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The Toxicity Effect of Certain Photosensitizing Compounds on Some Biological Aspects of Field Strain of *Agrotis ipsilon* (Hufnagel) Larvae

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ABSTRACT

The black cutworm, *Agrotis ipsilon* larvae is the most serious pest around the world; it attacked the plant in the growing stage of many crops such as cotton, potato, corn, and tomato causing great damage for them and others which led to reduce the yield. So many chemical insecticides are used for controlling this pest which may buildup resistance to these pesticides Therefore, it is needed to evaluate some chemical compounds that belonged to different categories such as safranin, methylene blue and bromophenol blue against the 4th larval instars of *A. ipsilon* using poison baits. The results clearly demonstrated that the tested compounds had, a stomach and contact toxicity through the larval feeding on treated baits also, all these toxicants exhibited antifeedant and starvation effects in addition resulting larval paralysis post-feeding. Results on the basis the medium lethal concentrations LC_{50} of the tested compounds varied on the larval stage, the promising treatment among the investigated compounds were safranin that showed the lowest $LC_{50,}$ which was more toxic as stomach poison followed by methylene blue and the lowest effect was noticed in case of bromophenol blue treatment. The effect of both LC_{50} and LC_{25}values of these compounds showed a remarkable significant increase in both larval and pupal durations with retardation in their development as well as an increase in the mortality percentage and the malformation for both the resulting pupae and adults. Also, the toxicants affected the fertility of adults. Therefore, these photosensitizers, compounds can be used for controlling the black cutworm on their hosts as toxic baits' alternatives to traditional chemical pesticides, for increasing their toxicity and decreasing insect-resistant build up as a method implementing the integrated pest control program.

INTRODUCTION

Agrotis ipsilon (Hufnagel), the black cutworm is a serious polyphagous underground pest. It has an economically important pest for many agricultural crops especially Potato plants which are attacked by the larvae in the early 4-6 pairs of growing leaves at that time. It must prevent the damage caused by this pest by effective control method (Abd-El-Aziz *et al.*, 2019). That led to the intensive used of many pesticides which affected the environmental balance and pollution. Therefore, damaged public health through

accidental exposure, its residues in the food or groundwater. Also, it led to the development of resistance to these insecticides. The conventional strategy needs to investigate a new type of insecticide to get a good source of new toxic substance for this insect pest control. Over the last few years various independent studies demonstrated that near-UV- or visible light-absorbing dyes that belong to various organic compounds overcome a photoinsecticidal effect contrary to a few insect species, any photoactive compound can add to the baits to facilitate feeding by the target pests and to prevent its feeding by non-target insects. Depending on the different constitutions of baits which are more specific according to insect species, that increase the potential for acting as a pesticide with no significant toxicological effect on the environment and human (Amor, *et al.,* 2000). Sunlight-activated photopesticides characterize a promising option to conventional chemical compounds and the mechanism of photodynamic activity described with Heitz (1995). Toxicity takes place with the dye at the cellular level as a vehicle for generating the singlet oxygen molecules. The insects which exposure to visible light post-treatment with photoactive compounds accumulates within them, caused destruction in the cuticle, midgut, following by stop feeding and finally death (Amor *et al.*, 1998). The target of the current study is to evaluate the toxicity of some photosensitizer compounds against the black cutworm *A. ipsilon* larvae and their effect on different biological aspects under laboratory conditions using a semi-field technique for the possible use with an alternative to chemical pesticides in Integrated Pest Management program. Accordingly, our studies could help in the way of using the photo treatment protocols to control *A. ipsilon* larvae in the laboratory further investigation need to stand on their mode of action, physiological effect and field applications. These photoactive compounds could initiate new generation development of insecticides, where would be saved for the environment and human at low cost.

MATERIALS AND METHODS

Rearing Insect:

Field strain of *Agrotis ipsilon* obtained from Giza Government used in this study. It has been reared in the laboratory in the absence of any insecticides as the methods described by Abdin, (1979) with some modification according to Abd-El-Aziz *et al.,* (2019). In the cotton leafworm department, Plant Protection Institute, Agriculture Research Center, the *A. ipsilon* reared for few generations to obtain a suitable number to carry out the experiments.

Chemical Compounds Used:

Three photosensitizer compounds, Safranin is a fluorescent dye, chemical formula; $C_{20}H_{19}CiN_4$ (350.84g /mol) M.wt. It is a biological stain used in histology and cytology. It was obtained from LOBA Chemie (Mumbai, India). Methylene blue, belonging to class phenothiazinium, chemical formula; $C_{16}H_{18}CiN_3S_4\ xH_2O$ (319.859 g/mol) M.wt. It plays an important role in staining the living cells Junqueira *et al.,* (2002). Bromophenol blue chemical formula; $C_{19}H_{10}\ Br_4\ O_5\ S$ its Molar mass: (669.99 g·/mol) M.wt. (Reasxys.) as the commercial powders were obtained from Alfa company, Benha, Egyptian international center for import.

