval instar treated with different concentrations of the three photosensitizing compounds were recorded after exposure to sun light for different periods.

Photodynamic effect of rose bengal:

Data obtained in Table (3) revealed that the first observation of the mortality was occurred among treated larvae fed on treated castor bean leaves with the lowest concentration of rose bengal ($26X10^{-5}$M) and exposed to sun light for 2 hours, where the mean mortality percentage was 3% and this value increased significantly to reach 15.50% mortality over 2 and half hours of exposure to sun light. At 4:00 hours of exposure to sun light, the mortality rate increased significantly to reach 37%.

At concentration $52X10^{-5}$ M, larval mortality was 1% after 1:50 hr. of exposure to sun light. After 2:00 hrs, larval mortality increased to 6% although this did not significant increase. The larval mortality increased significantly to reach 58.50% after 4:00 hrs. of exposure to sun light.

At concentration $77X10^{-5}$ M, larval mortality attained 1% after one hour of exposure to sun light. Larval mortality increased significantly to reach 54.50 % after 3:30 hrs. of exposure to sun light, more significant increase in the larval mortality which reached 79.50% after 4.00 hrs. of exposure to sun light.

Concerning the concentration $103X10^{-5}$ M, the incidence larval mortality resulted from treated the fourth larval instar recorded 3.50% after one hour of the exposure to sun light, then the larval mortality rates were increased.

Gradually as exposure period increased to reach its maximum mortality percentage which being 87.oo% after 4:00 hrs. of exposure to sunlight. Based on concentration $128X10^{-5}$ M, earliest larval mortality exhibited 6.50% after one hour of exposure to sun light, then the mortality rates significantly increased as the exposure periods to sun light increased to reach the highest mortality which recorded 92.50% after 4.00 hrs. of exposure to sun light.

Table 3. The photodynamic effect of different concentrations of rose bengal on the fourth larval instar of *S. littoralis*.

Sunlight exposure periods (hrs.)	Mortality percentage at indicated concentrations expressed as mole $26 X 10^{-5}$	$52 X 10^{-5}$	$77 X 10^{-5}$	$103 X 10^{-5}$	$128 X 10^{-5}$
0:30	0.00	0.00	0.00	0.00	0.00
1:00	0.00	0.00	1.00	3.50	6.50
1:30	0.00	1.00	7.00	10.50	19.00
2:00	3.00	6.00	22.00	23.00	34.50
2:30	15.50	27.00	38.00	45.00	55.00
3:00	20.00	30.50	40.00	55.00	66.50
3:30	30.00	38.00	54.50	63.00	77.50
4:00	37.00	58.50	79.50	87.00	92.50
Control	0.0	0.0	0.0	0.0	0.0

Photodynamic effect of eosin yellow lactone:

The mortality ratios of the fourth larval instar treated with different concentrations of eosin yellow lactone were recorded after exposure to sun light. Results represented in Table (4) showed that the lowest concentration $36X10^{-5}$ M showed slight mortality recorded 2% after 2:50 hrs. of sun light exposure. After 3:30 and 4:00 hours of sun exposure, the larval mortality increased significantly to 10.00 and 24%; respectively. Treated larvae with $72X10^{-5}$ M caused 6.50% mortality after 2hrs. and half of sun light exposure. By 3.00, 3.30 and 4hours of sun light exposure, the larval mortality increased gradually to 17.00, 23.50 and 44.50%; respectively.

At higher concentration $108X10^{-5}$ M eosin yellow lactone caused 12.00% mortality at 2:30 hours increased from 22.50% at 3:00 hours to 34.00% at 3hours and half of sun light exposure. After that, remarkable much increase in the treated larvae which reached to 59.50% at 4hours of sun light exposure.

Concerning the concentration $145X10^{-5}$ M, incidence larval mortality reached 6.50% at 2hours after sun light exposure. The mean dead treated larvae with eosin yellow lactone increased significantly from 27.00%

at 2:30 hrs to 36.50, 44.50 and 68.500% after 3:00, 3:30 and 4:00 hours of sun light exposure.

Table 4. The photodynamic effect of different concentrations of eosin yellow lactone on the 4th instar larvae of *S. littoralis*.

