EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

ENTOMOLOGY

A

ISSN
1687-8809

WWW.EAJBS.EG.NET

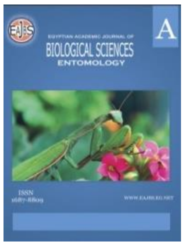
Vol. 14 No. 2 (2021)

Citation: *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 14(2) pp.47-58 (2021)*
DOI: 10.21608/EAJBSA.2021.168234

Egypt. Acad. J. Biolog. Sci., 14(2):47-58(2021)

Egyptian Academic Journal of Biological Sciences
A. Entomology

ISSN 1687- 8809
http://eajbsa.journals.ekb.eg/



Field Application and Biochemical Studies of Certain Selected Photosensitizer Against Black Cutworm, *Agrotis ipsilon*

Hanan S. Abd-El-Aziz[1] and Amal Th. Thabit[2]
1-Plant Protection Research Institute Agriculture Research Center, Dokki, Giza, Egypt.
2-Institute of Environmental Studies and Research, Ain Shams University
E-mail* : hananabdelaziz2020@yahoo.com - amal_hussien@iesr.asu.edu.eg

ARTICLE INFO
Article History
Received:17/3/2021
Accepted:5/5/2021
Keywords:
Black cutworm, methylene blue, safranin, bromophenol blue, protein, lipid, cholinesterase, acid and alkaline phosphatases.

ABSTRACT

Agrotis ipsilon larvae known as black cutworm is one of the most destructive and serious underground insect pests. Which attacks various field crops and causes great damage especially it prefers attacking the young plant in the growing stages. The extensive used of insecticides to control it to protect the yield from reduction led to build-up resistance to these insecticides. So, field experiments were approved to evaluate the effectiveness of certain photosensitizer compounds methylene blue, safranin, bromophenol blue belonging to various chemical groups against *Agrotis ipsilon* larvae in potato crop in El- Giza Governorate in Egypt for the duration of two successive seasons, February 2020 and 2021 under field conditions using double values of LC_{50} and LC_{90} of these compounds. A highly significant decrease in the population of *A. ipsilon* post 3 days of treatment with all tested photosensitize using poisonous baits and a significant difference between the two seasons. On the light of the median and quarter lethal effects of the three tested photosensitizer compounds, methylene blue, bromophenol blue and safranin on the total proteins, total lipids, cholinesterase, acid and alkaline phosphatase in the 4^{th} larval instars of the black cutworm, *A. ipsilon* the results proved that all tested compounds caused decreased in these biochemical contents except bromophenol blue treatment slightly increase in acid phosphatase at LC_{25} and methylene blue increased in total lipids content at both LC_{25} and LC_{50} relative to control.

INTRODUCTION

Black cutworm attacked many vegetables and cotton plants in the early development stage of plant with few leaves that led to reducing the crop yield. Many insecticides are used to protect the plants and overcome this problem, by avoiding the damage of plant which could happen by *A. ipsilon* larvae in this time and produce abundant yield with high quality than the infected fields (Abd-El-Aziz *et al.,* 2019). Due to the arbitrary expend of pesticides treatments produce harm by exiting residues in the food. Therefore, it is becoming clear that alternative pest managing tools are required, to reduce hazardous to the human, environment and non-target organisms, at the same time must use in the field application with the lowest cost (El-Ghobary, *et al.,* 2018). Photosensitizer compounds are considered an available substitute to conventional chemical compounds.

Which may be efficient as insecticide agents, with not mutagenic, non-poisonous, collects in the pest body and low impact on the environment (Lukšiene *et al*., 2007). Photosensitization as well as light, photosensitizer and oxygen is a possibly destructive event in biological systems due to some reactive oxygen species which able to damage numerous subcellular structures (Paillous and Fery- Forgues, 1994). Photodynamic procedures used in the plants as chemical protection weapons versus attack by herbivorous pests (Wainwright 2009). Recently, the probability of using some photoactive pesticides exists to be photodynamic type and used as a tool for controlling the several pests which inspected in field and laboratory (Heitz, 1987 and Lenke *et al*., 1987). The main point of this work is to evaluate the toxic effect of the double values of LC_{50} and LC_{90} of the three dyes safranin, bromophenol blue and methylene blue of the photosensitizing compounds against *A. ipsilon* pest in the field and the effect of median and quarter lethal concentrations and the change in some main components of cells as total protein, lipids, cholinesterase, acid and alkaline phosphatases.