Bait's Preparation:

The preparation of baits was carried out according to Balevski, *et al.*, (1974) with some modification by Abd-El-Aziz *et al.,* (2019) as applied in the field by mixing wheat bran with about 25 kg /feddan and about 8-10 liter of water, then add 1 kg of black honey (molasses, as attractant) and mix well until fermentation by leaving it overnight in dark and worm place then used in the experiments.

Bioassays of the Tested Photosensitizers to *A. ipsilon* Larvae:

The treatment of the photochemical process by means of reducing the population of the pest was examined in the laboratory using a semi-field technique from December 2019 to March 2020. The toxicity effect of the three photosensitizers; safranin, methylene blue and bromophenol blue against the 4th larval instar of *A. ipsilon* under laboratory and photo conditions was tested. Stock solution (4 g) of each compound was mixed with 100 ml (w: vol) of dechlorinated water to get a stock solution to use it in preparation of six serial concentrations of 0.0625, 0.125, 0.25, 0.5, 1 and 2 % of poisoned baits. One hundred of the starved 4th larval instars for about 6-8 hrs were divided into four replicates. Only five 4th larval instar in each Petri dish to good exposure to sunlight and avoid cannibalism and introduced to the baits treated with the different six concentrations of each compound, other groups fed on untreated baits as control and the Petri dishes took outdoors to direct exposure to sunlight for 48 hrs (daylight). The mortality was recorded after 1, 2, 24, 48 and 72 hrs then the LC_{50} values calculated post 2, 24, 48 and 72 hrs of treatment.

Effect of the Tested Photosensitizers on Some Biological Aspects of the *A. ipsilon*:

The LC_{50} and LC_{25} values of each photosensitizer post 48 hrs were prepared separately by mixing with the baits. Four hundred of the 4thlarval instar in four replicates were starved then left to feed on poisoned bait with direct exposure to sunlight (daylight) to simulate the field condition for two days, other groups were fed on untreated baits for control under the same condition. Then the remained larvae, transferred to other jars and feeding on untreated castor bean leaves, until pupation then adult emergence. Some biological aspects (larval duration, pupal duration, larval and pupal deformation, adult emergence, adult malformation, sex ratio, fertility and fecundity percentages of moths were investigated.

To the best of our knowledge, there is a lack of information about the control of *A. ipsilon* with photosensitizers, Particularly, few researches are dealing with photodynamic materials acting as pesticides against arthropod with economic importance particularly comparing with another natural control strategy with excepting research using rose bengal, safranin and tetramethrin against *H. dromedarii* ticks (Khater & Hendawy 2014 and Khater *et al.,* 2016), notably, safranin, a basic dye used mainly as a biological stain and redox indicator, was successfully tested as an acaricide for the first time. Therefore, the aim of this study is to compare the toxic effect and the impact of female reproductive potential of safranin, methylene blue and bromophenol blue on *A. ipsilon* females produced from treated larvae.

Statistical Analysis:

The mortality percent of the 4thlarval instar of *A. ipsilon* was calculated and corrected according to Abbott's formula (Abbott, 1925). The LC_{50}s % determined for established regression lines according to Finney, (1971). All obtained data for biological studies were statistically analyzed and calculated the variance ratios. The method of ANOVA by using (SPSS) computer program calculated at 5% level. The Toxicity index was calculated according to Sun (1950) for the direct comparison of the tested compounds where the most toxic compound has given 100 units on the toxicity index scale. Toxicity index = LC_{50} of the most effective compound ×100 / LC_{50} of the used compound. Relative potency was calculated according to the method (Zidan and Abdel-Mageed, 1988).

RESULTS AND DISCUSSION

A Toxicity of Safranin, Methylene Blue and Bromophenol Blue Against the 4th Larval Instars of *Agrotis ipsilon:*

The toxicity of the tested compounds as illustrated in Tables (1- 4), revealed that the larval mortality was increased gradually as exposure periods to sunlight increased to reach

their maximum with safranin even as LC_{50} values decreased. The LC_{50} values determined for the 4th larval instars of *A. ipsilon* could be arranged as descending orders, safranin, methylene blue and bromophenol blue, respectively. The larval mortality was recorded with the different time intervals post exposed to sunlight. The live and dead larvae were recorded, they were considered alive when a physical stimulation to brush occurred, while the dead were unable to move or their capability to right their bodies or any signs of life were regarded as dead. The obtained data of the LC_{50} values after 2, 24, 48 and 72 hrs showed that safranin was the most effective one with the lowest LC_{50} (6.56, 1.082, 0.336 and 0.107 ml%), respectively followed by methylene blue where the LC_{50} values (40.346, 2.314, 0.452 and 0.125ml%), respectively whereas the lowest effect was Bromophenol blue with (23.28, 5.162, 0.387 and 0.376 ml %), respectively.