Sunlight exposure periods (hrs.)	Mortality percentage at indicated concentrations expressed as mole				
	36 X 10^{-5}	72 X 10^{-5}	108 X 10^{-5}	145 X 10^{-5}	181 X 10^{-5}
0:30	0.00	0.00	0.00	0.00	0.00
1:00	0.00	0.00	0.00	0.00	0.00
1:30	0.00	0.00	0.00	0.00	0.00
2:00	0.00	0.00	0.00	6.50	13.00
2:30	2.00	6.50	12.00	27.00	29.50
3:00	5.50	17.00	22.50	36.50	38.50
3:30	10.00	23.50	34.00	44.50	59.50
4:00	24.00	44.50	59.50	68.50	79.00
Control	0.00	0.00	0.00	0.00	0.00

Data obtained in Table (4) showed that the highest concentration of eosin yellow lactone 181X10^{-5} m caused 38.500% mortality in the treated larvae after 3:00 hours of sun light exposure. The mean mortality rate in the treated fourth larval instar increased significantly from 59.50% after 3:30 hours to 79.00% after 4:00 hours of sun light exposure.

Photodynamic effect of methylene blue:

The mortality rates of the fourth larval instar treated with different concentrations of the photosensitizing methylene blue were determined after exposure to sun light.

Results summarized in Table (5) indicated that the lowest concentration of methylene blue 78X10^{-5} M caused 0.50% larval mortality after 2:00 hours of sun light exposure. The larval mortality increased gradually as sun light exposure periods increased to reach its maximum to 29.50% mortality after 4hours of sun light exposure. At concentration 156X10^{-5} M, the larval mortality was 2.00% after 2hours. The mortality rate in the fourth larval instar treated with methylene blue was increased significantly to 23.00% after 2:30 hours of sun light exposure. The larval mortality increased to 25.50% after 3:00 hours, but there were no significant differences between mortality rate at 2:30 and 3:oo hours. The highest mortality rate was 53:00% after 4:00 hours of sun light exposure.

In case of concentration 234X10^{-5} M, mortality percentage in the treated fourth larval instar of *S. littoralis* attained 1.50% at 1.30 hours. The larval mortality rate was increased significantly to 31.00% at 2:30 hours of sun light exposure. After that the rates of larval mortality treated with the photosensitizing methylene blue were gradually increased as the periods of sun light exposure increased to reach 38.00, 43.50 and 68.50% after 3:00, 3:30 and 4:00 hours of sun light exposure; respectively.

At concentration 313X10^{-5} M, the earliest larval mortality was 1.00% after 1:00 hour of sun light exposure, the dead larvae increased to 5% at 1:30 hours after exposure to sun light, but there was no significant difference between mortality at 1:00 hour and 1:30 hours. Gradual increase in the treated larvae with the photosensitizing methylene blue as the time of sun light exposure increased to reach its maximum 81.00% at 4 hours of sun light exposure.

At the highest concentration 391X10^{-5} M, the mortality rates in the treated fourth larval instar with the photosensitizing methylene blue increased from 5.50% at 1:00 hour to 8.50% after 1:30 hours of sun light exposure, but the was no significant difference between mortality after 1.00 hour and 1:30 hours. The larval mortality was increased significantly to 22.00, 42.50, 54.00 65.00 and 89.50% after 2:00, 2:30, 3:00, 3:30 and 4:00 hrs. of sun light exposure,respectively.

Table5. The photodynamic effect of different concentrations of methylene blue on the fourth larval instar of *S. littoralis*.

Sunlight exposure periods (hrs.)	Mortality percentage at indicated concentrations expressed as mole				
	78 X 10^{-5}	156 X 10^{-5}	234 X 10^{-5}	313 X 10^{-5}	391 X 10^{-5}
0:30	0.00	0.00	0.00	0.00	0.00
1:00	0.00	0.00	0.00	1.00	5.50
1:30	0.00	0.00	1.50	5.00	8.50
2:00	0.50	2.00	13.00	1.50	22.00
2:30	6.50	23.50	31.00	37.50	42.50
3:00	14.00	25.50	38.00	47.00	54.00
3:30	23.00	30.00	43.50	53.00	65.00
4:00	29.50	53.00	68.50	81.00	89.50
Control	0.00	0.00	0.00	0.00	0.00

Table 6. Median lethal time of photosensitizing compounds against the fourth larval instar of *S. littoralis*. exposed to sun light for different time intervals.

