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Photosensitizing Effect of Rose Bengal, Methylene Blue and Rhodamine on Cotton Leaf worm, Spodoptera littoralis Larvae

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#### **INTRODUCTION**

Photosensitizers are promising chemical compounds in agriculture sectors. In this investigation, our discussion is focused on three different photosensitizer and their effects on the main metabolites, total carbohydrates, total proteins, and total lipid were determined in the total body homogenates of *Spodoptera littoralis*. The results showed the highest decrease in the total lipid. On the other hand, total carbohydrates and protein showed a lower reduction in most treatments. The Rose Bengal photosensitizer was more effective on death percentages of *Spodoptera littoralis* and lightly played an active role in enzyme activity

ABSTRACT

Spodoptera littoralis is one of the most serious agricultural pests. It is responsible for significant yield losses Cotton is infested and common in Egypt. Ishaaya and Klein (1990) The intensive use of broad-spectrum insecticides against S. littoralis has led to the development of resistance and undesirable effect (Wu et al., 2007) Photosensitivity insecticide has attracted increasing attention as a new type of highly efficient and environment-friendly pesticide (Ben Amor and Jori 2000). The main groups of photodynamic sensitizers that have been used as photoinsecticides are the thiophenes, furanocoumarins, xanthenes, porphyrins, phenol-thiazines, thiophene, acridines, and quinines (Ben Amor and Jori, 2000). phloxin B and Xanthene dyes (Ben Amor and Jori, 2000, Schroder et al., 2001) xanthene (Sakurai and Heitz, 1982; Fondren and Heitz, 1978). porphyrin- compounds (Lukšiené et al., 2007) oxygen species (ROS) such as singlet oxygen has been identified as one of the major species responsible for biological damage caused by photosensitization (Weishaput, Goomer, & Dougherty, 1976). The generation of free radicals in the skin by solar ultraviolet light (UV) accelerates. The major difference between the application of photoactive dye and malathion (or other organophosphates) (Paillous&Fery-Forgues, 1994; Peiette, 1991; Sies, 1986). Pesticides formulations function only whereas the organophosphates are in contact with as well as stomach poisons. Krasnoff et al., (1994). The appearance of an efficient photo insecticidal agent as possible alternatives to traditional organophosphate (Rebeiz, et al., 1987; Heitz, 1997; Ben Amor et al., 1998). In this investigation, we tested three photosensitizers with and without photo radiation on different leaf worm stages

#### MATERIALS AND METHODS

#### Insects:

The Laboratory strain of the cotton leafworm, *Spodoptera littoralis* was reared in the laboratory as described by El-Defrawi, 1964, under constant conditions of  $25^{\circ C} \pm 1$  and  $70 \pm 5 \%$  R.H.

#### **Medium Lethal Concentration Detection:**

Semi-Artificial diet as described by Shorey and Hale 1956 was used, in order to establish the toxicity lines of all tested compounds, and diet were poured in plastic cubs with 10 cm diameters, each cub containing 3 larvae, in 6 different gradual concentrations from  $1x10^{-1}$  to  $1x10^{-6}$  each concentration consists from 20 replicates

#### **Bioassay:**

For the detection of the median lethal concentration (LC<sub>50</sub>) values of photosensitizer (Rose Bengal, Methylene Blue and Rhodamine) a semi-artificial diet were poured in plastic cubs in ten replicates for each concentration, three concentration were examined  $1 \times 10^{-2}$ ,  $1 \times 10^{-4}$  and  $1 \times 10^{-6}$ . I ml from each aqueous concentration of the tested compound then were left to dry. The treated diet was offered to newly hatched larvae, newly molted  $2^{nd}$  and  $4^{th}$  instars larvae for 24 hours in dark and exposed to sunlight for different durations (one, three, five, seven and nine hours). The average mortality percentage was corrected using Abbott's formula (1925). The corrected mortality percentage of each compound was statistically computed according to Finney (1971)

#### **Biochemical Study:**

The biochemical assay was done after *Spodoptera littoralis* were treated and exposed to different intervals to sunlight. The larvae were homogenates and after centrifugation, the supernatant was used directly for enzyme assay.