Similarity safranin recorded the lowest LC_{25} values compared with the other two compounds. The LC_{25} values were 1.293, 0.329, 0.141 and 0.053 ml % post 2, 24, 48 and 72 hrs, respectively for safranin then, 8.066, 1.307, 0.241 and 0.032 ml% in case of methylene blue while bromophenol recorded the highest LC_{25} values 8.571, 1.142 , 0.178 and 0.175 ml%, respectively at the same time intervals.

It was cleared in the primary studies the source of light was serious to the sensation of dye as an effectual agent for pest control. Where the effectual of natural sunlight may be more than the artificial light due to its intensity and photons of suitable wavelength to easily realize by dyes to cause toxicity. The characteristic color of methylene blue is caused by the strong absorption band in the 550-700 nm region (Junqueira *et al.*, 2002), that harmonized with Ochsner (1997) the photosensitizer absorbs the energy absolutely from light source then transferred to oxygen molecules to produce an activated type of singlet oxygen which could oxidize directly in a biological cell due to its electron-rich double bonds and electrophilic which may be considered the main reason for cytotoxic then lead to cell death. Clement *et al.*, (1980) described that the larval mortality of the black cutworm increases as concentration increase to reach its maximum at 5×10^{-3} M of rose bengal than others. These results were consistent with the result observed by Khater *et al.,* (2016), on ticks LC_{50} values of Safranin post-treatment for 8 and 24 hrs were 0.78 and 0.20 %, respectively. In addition to Khater and Hendawy (2014), the LC_{50} was 0.35 and 0.15 %, respectively in the same tested insect and time. Methylene blue was more toxic than hematomporphyrin on *S. littoralis* (Abd El-Naby, 2007). In contrast, El - Ghobary, *et al*., (2018) found that methylene blue was being less effective against the 4th larval instar of *S. littoralis* by recording larval mortality 29.50 % with concentrations $78X10^{-5}$ M and 89.50 % with $391X10^{-5}$ M. in these fields of study, Junqueira *et al.* (2002) and Tardivo *et al*. (2015) recorded that rose bengal was the most poisonous photosensitizer of used compound followed by eosin yellow. In addition, methylene blue is playing an important role in photodynamic therapy as a drug in vivo and in vitro of the living cells. It capable to regard many diseases as virus and fungal infections with low toxicity.

Based on, the LC_{50} of each compound toxicity index after exposure time 2, 24, 48 and 72hrs hrs. were tabulated in Tables (1-4) reflected that safranin exhibited the highest larvicidal effect and given an arbitrary value of 100 units with all tested time, that may be due to more rapid metabolism of it or the visible light-absorbing polycyclic dyes which need to the occurrence of singlet oxygen. Its action as phytotoxins versus a diversity of poisonous insects (Heitz, 1987).The toxicity index values of the other compounds varied, where bromophenol was 28.18, 20.96, 86.91 and 28.46% while methylene blue exhibited larvicidal activity as toxicity index with 16.26, 46.76, 74.33 and 85.6 % at 2, 24, 48 and 72 hrs as toxic as the toxicity of safranin, respectively. Because most bromophenols reveal strong poisonous effects mainly, according to the degree of bromination and the method of eliminating 4-bromophenol in aqueous liquid is necessary and more effective (Dandan Xua, 2018).

Table 1: Susceptibility of the 4th larval instars of *Agrotis ipsilon* to safranin, methylene blue and bromophenol blue post 2 hrs.

Tested compound	Safranin		Methylene blue		Bromophenol blue	
	LC_{25} (ml %)	LC_{50} (ml %)	LC_{25} (ml %)	LC_{50} (ml %)	LC_{25} (ml %)	LC_{50} (ml %)
	1.293	6.56	8.066	40.346	8.571	23.28
Slope ±S. E	0.956 ± 0.084		0.965 ± 0.198		1.554 ± 0.462	
95% Fiduciual limits (lower-upper)	1.0478-1.6741	4.425-11.261	4.1965-35.0438	13.496 -507.809	4.2511-110.1803	8.097 - 1188.082
Toxicity index		100		16.26		28.18
Relative potency		6.51		1		1.73

Toxicity index = LC_{50} of the most toxic compound ×100 / $LC_{50}$of the tested compound.
Relative potency = LC_{50} of the least toxic compound / $LC_{50}$of the tested compound.

Table 2: Susceptibility of the 4th larval instars of *A. ipsilon* to safranin, methylene blue and bromophenol blue post 24 hrs.