Photosensitizing compounds	Conc. (M)	LT_{50} (hrs.)
	26 X 10^{-5}	>4:00
	52 X 10^{-5}	3:50
Rose bengal	77 X 10^{-5}	3:25
	103 X 10^{-5}	2:45
	128 X 10^{-5}	2:20
	36 X 10^{-5}	>4:00
	72 X 10^{-5}	>4:00
Eosin yellow lactone	108 X 10^{-5}	3:50
	145 X 10^{-5}	3:38
	181 X 10^{-5}	3:25
	78 X 10^{-5}	>4:00
	156 X 10^{-5}	3:55
Methylene blue	234 X 10^{-5}	3:38
	313 X 10^{-5}	3:25
	391 X 10^{-5}	2:50

Percentages of *S. littoralis* fourth larval instar exposed to sun light for different time intervals.

It is interest 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's control (Mcquate *et al.*, 2005, Khater and Hendawy, 2014 and Attia 2016).

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

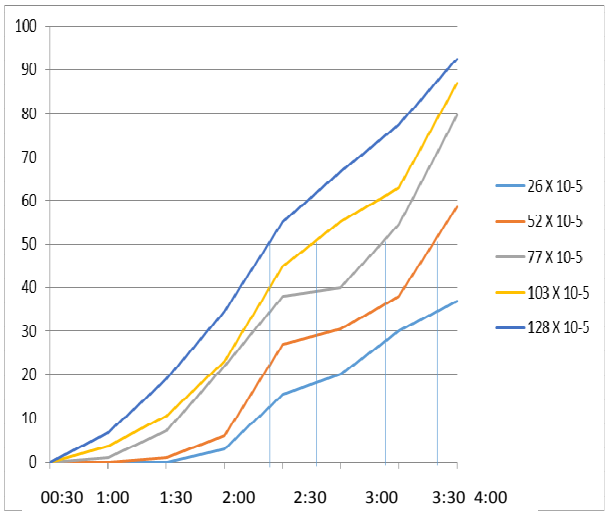

Fig. 1 Effect of different concentrations of rose bengal on the mortality percentages of *S. littoralis* fourth larval instar exposed to sun light for different time intervals.

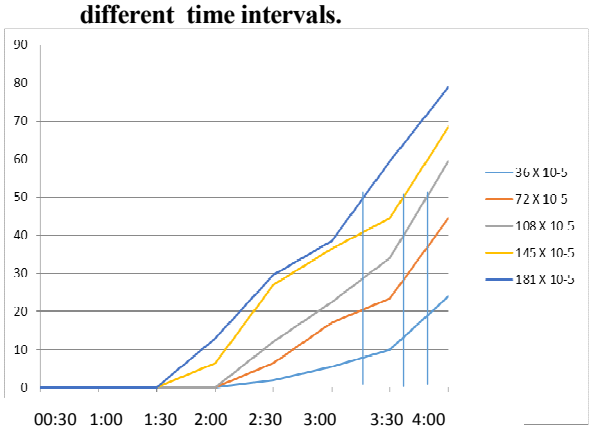

Fig. 2. Effect of different concentrations of eosin yellow lactone on the mortality

Concerning the toxicity of the three photosensitizing compounds used against the pest under the study, the 4th instar larvae of *S. littoralis* were allowed to feed on different concentrations of the tested photosensitizer compound; rose bengal, eosin yellow lactone an methylene blue for different periods of sun light exposure. According aforementioned results, the treated larvae showed different susceptibility to the tested compounds. The LC_{50} values ranged between $136X10^{-5}$ M and $37X10^{-5}$ M. This agrees to the previously findings of several authors working with different photosensitizing compounds in both laboratory and field application (Meyer *et al.*,1985 and Attia, 2016).

As revealed from the obtained results, rose bengal was the most promising compound, followed by eosin yellow lactone. This result had been detected by several investigators other insects (Aref, 2010 and Attia, 2016). On the other hand, methylene blue exerts as extremely low toxic activity against the cotton leaf worm larvae; this result is in agreement with Lukoiene *et al.* (2005) on *Liriomyza bryoniae* and Attia (2016) on house fly adults. On contrary, Graham *et al.* (1972) recorded that methylene blue was very toxic against yellow mealworm larvae, *Tenebrio moliator*. These differences in the results may be owing to different insect species used. Mangan and Moreno (2001) mentioned that the efficiency of the photosensitizers used as pesticides depends on the feeding intensity, sun light exposure and ingestion of the target insect species.