#### **Total Proteins:**

Sample solutions 50  $\mu$ l were pipetted into a test tube and the volume was adjusted to 0.1 ml with phosphate buffer (pH 7.0). 5 ml of protein reagent were added to the test tube and the contents were mixed. The absorbance at 600 nm was measured after 2 min. and before 1 hr against blank prepared from 0.1 ml of phosphate buffer (pH 7.0) and 5 ml of protein reagent. The weight of the protein was compared against the corresponding absorbance resulting in a standard curve used to determine the protein in unknown samples. They were determined by the method of Bradford (1976).

#### **Total Carbohydrates:**

Total carbohydrates were determined as described by Singh and Sinha (1977) where Sample solution 100 ml was diluted to one ml with  $H_2O$ , then 5 ml enthrone reagent. A blank containing 1.1 ml of  $H_2O$  and 5 ml of enthrone reagent was placed.

#### **Total Soluble Lipids:**

Total lipids were estimated according to Knight *et al.*, (1972) using phosphovanillin reagent. Where solution 250 ml was added to concentrated sulfuric acid (5 ml) in a test tube and heated in a boiling water bath for 10 min. After cooling to room temperature, the digest (500 ml) was added to the phosphovanillin reagent (6.0 ml). After 45 min., the developed effect of Chlorophyllin Compound (Photosensitizer) on main metabolites level of *Spodoptera littoralis*. The color was measured at 550 nm against a reagent blank prepared from 500 distilled water and 6.0 ml phosphovanillin reagent. The result is expressed as mg lipid/insect.

#### **RESULTS AND DISCUSSION**

#### The Effect of The Photosensitizers on Spodoptera littoralis Larvae:

Preliminary assays were performed with larvae of *Spodoptera littoralis* to select the most effective photosensitizers and to determine whether photosynthesizes have an effect on the lifespan of insects as a chemical or not. The control as it is predictable gave zero death percentage, as the data illustrated in the previous all used photosynthesizes had a toxic effect, besides the neonates were the most sensitive instar. The sensitivity was enlarged in the case of RHB (Rose Bengal) where it gave 81.67, 51.66 and 35% death with the neonate, second and fourth instars, respectively. Both Methylene Blue and RHB (Rhodamine B) gave lower responses as follows 33.44, 16.66 and 3.33 % death for Methylene Blue and 36.67, 6.66 and 10.34 % death with RHB with the same previous instars when the highest concentration of photosynthesizes was used. Six concentrations of each photosensitizer were examined due to determine  $LC_{50}$  where it was found that it was the greatest in the case of RHB where it gave 6382.126, 40.968 and 8.726 and 12904.46, 34456.56 and 765523.34 in Case of using Methylene Blue and finally it recorded 13112.90, 8977627.98 and 13987657.89 ppm with RHB all previous with the neonate, second and fourth instars, respectively (Table 1).

**Table 1:** Effect of different concentrations of Rose Bengal, Methylene Blue and Rhodamine on percentage mortality, LC<sub>50</sub>, LC<sub>90</sub>, Slope and Relative Potency against three different larval stages of *Spodoptera Littoralis*.