Tested compound	Safranin		Methylene blue		Bromophenol blue	
	LC_{25} (ml %)	LC_{50} (ml %)	LC_{25} (ml %)	LC_{50} (ml %)	LC_{25} (ml %)	LC_{50} (ml %)
	0.329	1.082	1.307	2.314	1.142	5.162
Slope ±S. E	1.305 ± 0.072		2.72± 0.209		1.03±0.084	
95% Fiduciual limits (lower-upper)	0.1269-0.5774	0.731- 3.429	0.8639-2.152	1.98-6.218	0.9485-1.4239	3.681-8.112
Toxicity index		100		46.76		20.96
Relative potency		4.77		2.23		1

Toxicity index = LC_{50} of the most toxic compound ×100 / $LC_{50}$of the tested compound.
Relative potency = LC_{50} of the least toxic compound / $LC_{50}$of the tested compound.

Table 3: Susceptibility of the 4th larval instars of *A. ipsilon* to safranin, methylene blue and bromophenol blue post 48 hrs.

Tested compound	Safranin		Methylene blue		Bromophenol blue	
	LC_{25} (ml %)	LC_{50} (ml %)	LC_{25} (ml %)	LC_{50} (ml %)	LC_{25} (ml %)	LC_{50} (ml %)
	0.1409	0.336	0.2413	0.4519	0.1783	0.3865
Slope ±S. E	1.7868 ± 0.2372		2.475± 0.2624		2.008 ± 0.3292	
95% Fiduciual limits (lower-upper)	0.0887-0.1912	0.2591-0.4223	0.1756-0.3052	0.3644-0.546	0.0961-0.2527	0.2793-0.488
Toxicity index		100		74.33		86.91
Relative potency		1.35		1		1.17

Toxicity index = LC_{50} of the most toxic compound ×100 / $LC_{50}$of the tested compound.
Relative potency = LC_{50} of the least toxic compound / $LC_{50}$of the tested compound.

Table 4: Susceptibility of the 4th larval instars of *A. ipsilon* to safranin, methylene blue and bromophenol blue post 72 hrs.

Tested compound	Safranin		Methylene blue		Bromophenol blue	
	LC_{25} (ml %)	LC_{50} (ml %)	LC_{25} (ml %)	LC_{50} (ml %)	LC_{25} (ml %)	LC_{50} (ml %)
	0.053	0.107	0.032	0.125	0.175	0.376
Slope ±S. E	2.177± 0.107		1.139 ± 0.068		2.027 ± 0.081	
95% Fiduciual limits (lower-upper)	0.0138-0.0541	0.044-0.158	0.0171-0.0445	0.086 -0.164	0.0977-0.2372	0.245-0.582
Toxicity index		100		85.6		28.46
Relative potency		3.51		3.01		1

Toxicity index = LC_{50} of the most toxic compound ×100 / $LC_{50}$of the tested compound.
Relative potency = LC_{50} of the least toxic compound / $LC_{50}$of the tested compound.

In the current study, using the relative potency level as a suitable process in comparing the toxicity degree of the variables examined compounds expressed as the number of folds, at the requisite toxicity level, compared with the lowest effective toxicant integrated into the estimation in the study. As regards the relative potency levels depend on the LC_{50} values as reported in the same Tables, the larvicidal activity values of safranin and bromophenol were 6.51 and 1.73 folds after 2 hrs; even as 1.35 and 1.17 folds in case of 48 hrs., respectively as toxic as the larvicidal effect of methylene blue. While as regards the toxicity of bromophenol blue the relative potency after 24 hrs was 4.77 and 2.23 for safranin and methylene blue, respectively while in the case of 72 hrs the potency was 3.51 and 3.01 times as toxicity as the toxic effect of bromophenol blue, respectively.

Our results are in harmony with the authors El-Ghobary, *et al.*, (2018) the toxicity index of methylene blue and eosin yellow lactone was 35.78 & 45.68 %, respectively as toxic as rose bengal on *S. littoralis* in addition to, Khater *et al.*, (2016), found that safranin to be 10, 38 and 73 times more effective than tetramethrin against ticks post 8, 6 and 24 hrs of treatment, respectively comparing with 33 times post 2 hrs.

Effect of the Tested Photosensitizers On Some Biological Aspects of the *A. ipsilon* on:

Larval Mortality:

The effect of the LC_{50} and LC_{25} values of photosensitizing compounds on *A. ipsilon* larvae**,** under sunlight condition, the larval mortality, pupation, adult emergency, fecundity and fertility of *A. ipsilon* was presented in Table 5. The results showed that the accumulative mortality was 98, 92 and 88% as a result of treatment with LC_{50s} of safranin, methylene blue and bromophenol blue, respectively. While LC_{25}s recorded 94, 76 and 68%, respectively compared with 1.47% in the control group. The efficiency of photosensitizers related to mortality increased as the concentration of used dyes and the exposure time to sunlight post-treatment increased.