MATERIALS AND METHODS

Insect Culture:

Field strain of *Agrotis ipsilon* obtained from El-Giza government used in this investigation and reared in the laboratory without contamination with any insecticides for generations to get the correct number to carry out the experiments as discussed by Abdin, (1979) and modified by Abd-El-Aziz *et al.,* (2019). In the Agriculture Research Center, Plant Protection Institute, Department of cotton leafworm.

The Used Photosensitizer Compounds:

1-Methylene blue Molar mass: (319.859 g $mole^{-1}$; M.wt.) chemical formula; $C_{16}H_{18}CiN_3 S_4 xH_2O$. is a phenothiazinium compound, 3, 7-bis (Dimethyl amino)-phenothiazin-5-ium chloride.

2- Bromophenol blue Molar mass :(669.99 g $mole^{-1}$; M.wt.) chemical formula; $C_{19}H_{10} Br_4 O_5 S$.3',3'',5',5''-teterabromophenolsulfonphthalein (Reasxys.).

3-Safranin, Molar mass: 350.84 g $mole^{-1}$; (M.wt.) chemical formula $C_{20}H_{19} CiN_4$. is the azonium compound of symmetrical 2,8- dimethyl- 3,7- diaminophenazine. Sometimes described as dimethyl safranin soluble in water (Rosenberg L, 1971), which obtained from LOBA Chemie (Mumbai, India).

The Preparation of Baits:

The bait's prepared according to Balevski, *et al*. (1974) and modified via mixing wheat bran (25 kg/ feddan) with approximately (8-10 liter) of water, 1 kg black honey (attractive substance) was added and mix well after that kept it in a warm place overnight in dark until fermentation. Then, the baits mix with LC_{50} and LC_{25} values of photosensitizers for biochemical analysis. In addition, double values of LC_{50} and LC_{90} are used for field application.

Biochemical Studies Using Semi-Field Technique:

The biochemical analysis carried out on the whole-body tissues of larvae post-treatment the 4^{th} larval instars with both LC_{50} and LC_{25} values of the three tested dyes post 48 hrs (gotten from our previous data; Abd-El-Aziz and Thabit, 2021). By homogenate of untreated and treated larvae (1g tissues/ ml) in 0.9% saline solution and centrifuged in refrigerating centrifuge for 10 min at 5000 rpm. After that, the separated supernatant was kept into three replicates inside a deep-freezer at -20 C^{o} in Eppendorf tube till chemical analysis was examined.

The measurement of different biochemical analyses using colorimetric methods by kits obtained from BioDiagnostic company, Diagnostic and research reagents, Egypt where total lipids kit measured according to Bailey *et al.* (1975) method. Total protein kit based on Biuret reaction (Gornal *et al.,* 1949). The ChE activity was estimated based on the method of Knedel and Bottger (1967). The activity of acid phosphatases was measured according to Laufer and Schin (1971) method and alkaline phosphatase according to Belfield and Goldberg (1971). These analyses were carried out in the central laboratory, Plant Protection Research Institute, Dokki, Giza, Egypt.

Field Applications:

Field applications were carried out in El Giza Government at Kafr Turky village, in a field cultivated with potatoes around sixteen karats (2800 m^2) using a complete randomized block pattern by four replicates. The experiment area split into plots of about 350 m^2 for each treatment, that divided into four portions, first one represented the control (using baits only), each portion of the others divided into 4 replicates and applied with the double values of both LC_{50} (0.904, 0.774 and 0.672) ml% and LC_{90} (3.362, 3.504 and 2.978) ml% of methylene blue, bromophenol blue and safranin post 48 hrs, respectively which were gotten from our previous studies (Abd-El-Aziz and Thabit, 2021). The application was in the early morning with the sunrise by laying a suitable number of poisonous baits under plants, during two consecutive seasons in February 2020 and 2021. First, counting the number of cutting leaves, or the larvae per damaged plant by feeding justified before application and post 3 days of application haphazardly in each replicate. The reduction in the population of *A. ipsilon* larval was estimated.

Statistical Analysis:

The mortality of the tested larvae was corrected according to Abbott's formula (Abbott, 1925) and the LC_{25}, LC_{50} and LC_{90} % values calculated using probit analysis (Finney,1971). Also, the obtained data were investigated using one-way analysis of variance (ANOVA) by SPSS computer program, the means using Duncan's Multiple Range condition ($P<0.05$) between all usages via examined days. Reduction % related to each treatment and control calculated according to Handreson and Telton, (1955) equation.