Photosensitizing compounds	Concentrations	Percentage mortality		
	(molar)	N	2 <sup>rd</sup>	4 <sup>th</sup>
	1 X 10 <sup>-6</sup>	15.00 (6/60)	5.08 (3/59)	1.66 (1/60)
	1 X 10 -5	46.7 (28/60)	30.50 (18/59)	15.00 (9/60)
Rose Bengal (RHB)	1 X 10 -4	38.4 (23/60)	35.00 (21/60)	21.66 (13/60)
Nose bengal (MID)	1 X 10 -3	55.00 (33/60)	38.33 (23/60)	28.33 (17/60)
	1 X 10 -2	56.67 (34/60)	45.00 (27/60)	32.75 (19/58)
	1 X 10 -1	81.67 (49/60)	51.66 (31/60)	35.00 (21/60)
	Control	0.00 (0/60)	0.00 (0/60)	0.00 (0/60)
LC50		6382.126	40.968	8.726
LC <sub>90</sub>		6.39	0.0001	0.0003
Slope		- 0.4273	- 0.2346	- 0.2848
	1 X 10 -6	6.67 (4/60)	0.00 (0/60)	0.00 (0/60)
	1 X 10 -5	15.00 (9/60)	3.33 (2/60)	0.00 (0/60)
	1 X 10 <sup>-4</sup>	28.9 (18/60)	8.33 (5/60)	1.66 (1/60)
Methylene Blue	1 X 10 -3	31.67 (19/60)	11.66 (7/60)	0.00 (0/60)
	1 X 10 -2	31.67 (19/60)	18.96 (11/58)	1.66 (1/60)
	1 X 10 -1	33.34 (20/60)	16.66 (10/60)	3.33 (2/59)
	Control	0.00 (0/60)	0.00 (0/60)	0.00 (0/60)
LC50			34456.67	765523.34
LC <sub>90</sub>		2345679.56	7388765.12	9876546.45
Slope		- 0.2081	- 0.2743	- 0.4563
	1 X 10 <sup>-6</sup>	6.67 (4/60)	0.00 (0/60)	0.00 (0/60)
	1 X 10 <sup>-5</sup>	15.00 (9/60)	1.66 (1/60)	0.00 (0/60)
	1 X 10-4	23.73 (15/60)	5.08 (3/59)	1.66 (1/60)
Rhodamine B (RHB)	1 X 10 <sup>-3</sup>	31.67 (19/60)	1.66 (1/60)	3.33 (2/60)
	1 X 10 <sup>-2</sup>	35.00 (21/60)	5.08 (3/59)	8.47 (5/59)
	1 X 10 <sup>-1</sup>	36.67 (22/60)	6.66 (4/60)	10.34(6/58)
	Control	0.00 (0/60)	0.00 (0/60)	0.00 (0/60)
LC50		13112.90	8977627.98	13987657.89
LC <sub>90</sub>		2411235.45	45678976543.9	987654398.9
Slope		- 0.36678	0.875611	- 0.987655

## The Effect of The Natural Sunlight on Photosensitizer's Ability to Reduce *Spodoptera littoralis* Larvae:

In order to determine the efficacy of these chemicals on the ability to reduce the target insect pest, five different times were used (1, 3, 5, 7 and 9 hours) and the effect on the three instars (newly hatched larvae,  $2^{nd}$  and  $4^{th}$ ) for each chemical compound.

Rose Bengal (Table 2) showed the highest mortal effect on all stages where it gave 96.66, 80.00 and 63.33 death % nine hours post-application with newly hatched,  $2^{nd}$  and  $4^{th}$  larvae respectively where it gave 53.33, 40.00 and 33.33 death % with Methylene Blue (Table 3) and 73. 33, 55.17 and 53.33 death % when RHB was applied (Table 4) with the highest concentrations that were tested 1x10-2, both other concentrations of each photosensitizer gave the same trend and the Rose Bengal was the most effective.