Pupation:

The treatment with LC_{50} and LC_{25} have markedly reduced the metamorphosis of treated larvae to pupae in case of treatment with LC_{50} than $LC_{25}$of the tested compounds. Where the lowest pupation was with safranin 2 and 6%, and slightly higher 24 and 32% in case of treatment with methylene blue and bromophenol blue, respectively at LC_{25}. whereas 8 and 12% at LC_{50}s of the same compounds, respectively compared with 98.53% in control.

Adult Emergence:

The same table (5) showed that all the tested compounds affected on the adult emergence it obvious that safranin was the most effective compound where didn't record any emerged adult in case of pretreated 4^{th} instars with both LC_{50} and LC_{25} levels. Basing on the total number of treated larvae, the adult emergence was reduced to 6 and 16% in the case of methylene blue, while bromophenol blue records slightly increase to 10 and 26% with LC_{50} and LC_{25} values, respectively compared with 92.65% in control.

Based on the total number of formed pupae; the adult emerged % were 0, 75 and 83.33% as a result of treatment with LC_{50} values of safranin, methylene blue and bromophenol blue, respectively. While with Lc_{25} values the adult emerged % were 0, 66.66 and 81.25% for safranin, methylene blue and bromophenol blue, respectively compared with control which being 94.03%.

Fecundity and Fertility of *A. ipsilon* Adult*:*

According to the previous result of safranin as shown in Table, 5; there wasn't any emerged adult therefore, the treatment caused greatly effectual on fecundity and fertility of the treated larvae. While methylene blue and bromophenol blue showed a highly significant reduction in the number of eggs laying per female which record 148 and 210.67 eggs comparing with untreated control that exhibited 395 eggs in case of LC_{50} treatments and LC_{25} recorded a slight increase in the number of deposit eggs than $LC_{50,}$ to be in range

(168.67) and (270.33) with methylene blue and bromophenol blue, respectively compared with control (395 eggs).

The fertility of adults measured with hatchability of egg deposit, all treatments with LC_{50} showed highly sterility of all produced moths with no egg hatched compare with 89.11% of untreated control. While LC_{25} showed slight hatchability 17.79 % and 27.22% with methylene blue and bromophenol blue, respectively compared with 89.11% eggs in control, that in harmony with Khater and Hendawy (2014)where the hatchability percentage of untreated control was about 92 %. These results cleared that the main mode of action of these compounds was not only direct mortality but they had a latent effect by reducing pupation, adult emergence, fertility and fecundity of produced adults. Where although some larvae succeed to form pupae but can't produce adults while almost all of them failed to deposit eggs or hatch.

Table 5: Effect of LC_{50} and LC_{25} values of safranin, methylene blue and bromophenol blue on larval mortality, pupation, emergency, fecundity and fertility of *A. ipsilon* adult

Tested compounds	Accumulative larval mortality %	Pupation %	Emergency % Based on the total. no of larvae	Emergency % Based on the total. no of pupae	Fecundity mean±S.E	Fertility (Hatchability %)
	LC_{50} (ml %)					
safranin	98	2	0	0	-	-
methylene blue	92	8	6	75	$148^{c} \pm 11.93$	Zero
bromophenol blue	88	12	10	83.33	$210.67^{b} \pm 22.48$	Zero
Control	1.47	98.53	92.65	94.03	$395^{a} \pm 15.62$	89.11
F values					0.0001 ***	
L.S.D.					59.66	
	LC_{25} (ml %)					
safranin	94	6	0	-	-	-
methylene blue	76	24	16	66.66	$168.67^{c} \pm 25.69$	17.79
bromophenol blue	68	32	26	81.25	$270.33^{b} \pm 10.48$	27.22
Control	1. 47	98.53	92.65	94.03	$395^{a} \pm 15.62$	89.11
F values					0.0004***	
L.S.D.					63.62	

Means with the same letter are not significantly different at $p < 0.05$.

Effect of the LC_{50} and LC_{25} Values Of The Tested Photosensitizers On Larval Duration, Pupal Duration And Adult Longevity of *A. ipsilon*:

Larval Duration:

The data tabulated in Table (6) reflected the effect of LC_{50} and LC_{25} values of safranin, methylene blue and bromophenol blue on the different duration post-treatment. The larval duration preliminary from initial larval instar treated up to form pupae. The results showed highly significant prolongation in larval periods post-treatment as 4^{th} larval instars with both LC_{50} and LC_{25} in spite of control where there was no significant difference between them. Where the most prolonged one was safranin, then methylene blue and bromophenol blue. In case of treatment with LC_{50} values, larval periods post-treatment was in the range 27.50, 26.67 and 25.67 days for safranin, methylene blue and bromophenol blue, respectively compared with control (15.5 days). In the same trend highly significant prolongation in larval period with LC_{25} values between all treated and untreated larvae while there was no significant difference between methylene blue and bromophenol blue. The larval period attended 30.5, 24.5 and 25.5 days for safranin, methylene blue and bromophenol blue, respectively compared with 15.5 days for control.