RESULTS AND DISCUSSION

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

The data tabulated in Table (1) represented the LC_{25}, LC_{50} and LC_{90} values of the three tested photosensitizers, the efficiency of various used concentrations of methylene blue, bromophenol blue and safranin against the 4th larval instars of *Agrotis ipsilon* post 48 hrs of the exposure to sunlight. The tested compounds had noxious effects where the LC_{25} values were 0.241, 0.178 and 0.141 ml % and LC_{50} values were 0.452,0.387 and 0.336 ml % of methylene blue, bromophenol blue and safranin, respectively (obtained from our previous study, Abd-El-Aziz and Thabit, 2021). The LC_{90} values were 1.489, 1.752 and 1.681 ml %, respectively with the same compounds. The most poisonous compound based on the lowest LC_{25}, LC_{50} and LC_{90} was observed in the 4th instars larvae post-feeding could be arranged as descending orders, as safranin then methylene blue and bromophenol blue compounds. These results in harmony with Khater *et al*., (2016) compare the potency of LC_{50} and LC_{90} of safranin post 2 hrs was 33 and 22 times more than tetramethrin. LT_{50} and LT_{90} were 2.17 and 31.50 h, respectively. The LT_{50} were 0.80 and 2.17 h, respectively at concentration4 % post 48 h of treatment with safranin and tetramethrin. Also, Abd El-Naby, (2019) showed the effectiveness of photosensitizers as insecticides influenced by various factors as exposure time to light and photosensitizers concentration and a direct relationship with the exposure time and between concentration with mortality. Which led to an increase in the death percentage and the photosensitizer magnesium chlorophyllin more effective than copper chlorophyllin.

Table 1: The LC_{25}, LC_{50} and LC_{90} values of *Agrotis ipsilon* post 48 hrs. of treatment with methylene blue, bromophenol blue and safranin

Tested compound	Methylene blue			Bromophenol blue			Safranin		
	LC_{25} (ml %)	LC_{50} (ml %)	LC_{90} (ml %)	LC_{25} (ml %)	LC_{50} (ml %)	LC_{90} (ml %)	LC_{25} (ml %)	LC_{50} (ml %)	LC_{90} (ml %)
	0.241	0.452	1.681	0.178	0.387	1.752	0.141	0.336	1.489
Slope ±S. E	2.475± 0.2624			2.008 ± 0.3292			1.7868 ± 0.2372		
95% Fiduciual limits (lower-upper)	0.1756-0.3052	0.3644-0.546	1.2089-2.99	0.0961-0.2527	0.2793-0.488	1.2206-3.0982	0.0887-0.1912	0.2591-0.4223	1.1777-2.0423

Field Experimental:

The effect of three photosensitizers (methylene blue, bromophenol blue and safranin) using double concentrations of LC_{50} values (0.904, 0.774 and 0.672) ml % and LC_{90} values (3.362, 3.504 and 2.978) ml % baits, initially used in the field to evaluate their ability to reduce *A. ipsilon* population in the potatoes fields, at Kafr Turky village, El Giza Government under field conditions (daylight) during two consecutive seasons. The data in Table (2) revealed a significant reduction in the black cutworm population with all used concentrations of three photosensitizers between them and not a significant difference between the two seasons revealed to control. Where the reduction % was (79.27, 75.96 and 84.55) % and (79.95, 74.1 and 84.93) % post three days of application with double LC_{50}s of methylene blue bromophenol blue and safranin during the two seasons 2020 and 2021, respectively. As well as, the treatment with double LC_{90} caused significant reduction as (83.84, 80.94 and 89.87) and (82.16, 80.56 and 88.42) % at 2020 and 2021 in the population of *A. ipsilon* treated with methylene blue, bromophenol blue and safranin in potato fields, respectively. Our data based on the mean of reduction post-treatment (79.61, 75.03 and 84.74%) and (83, 80.75 and 89.15%) with double values of LC_{50} and LC_{90}, respectively where there is a significant difference between the three compounds in the population during the two tested seasons which indicated that safranin was the most efficient compound versus the *A.ipsilon* larvae with the highest reduction and relatively low values compared to other tested photosensitizers that make it an excellent control in an integrated pest management system followed by methylene blue and the lowest effect bromophenol blue. There is little information on the field application with these photosensitizers the subsequent introduction to visible light, stimulates toxic photochemical effects and accumulative in the cell causing death (Lukšiene *et al*., 2007). Abd El-Naby, (2019) studied the effect of two photosensitizers at concentrations10^{-3} and 10^{-2} M caused a highly significant reduction in *S. littoralis* population in the tomato field during seasons 2015 and 2016 and magnesium chlorophyllin more effective than copper chlorophyllin. In addition, the reduction in *S. littoralis* in the cotton field ranged between 74.2 and 90.2% at the first season and between 64.9% and 94.8% in the second season post-treatment (Ahmed *et al.*, 2018).