Sunlight exposure periods (hours)	Concentrations of Rose Bengal mean no of dead larvae ± se (% of mortality) against						
	newly hatched larvae						
	Control	1x 10 -4	1x 10 -2				
1	0.00 (0/30)	16.66 (5/30)	36.66 (11/30)	43.33 (13/30)			
3	0.00 (0/30)	33.33 (10/30)	50.00 (15/30)	65.51 (19/29)			
5	0.00 (0/30)	43.33 (13/30)	60.00 (18/30)	83.33 (25/30)			
7	0.00 (0/30) 63.33 (19/3		65.51 (19/29)	90.00 (27/30)			
9	0.00 (0/30)	96.66 (29/30)					
LIT <sub>50</sub> (hr.)	0.6654 0.4727 1.3047						
	2 <sup>nd</sup> larvae instar						
1	0.00 (0/30)	0.00 (0/30)	10.00 (3/30)	23.33 (7/30)			
3	0.00 (0/30) 0.00 (0/30	0.00 (0/30)	23.33 (7/30) 43.33 (13/30) 58.62 (17/29)	51.72 (15/29) 56.66 (17/30) 63.33 (19/30)			
5	0.00 (0/30)	0.00 (0/30)					
7	0.00 (0/30)	30.00 (9/30)					
9	0.00 (0/30)	36.66 (11/30)	63.33 (19/30)	80.00 (24/30)			
LIT <sub>50</sub> (hr.)	0.4237 0.8264 0.945						
	4 <sup>th</sup> larvae instar						
1	0.00 (0/30)	0.00 (0/30)	10.00 (3/30)	36.66 (11/30)			
3	0.00 (0/30)	0.00 (0/30)	23.33 (7/30)	55.17 (16/29)			
5	0.00 (0/30)	0.00 (0/30)	23.33 (7/30)	60.00 (18/30)			
7	0.00 (0/30)	0.00 (0/30)	31.03 (9/29)	70.00 (21/30)			
9	0.00 (0/30) 3.33 (1/30) 36.66 (11/30) 63.33 (19/						
LIT <sub>50</sub> (hr.)	0.2141 0.3178 0.4268						

**Table 2:** Photodynamic effect of Rose Bengal against newly hatched, 2<sup>nd</sup> and 4<sup>th</sup> larval stages of *Spodoptera littoralis*.

Table 3: Photodynamic effect of Methylene	Blue against newly hatched, 2 <sup>nd</sup> and 4 <sup>th</sup> larval
stages of Spodoptera littoralis.	

Sunlight exposure periods (hours)	Concentrations of Methylene blue mean no of dead larvae ± se (% of mortality) against				
	newly hatched larvae				
	Control	1x 10 -6	1x 10 -4	1x 10 -2	
1	0.00 (0/30)	0.00 (0/30)	0.00 (0/30)	30.00 (9/30)	
3	0.00 (0/30)	0.00 (0/30)	10 (3/30) 23.33 (7/30)	41.37 (12/29)	
5	0.00 (0/30)	20.00 (6/30)		53.33 (16/30)	
7	0.00 (0/30)	26.66 (8/30)	27.58 (8/29)	46.66 (14/30)	
9	0.00 (0/30)	33.33 (10/30)	40.00 (12/30)	53.33 (16/30)	
LIT <sub>50</sub> (hr.)		0.424			
	2 <sup>nd</sup> larvae instar				
1	0.00 (0/30)	0.00 (0/30)	0.00 (0/30)	16.66 (5/30)	
3	0.00 (0/30)	10.00 (3/30)	0.00 (0/30)	20.68 (6/29)	
5			20.00 (6/30)	20.00 (6/30)	
7	0.00 (0/30)	20.00 (9/30)	24.13 (7/29)	26.66 (8/30)	
9	0.00 (0/30)	10.00 (3/30)	20.00 (6/30)	40.00 (12/30)	
LIT <sub>50</sub> (hr.)		0.091			
	4 <sup>th</sup> larvae instar				
1	0.00 (0/30)	0.00 (0/30)	0.00 (0/30)	10.00 (3/30)	
3	0.00 (0/30)	0.00 (0/30)	10.00 (3/30)	20.68 (6/29)	
5	0.00 (0/30)	0.00 (0/30)	16.66 (5/30)	20.00 (6/30)	
7	0.00 (0/30)	10.00 (3/30)	17.24 (5/29)	26.66 (8/30)	
9	0.00 (0/30)	0.00 (0/30)	23.33 (7/30)	33.33 (10/30)	
LIT <sub>so</sub> (hr.)	0.072 0.034 0.031				