Pupal Duration:

The data presented in the same Table (6) stated that safranin didn't record any emerged adults with LC_{50} treatment. While both the tested compounds methylene blue and bromophenol blue showed a highly significant extension in pupal duration for LC_{50}s treatment compare with control and there was no significant difference between each other. The most extended pupae resulted from methylene blue (22.33 days) followed by 21.33 days for bromophenol blue while the pupae of safranin spent 19.67 days and failed to emerge adults comparing with control (14.0 days).

Table 6: Effect of LC_{50} and LC_{25} values of safranin, methylene blue and bromophenol blue on larval, pupal durations and adult longevity of *A. ipsilon.*

Tested compounds	Larval period post-treatment (day) Mean ± S. E	Pupal duration(day) Mean ± S. E	Adult longevity (day) Mean ±S. E
	LC_{50}		
Safranin	$27.50^{a}\pm 0.29$	-	-
Methylene Blue	$26.67^{a}\pm 0.88$	$22.33^{a}\pm 0.33$	$7.67^{b}\pm 0.33$
Bromophenol blue	$25.67^{a}\pm 1.76$	$21.33^{a}\pm 2.33$	$7.0^{b}\pm 1.0$
Control	$15.50^{b}\pm 0.87$	$14.0^{b}\pm 1.0$	$12.33^{a}\pm 1.2$
F values	0.0002***	0.0191*	0.0125*
L.S.D.	3.54	5.49	3.19
	LC_{25}		
Safranin	$30.5^{a}\pm 0.87$	$19.67^{a}\pm 1.76$	-
Methylene Blue	$24.5^{b}\pm 1.15$	$20.33^{a}\pm 0.88$	$8.67^{b}\pm 0.88$
Bromophenol blue	$25.5^{b}\pm 1.04$	$18.33^{ab}\pm 1.45$	$10.67^{ab}\pm 0.88$
Control	$15.5^{c}\pm 0.87$	$14.0^{b}\pm 1.0$	$12.33^{a}\pm 1.2$
F values	0.0000***	0.0815^{ns}	0.1044^{ns}
L.S.D.	3.23	5.16	3.46

Means with the same letter are not significantly different at $p < 0.05$.

Adult Longevity:

There is a significant reduction in adult longevity treated with Lc_{50} of methylene blue and bromophenol blue, which being 7.67 and 7.0 days compared with 12.33 days of untreated control. On the other hand, there was no significant difference in adult longevity was observed between methylene blue and bromophenol blue 8.67 and 10.67 days in spite of 12.33 days of control.

It could be concluded that all tested Photosensitizer treatments recorded a highly significant increase in larval durations at both LC_{50} and LC_{25} in addition to a significant increase in pupal duration with LC_{50} and not significant with LC_{25} values. While there was a significant decrease in adult longevity with LC_{50} and not significant with LC_{25} values.

Generally, before treatment the larvae should be starved for at least 6-8 hrs to facilitate and ensure increasing the amount of food taken with dyes and accelerate its entry into the larval tissues, this phenomenon was agreed with reported by Lukðienë *et al.,* (2005). The obtained results indicated that toxicity greatly varied according to the chemical structure of treated dyes. Examination of the susceptibility of the black cutworm, *A. ipsilon* larvae on the chosen photosensitizer compounds proved that safranin was the most efficient dye at both LC_{50} and LC_{25} values follow by methylene blue while bromophenol blue was the lowest efficient one. The prolongation in the larval, pupal durations and decreased in adult's longevity may be due to disturbance in metamorphosis which led to significantly extend the

instar period with weakness which could be attacked with the various enemy and finally dead, delay emerged molts and the effect on the sex ratio led to disturbance in a successive generation so can be used in IPM program. The numbers of survived and ovipositing females, deposited eggs /female and hatched larvae were noted with them that in harmony with the observation of Abdel-Aziz et *al.,* (2013) the treatment with plant oils recorded significant prolongation in larval and pupal durations for both 2nd and 4th instars of *S. littoralis* even as a reduction in adult Longevity of both sexes was observed. Khater *et al.,* (2016), the treatment with safranin and tetramethrin with low concentrations showed a significant reduction in the female's fecundity; expressed as eggs lying per female and the hatchability. Based on photochemical reactions, the photosensitizer effect was accumulated post-exposure to visible light led to the death in the insect body (Khater and Hendawy 2014). Phototoxicity takes place as a catalyst at the cellular stage to induct oxidation reactions that destroy the cell as oxidative stress which led to larval death (Hamblin, 2016). Or the appearance of different malformation in larval, pupal and adult stages which reflect the disturbance in growth and fecundity (Almeida, *et al.,* 2009), which led to reduce the population of the insect pest which doesn't obtain resistance versus photoactive compounds. *A. ipsilon* buildup resistance to numerous pesticides used improved that requiring big efforts to alternative methods for control it. Various authors on several photosensitizer and insects were reported photosensitizer compounds could be used in substitute of conventional chemical compounds in the pest (Khater and Hendawy 2014).