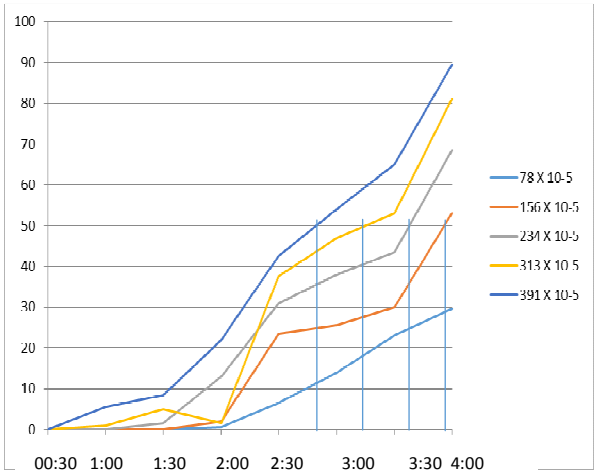

Fig. 3. Effect of different concentrations of methylene blue on the mortality

According to the chemical structure of the tested compounds, rose bengal has the highest number of halogen atoms included 4 iodine and 4 chlorine atoms and methylene blue has the lowest number included one chlorine atom. The photosensitizer compounds with greatest number of the halogen atom substituents yield greater toxicity, therefore, the halogen atoms amplify the reactions (Heitz and Downum, 1995 and 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 the exposure to sun light after treatments. As the concentration of the compound and the time of exposure increase, the percentage of the larval mortality increase. The LT_{50} (median lethal time) values of rose bengal ranged between> 4hrs. at $26X10^{-5}$ M and 2:20 hrs.at concentration $128X10^{-5}$ M, whereas the LT_{50} values of eosin yellow lactone ranged between > 4 hrs. at concentration $36X10^{-5}$ M and 3:25 hrs. at concentration $181X10^{-5}$ M; on the other hand the LT_{50} values of methylene blue ranged between > 4 hrs. at concentration $78X10^{-5}$ M and 2:5 hrs. at concentration $391X10^{-5}$ M.

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.

3.Biochemical studies:

In the present study total protein, lipids and carbohydrates were quantitavely estimated in the fourth larval instar treated with the median lethal concentrations (LC_{50}) of the tested photosensitizers as compared with the untreated one. The obtained results are represented in Table (7).

Total protein:

As summarized in Tab (7), the results showed significant reduction in the protein content at rose bengal and eosin yellow lactone, where the corresponding amounts of the total protein were 34.80 and 35.53 mg/g.b.wt. with change -11.68 and -9.82%; respectively compared with untreated larvae. On the other hand, in case of methylene blue treatment recorded, the amount of total protein increased to 40.17mg/g.b.wt. with slight change+1.95% compared with the untreated larvae, but there is no significant difference was observed between the amount of total protein in treated larvae and control.

Protein is the most characteristic compounds of living matter. The reduction in protein content resulted from damage of protein molecule and alteration of certain amino acid side chains due to compound sensitized photo oxidation, which lead to alteration in its properties to the point where it can no longer serve its usual purpose; photo oxidation of tyrosine with methylene blue involves rupture of the ring and formation of CO_2 (Attia, 2016). Histidine gives acetyl urea on photo oxidation with methylene blue. Methionine gives methionine sulfoxide with methylene blue and rose bengal (Spikes and Macknight, 1970).

Table 7. Amounts of total protein, total lipids and total carbohydrates contents in the fourth larval instar of *S. littoralis* treated with LC_{50} of three photosensitizing compounds.

Photosensitizing compounds	Total Protein (mg/g.b.wt.) Mean	Change %	Total Lipids (mg/g.b.wt.) Mean	Change %	Total Carbohydrates Change %age	Change %
Rose bengal	34.80 b	-11.68	4.89 c	-40.37	10.60 d	-47.55
Eosin yellow lactone	35.53 b	-9.82	6.78 b	-17.31	13.87 c	-32.37
Methylene blue	40.17 a	+1.95	6.70 b	-18.29	15.97 b	-20.98
Control	39.40 a	-	8.20 a	-	20.21 a	-
L.S.D	1.399		0.299		0.512	

The obtained results are accordance with Broome *et al.* (1976) who reported that rose bengal at concentration $5X10^{-3}$ M decreased the total protein content in 4-days-old adults of the boll weevil, *Anthonomus grandis* by 41%. The results are in agreement with Attia (2016) who indicated that the protein contents of the treated house fly adults with LC_{50} value of rose bengal and the untreated one was significantly differed in terms of the optical density measurements.