Table 2: The field evaluation of *A. ipsilon* larvae post 3 days of application with two folds of LC_{50} and LC_{90} values of methylene blue, bromophenol blue and safranin in potato field in Giza Governorate during season 2020 and 2021.

Tested compound	LC_{50} x2 (ml %)	Total number of larvae, damage or cutting leaves during season 2020						LC_{90} x2 (ml %)	Mean of reduction % in two seasons based on LC_{50}
		Before	After 3 days	Reduction%	Before	After 3 days	Reduction%		
Methylene blue	0.904	98	13	79.27	87	9	83.84	3.362	79.61
Bromophenol blue	0.774	78	12	75.96	82	10	80.94	3.504	75.03
Safranin	0.672	91	9	84.55	108	7	89.87	2.978	84.74
Control		50	32		50	32			
Tested compound	LC_{50} x2 (ml %)	Total number of larvae, damage or cutting leaves during season 2020						LC_{90} x2 (ml %)	Mean of reduction % in two seasons based on LC_{90}
		Before	After 3 days	Reduction%	Before	After 3 days	Reduction%		
Methylene blue	0.904	92	12	79.95	112	14	82.16	3.362	83
Bromophenol blue	0.774	89	15	74.1	95	12	80.56	3.504	80.75
Safranin	0.672	102	10	84.93	146	11	88.42	2.978	89.15
Control		63	41		63	41			

Mean with the same letter are not significantly different. *** high significant

Biochemical studies using the semi-field technique.

In the current study, total proteins, lipids, cholinesterase, acid and alkaline phosphatases were estimated in the 4th larval instars *A. ipsilon* treated with the median and quarter lethal concentrations of the three investigated photosensitizers; methylene blue, bromophenol blue and safranin comparing with the untreated control (Tables, 3-7). Data presented in Table (3) showed a highly significant decrease in the total protein content in the larval homogenate treated with LC_{50}s of methylene blue, bromophenol blue and safranin (0.76, 0.64 and 0.56 g/dl), respectively compared with the untreated control (2.17 g/dl) and not a significant difference between each other. Reduction % in total proteins content were -64.82, -70.55 and -74.01% related to control. In case of the treatment at LC_{25}s value, total proteins content observed a highly significant decrease to reach 0.91, 1.12 and 0.71 g/dl compared with control (2.17 g /dl) for methylene blue, bromophenol blue and safranin, respectively. The change in total protein content was reduced by -58.16, -48.19 and -67.07 % related to control. From the previous results, it might be concluded that safranin had a high inhibition effect in the protein synthesis in the larval homogenate in both LC_{25}s and LC_{50}s treatments followed by methylene blue then bromophenol blue. That indicated the reduction in total protein content of the treated larvae related to the toxic effect of these compounds via disruption and imbalance in the hormonal system of insect, ecdysteroid and juvenile hormone or interfered with protein synthesis that explains the appearance of the malformation in larva, pupa and adults as observed by our previous study Abd -El-Aziz and Thabit (2021) where no moths have emerged with safranin in addition, low emerged moth and high malformation and increasing infertility with the other compounds. Or correlated to interruption in structure and the enzymatic system of insect which accountable for the metabolism of proteins and lipids (Khalaf, 1998). Where proteins are the most attribute compounds of the living cell. The decrease in total protein content is a result of protein damage molecule and change of particular amino acid of side chains. Photooxidation of tyrosine with methylene blue involves the break of the circle and configuration of CO_2 (Attia, 2016). Our result agrees with El–Ghobary, *et al.,* (2018) who proved that the treatment with LC_{50} of photosensitizer compounds caused decreased in the total lipids, total protein and total carbohydrates content of *S. littoralis*, while methylene blue slightly increased total proteins compared with control. Other studies in the same field mentioned the effect of LC_{50} value of rose bengal on the adults' flies and the untreated control showed a significant decrease in the total protein content (Attia, 2016). Variations in protein level maybe reflect the stability between transport, storage, synthesis and degradation of structural and nutrients functional throughout ontogeny in addition to the response to certain biological situations (Shoukry *et al*., 2003). Also, the disturbance in proteins and lipids improved as a significant increase in larval and pupal durations with delay in their growth, increase in the larval mortality, pupa, adult malformation and infertility of adults resulted from treatment with safranin, methylene blue and bromophenol blue (Abd -El-Aziz and Thabit, 2021).