Effect of the LC_{50} and LC_{25} Values of the Tested Photosensitizers on Sex Ratio and Morphological Abnormalities in All Generation of *A. ipsilon*

The tested compounds showed a different effect on the sex ratio, morphological abnormalities in *A. ipsilon* (Table 7 and Figs 1-3) with both treatments the most disturbed product was bromophenol blue, by producing males over females about (4 folds) and females over males by about (2 folds) as a result of treatment with LC_{50} and LC_{25} values, respectively. Also, methylene blue produced males over females about 1.5 folds with LC_{25} values. While there was no effect with LC_{50} treatment. In contrast, no moths were produced after safranin treatment compared with a high proportion of female production in control (1: 0.97).

Treatment of the 4th larval instar *A. ipsilon* with LC_{50} and LC_{25} values of tested dyes, safranin, methylene blue and bromophenol blue demonstrated some morphological abnormalities. The most effective one was safranin with 100 % deformed pupa with no moth emerged with both treatments, followed by methylene blue with 66.66 and 62.5% for adult malformation while pupal deformation were 25 and 33.33% to form total malformation 75 and 74.99 % with LC_{50} and LC_{25} values, respectively. In contrast, bromophenol blue was the least effective with 20 and 38. 46% for adult malformation and 16.66 and 18.75% for deformed pupae to record the lowest total malformation 33.33 and 50 % LC_{50} and LC_{25} values, respectively in spite of control 1. 47, 4.48 and 5.97 % for adult malformation, pupal deformation and total malformation, respectively. The previous results showed malformation especially in produced moths as failed to emergence by deformed pupa, pupa-adult intermediate, or with malformed wings that led to debility to fly and crawl causing failure in mating in addition, overcome male upon female. These results are interesting to decrease build up the population. Also, the dead larvae noted to be very small, failed to molt or take off the old cuticle compare with the control that may be due to pigmentation of the larval body with dyes which was shown by necked eyes particularly digestive tract as the internal lining of the alimentary canal was adhering with dye particles and the larvae couldn´t relief the fed amount of dye even afterward long time as shown in (Figs 1-3). Which translated as the anti-feeding effect, it was cleared in the primary studies the source of light was serious to the sensation of dye as an effectual agent for pest control. Where the effectual of natural sunlight may be more than the artificial light due to its intensity and photons of

suitable wavelength to easily realize by dyes to cause toxicity. Also, Younis, *et al.,* (2020) sunlight is a critical factor for the stimulation of used photosensitizer, rose bengal which is greatly efficient at low concentration and little exposure time. That harmonized with Lukðienë *et al.,* (2005) the leafminer feeding for 5 hrs under the red light and 15 hrs in the dark at 22 ± 1 °C on hematoporphyrin dimethyl ether resulted in males less sensitive than females. In this field of investigation, rose bengal and erythrosin B inducted physiological and morphological abnormalities to larval, pupal and adult stage of *Musca autumnalis* (Fairbrother *et al*., 1981) and Abdel-Aziz *et al.,* (2013) demonstrated the highest deformation (66.1%) in larval and pupal stages of *S. littoralis* in addition, several degrees of adult malformations (15%).

Finally, the larvicidal effect of safranin was clearly in this study which was greatly effectual with all used concentrations than methylene blue or bromophenol blue and considered promising as photodynamic effect compared to the others. Photosensitizers could be integrated into programs of pest management of Lepidoptera, particularly as lacking biocontrol agent (Martin *et al.,*1998). It was very important to respect the performance of these Photosensitizers compounds under field conditions and civilizing their photostability.

Table 7: Effect of LC_{50} and LC_{25} values of safranin, methylene blue and bromophenol blue on sex ratio and malformation of pupa and adult of *A. ipsilon*.