Sunlight exposure periods (hours)	Concentrations of Rhodamine RHB mean no of dead larvae ± se (% of mortality) against					
	Newly hatched larvae					
	Control 1x 10 <sup>-6</sup> 1x 10 <sup>-4</sup>		1x 10 <sup>-2</sup>			
1	0.00 (0/30)	33.33 (10/30)	30.00 (9/30)	36.66 (11/30)		
3	0.00 (0/30)	40.00 (12/30)	36.66 (11/30)	44.82 (13/29)		
5	0.00 (0/30)	50.00 (15/30)	53.33 (16/30)	63.33 (19/30)		
7	0.00 (0/30)	56.66 (17/30)	58.62 (17/29)	70.00 (21/30)		
9	0.00 (0/30)	60.00 (18/30)	63.33 (19/30)	73.33 (22/30)		
LIT <sub>50</sub> (hr.)		0.588	0.392	1.011		
	2 <sup>nd</sup> larvae instar					
1	0.00 (0/30)	30.00 (9/30)	40.00 (12/30) 36.66 (11/30) 43.33 (13/30)	23.33 (7/30)		
3	0.00 (0/30)	37.03 (10/27)		41.37(12/29)		
5	0.00 (0/30)	33.33 (10/30)		50.00(14/28)		
7	0.00 (0/30)	39.28 (11/28)	51.72 (15/29)	50.00(15/30)		
9	9 0.00 (0/30)		56.66(17/30)	55.17(16/29)		
LIT <sub>50</sub> (hr.)		0.4112	0.723	0.817		
		4 <sup>th</sup> larv	/ae instar			
1	0.00 (0/30)	0.00 (0/30)	50.00 (15/30)	20.00 (6/30)		
3	0.00 (0/30)	0.00 (0/30)	40.00 (12/30)	27.58 (8/29)		
5	0.00 (0/30)	0.00 (0/32)	26.66 (8/30)	30.00 (9/30)		
7	0.00 (0/30)	3.33 (1/30)	31.03 (9/29)	44.82 (13/29)		
9	0.00 (0/30)	10.00 (3/30)	43.33 (13/30)	53.33 (16/30)		
LIT <sub>50</sub> (hr.)	0.210 0.2494 0.329					

**Table (4):** Photodynamic effect of Rhodamine RHB against newly hatched, 2<sup>nd</sup> and 4<sup>th</sup> larval stages of *Spodoptera littoralis*.

## 3- The effect of photosensitizers on the total chemical composition of *Spodoptera littoralis* larvae.

This part was studied thoroughly in order to explain the advantage found when Rose Bengal was used, the results conducted that total protein was decreased in I case of RHB reaction where it gave 37.01 in comparison to control (42.63) the same trend was found in other tested materials, (Table 5). Both total carbohydrates and lipids gave the same trends but it was found that only in RHB larvae failed to store lipids where it was significantly decreased to half in comparison with control (Table 5). The fat body is the main cell responsible for energy metabolism and conversation and storage of fat (Arrese and Soulages 2010). The decrease in the levels of lipid found in our investigation was interesting in using RHB. Photosensitizerare compounds are found to produce reactive oxygen species (ROS) upon irradiation, which cause cell death (Vatansever *et al.*, 2013).

**Table (5):** Percentage of total protein, lipids and carbohydrates on *Spodoptera littoralis* homogenate after treated with  $LC_{50}$  of photosensitizer (Rose Bengal-Methylene Blue and Rhodamine) and exposed to sunlight for different times.