Total lipids:

Results represented in Table (7) summarized the effect of the three tested photosensitizer compound that mentioned previously on the total lipids content in the fourth larval instar treated with LC_{50} values of the used compounds. The data showed significant reduction in the total lipids content with all tested compounds compared with control. The three tested compounds resulted reduction reaching -40.37%, -17.31 and -18.29% for rose bengal, eosin yellow lactone and methylene blue, respectively as compared with control.

Lipids are essential structural component of the cell membrane and cuticle. They provide a rich source of metabolic energy. They facilitate water conversation both by formation of an impermeable cuticle barrier and by yielding metabolic water upon oxidation Abdel-Aziz and El-Gohary (2013). Polyunsaturated fats appeared to be photooxidized largely by singlet oxygen giving mono and dihydroperoxides; these initial products can undergo dark autoxidation of with further degradation and formation of more complex mixtures of reaction products (Heitz and Downum, 1995 and Attia, 2016).

Total carbohydrates:

As shown in Table (7), The most effective photosensitizer compound was rose bengal, where it caused highest reduction in the total carbohydrates content (-47.55%) relative to control, while methylene blue caused the lowest reduction in the total carbohydrate content (-20.98%) relative to control, on the other hand, eosin yellow lactone caused middle situation of reduction in the total carbohydrates content (-32.37%) compared with control.

Carbohydrates content are major component necessary for growth and development. So, the change in the activity as a result of treatment and the function in total carbohydrates content may be due to metabolism disturbance. The reduction in carbohydrates may inhibit all functions in all insect tissues during metamorphosis period specially cuticle. Also, it is necessary for male and female reproductive system and the development of embryo whereas carbohydrates yolk in the oocyte is essential step for successful development of the embryo also, sugar is very important constituent of the testes and seminal plasma in male (Chippendal, 1978). On contrary, Attia (2016) reported that total carbohydrates level increased in the treated adult house flies with The LC_{50} value of rose bengal, although this did not a significant change.

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فاعلية بعض المركبات المستحثة بالضوء ضد دودة ورق القطن وعلاقتها ببعض النظم البيوكميائية

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تهدف الدراسة الحالية الي تقييم التأثيرات السمية والبيوكميائية لثلاث مركبات مستحثة بالضوء وهي روز بنجال, ايوسين بيلو لاكتون و ميثلين بلو علي العمر اليرقي الرابع لدودة ورق القطن . اوضحت النتائج ان مركب الروز بنجال هو اكثر المركبات المختبرة سمية يليه الايوسين بيلو لاكتون ثم الميثلين بلو حيث سجلت LC_{50} القيم التالية ٣٧ x ١٠$^{-٥}$, ٨١ x ١٠$^{-٥}$ و ١٣٦ x ١٠$^{-٥}$ مولر على الترتيب. فيما يتعلق بالعملية المستحثة بالضوء للثلاث مركبات كانت قيمة LT_{50} لأقل تركيز للروز بنجال ,الايوسين بيلو لاكتون والميثلين بلو اكثر من اربعة ساعات لكل منها. وعلى الجانب الاخر كانت قيم LT_{50} لاعلى تركيز ٢,٢ , ٣,٢٥ و٢٥,٠ على الترتيب. يدل ذلك على ان مركب الروز بنجال هو المركب الاكثر فاعلية. اما التأثيرات نصف المميتة المتوسطة للثلاث مركبات المستحثة بالضوء موضع الدراسة على مستويات البروتين ,اللبيدات والكربوهيدرات الكلية على اليرقات المختبرة اثبتت النتائج ان هذه المركبات ادت الى نقص المحتويات البيوكميائية بإستثناء الميثلين بلو الذي ادى الى زيادة طفيفة في المحتوى الكلي للبروتين منسوبا الى المقارنة.