Tested compounds	Sex ratio		Adult malformation %		Pupal deformation D. P+M.P+P.ad %		Total malformation %	
	♀	♂	Based on total no. of pupae	Based on total no. of adult emergence	Based on total no. of larvae	Based on total no. of pupae	Based on total no. of larvae	During generation
	LC_{50}							
Safranin					2	100	2	100
Methylene Blue	1	1	50	66.66	2	25	6	75
Bromophenol blue	1	4	16.66	20	2	16.66	4	33.33
Control	1	0.97	1.49	1.47	4.41	4.48	5.88	5.97
	LC_{25}							
Safranin	-	-	-		6	100	6	100
Methylene Blue	1	1.5	41.66	62.5	8	33.33	18	74.99
Bromophenol blue	2	1	31.25	38. 46	6	18.75	16	50
Control	1	0.97	1.49	1.47	4.41	4.48	5.88	5.97

M.P: malformed pupa; P-ad: pupa - adult. D.P: dead pupae

Fig1:. Control larva, pupa and adults of *Agrotis ipsilon*

Fig.2:Total malformations in *Agrotis ipsilon* post treatnent with safranin.

Fig3: Total malformations in *Agrotis ipsilon* post treatnent with methylene blue

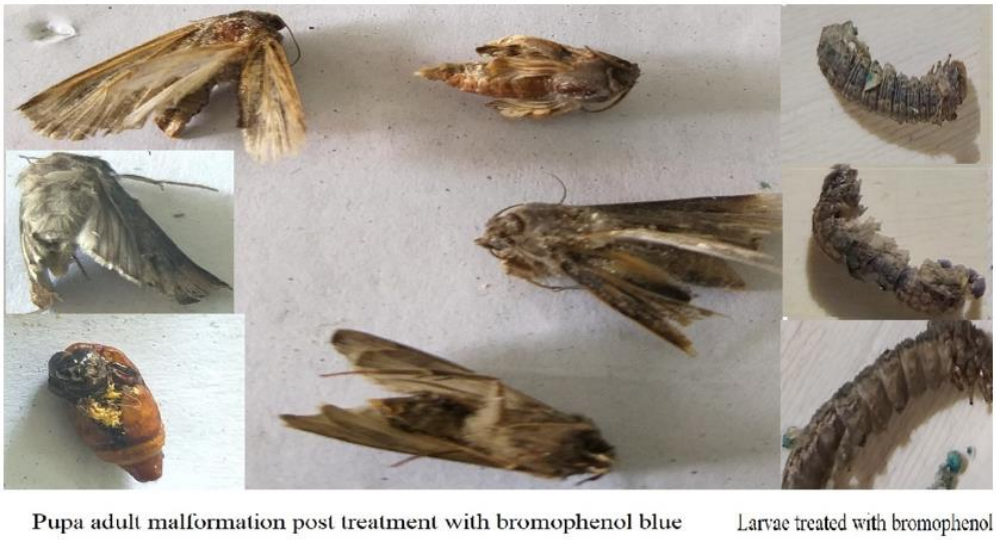

Fig.4: Total malformations in *Agrotis ipsilon* post treatnent with bromophenol blue

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ARABIC SUMMARY

تاثير سميه بعض المستحسات الضوئيه على بعض النواحى البيولوجية ليرقات السلالة الحقلية للدودة القارضة السوداء

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تعتبر يرقات ال *Agrotis ipsilon* من اخطر الافات حول العالم فهى تهاجم النبات فى مراحل النمو كمحاصيل القطن البطاطس والدرة و الطماطم مما تتسبب فى اضرار لها ولغيرها مما يؤدى الى تقليل انتاجية المحصول. تستخدم من المبيدات الحشريه الكيميائيه لمكافحتها والتى قد تؤدى الى زياده المقاومه لها لدلك نحاول تقيم بعض المركبات الكيميائيه التى تنتمى الى فئات مختلفه مثل safranin, methylene blue and bromophenol blue ضد الطور اليرقى الرابع كطعوم سامه وقد اظهرت النتائج ان المركبات المختبره لها سميه معديه وملامسه عن طريق تغديه اليرقات على الطعم السام كل هدا اظهر تاتيرات مضاده للتغديه والتجويع بالاضافه الى شلل اليرقات بعد التغديه بناءاعلى تاثير التركيزات النصف مميته LC_{50} للمركبات المختبره فقد اظهرت اختلافا فى مرحله اليرقات و كانت افضل معامله بين المركبات الى تم فحصها باقل تركيز مميت والدى كان اكثر سميه كسم معدى يليه methylene blue واقل تاثيرا كان methylene blue وقد وجد ان تاثير المعامله ب LC_{50} , LC_{25} هده المركبات ادى الى زياده ملحوظه فى فتره العمر اليرقى و طور العدراء مع تاخر نموها وزياده نسبه الموت والتشوهات فى كل من الشرانق الناتجه والفراشات كما تاثرت خصوبه الفراشات ز لدلك يمكن استخدام هده المستحسات الضوئيه فى مكافحه الدوده القارضه على عوائلها كطعوم سامه لزياده سميتها كبدائل للمبيدات الكيميائيه ولتقليل تزايد مقاومه الحشرات كطريقه فى برنامج المكافحه المتكامله للافات