, i 6							
Compound	Total protein (mg protein/g b.wt.)		Total lipid (mg lipids/g b.wt.)		Total carbohydrates (mg glucose/g b.wt.)		
		2 <sup>nd</sup> Instars larvae	%	2 <sup>nd</sup> Instars larvae	%	2 <sup>nd</sup> Instars larvae	%
1	**R Blh	$45.54 \pm 0.73$	106.82	$28.23 \pm 0.45$	28.27	$11.01 \pm 0.85$	32.57
2	R B2h	$43.46 \pm 0.39$	101.94	$66.90 \pm 0.37$	67.01	$16.48\pm0.12$	48.75
3	R BDark	$37.01 \pm 0.73$	86.81	$45.33 \pm 0.69$	45.40	$27.95\pm0.98$	82.69
4	Control	$42.63 \pm 0.90$	100.00	$99.83 \pm 0.87$	100.0	$33.80\pm0.00$	100.00
1	***M B1h	$48.55 \pm 0.84$	92.24	$99.31 \pm 0.07$	105.97	$29.12 \pm 0.36$	63.58
2	MB2h	$38.43 \pm 0.44$	73.01	$89.33 \pm 0.07$	95.32	$30.31 \pm 0.78$	66.17
3	M BDark	$46.99 \pm 0.88$	89.28	$91.72 \pm 0.66$	97.87	$44.88 \pm 0.74$	97.99
4	Control	$52.63 \pm 1.00$	100.00	93.71± 0.54	100.00	$45.80 \pm 0.72$	100.00
1	****R 1h	$48.55 \pm 0.34$	111.27	97.23 ± 0.68	98.38	$39.12 \pm 0.93$	93.58
2	R2h	$38.43 \pm 0.64$	88.08	$99.90\pm0.71$	101.98	$33.31 \pm 0.38$	79.68
3	R Dark	36.99 ± 0.77	84.78	87.33 ± 0.34	88.36	41.88 ± 0.59	100.19
4	Control	$43.63 \pm 0.88$	100.00	$98.83 \pm 0.38$	100.00	$41.80 \pm 0.44$	100.00

\* % = percentage relative to control \*\* Bengal \*\*\* Methylene blue \*\*\*\* Rhodamine

In this study, we have examined the systemic effect of three important photosensitizers on three *Spodoptera littoralis* larval instars; we found that *Spodoptera littoralis* sensitive to RHB in a way that it can be used in control programs for this pest and other pests in the same order where it represents model pest. The efficacy of photoactive compounds depends on the insect's feeding intensity and ingestion of the dye (Ben Amor and Jori, 2000) here we disagree with (Bradford 1976) who reported that photosensitized insects show large differences from controls. We recommend further investigations on open field studies and combination with other pesticides and toxicological studies in the future in order to detect the economic benefit of this product.

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#### **ARABIC SUMMARY**

تأثير المواد المستحثة للضوء مثل Rhodamine - Methylene Blue - Rose Bengal على يرقات دودة ورق القطن Spodoptera littoralis

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المواد المستحة للضوء هي مركبات كيميائية واعدة في مجال مكافحة الأفات الزراعة. وفي هذا البحث تركزت الدراسة على إستخدام ثلاثة أنواع من المركبات من المحسّسات الضوئية المختلفة وهما Rhodamine - Methylene Blue - Rose Bengal وذلـــــك علــــي يرقــــات دودة ورق القطنSpodoptera littoralis حيث تم دراسة تأثير ها على كلامن "المستقلبات الرئيسية - نسبة الكربو هيدرات الكلية - البروتينات الكلية - ونسبة محتوى إجمالي الدهون في يرقـات دودة ورق القطن Spodoptera littoralis.

أظهرت النتائج أنَّة كان<sup>1</sup>ت هناك أعلى نسبة إنخفاض في محتوى الدهون الكلية ومن ناحية أخرى الى أن الكربو هيدرات الكلية ومحتوى البروتين حدث لها إنخفاضًا ولكن بنسبة أقل وذلك في معظم المعاملات، كما أظهرت الدراسة إلى أن المحسس الضوئي Rose Bengal كان أكثر فاعلية في حدوث النسب المئوية للموت ليرقات دودة ورق القطن Ittoralis وكما لعب دورًا رئيسيا فى نشاط بعض الإنزيمات.