Table 3: The amounts and the changes % in the activity of total proteins of *A. ipsilon* larvae post-treatment with LC_{25} and LC_{50} values of methylene blue, bromophenol blue and safranin.

Treatments	Total proteins (g/dl)			
	LC_{50}		LC_{25}	
	Mean ± S. E	*Change%	Mean ± S. E	*Change%
Methylene blue	$0.76^{b} \pm 0.04$	-64.82	$0.91^{c} \pm 0.05$	-58.16
Bromophenol blue	$0.64^{b} \pm 0.05$	-70.55	$1.12^{b} \pm 0.05$	-48.19
Safranin	$0.56^{b} \pm 0.12$	-74.01	$0.71^{c} \pm 0.05$	-67.07
Control	$2.17^{a} \pm 0.09$			
F value	0.0000***		0.0000***	
L.S. $D_{0.05}$	0.2667		0.2012	

Mean in a column followed by the same letter are not significantly different (p <0.05).
*** high significant. *Change% = (Treated-Control) / Control) x 100. S.E: Standard error

Total Lipids:

Results showed in Table (4) summarized the effect of LC_{50} and LC_{25} values of methylene blue, bromophenol blue and safranin on the total lipids content in the 4th larval instars, *A. ipsilon*. A significant increase in the total lipids was exhibited by methylene blue (105.23 and 100.58 mg/dl) at both LC_{50} and LC_{25}, respectively compare with (74.56 mg/dl) untreated control and the corresponding increasing % were 41.13 and 34.89%. An LC_{50} of bromophenol blue slightly significant decrease in total lipids was observed post-treatment (72.38 mg/dl), while a highly significant decrease was recorded with LC_{25} (29.09 mg/dl) compare with control (74.56 mg/dl). The highest change % in total lipids was (-60.99 %) at LC_{25} while the lowest change % -2.93 at LC_{50} with regard to control. In case of safranin, treatments exhibited different effects were, a highly significant decrease in total lipids (39.47 mg/dl) at LC_{25} and not a significant decrease (55.72 mg/dl) at LC_{50} with regard to control (74.56 mg/dl). In addition, reduction % (-47.07 and -25.27%) at LC_{25} and LC_{50} treatments, respectively. Our results agree with El–Ghobary, *et al.,* (2018) who stated that the treatment with rose bengal, eosin yellow lactone and methylene blue, showed a significant reduction -40.37%, -17.31 and -18.29%, respectively in total lipids compared with control. The total lipids are essential as a resource of energy in insects. where obtained by synthesizing them from internal bodies or from food (Gilbert, 1967). Sharaby and EL-Dosary (2016) reported that the total lipid content was increased in red Palm Weevil while acetylcholinestrase or acid phosphate enzyme either in haemolymph or in body tissues insignificantly affected. Photosensitization engaged the activation of light-sensitive compounds to make chemical reactions that damage or destroy cells, in some cases the excited photosensitizer changed into a noxious photoproduct (Spikes, 1985). The chemical structure of dye revealed its activity because it can adjust the nature of the subcellular photo injury sites and referred to the reality that the reaction of one atom in oxygen molecular is reduced to water and insects improved detoxification via activation of some enzyme in a varied range of unknown chemicals as insecticides and drugs (Ben Amor and Jori, 2000 and Hodgson, 1983).

Table 4: The amounts and the changes % in the activity of total lipids of *A. ipsilon* larvae post-treatment with LC_{25} and LC_{50} values of methylene blue, bromophenol blue and safranin.

Treatments	Total lipids (mg/dl)			
	LC_{50}		LC_{25}	
	Mean ± S. E	*Change%	Mean ± S. E	*Change%
Methylene blue	$105.23^{a} \pm 12.0$	41.13	$100.58^{a} \pm 3.03$	34.89
Bromophenol blue	$72.38^{b} \pm 2.51$	-2.93	$29.09^{d} \pm 1.11$	-60.99
Safranin	$55.72^{b} \pm 3.05$	-25.27	$39.47^{c} \pm 1.36$	-47.07
Control	$74.56^{b} \pm 3.58$			
F value	0.0046**		0.0000***	
$L.S.D_{0.05}$	21.419		8.168	

Mean in a column followed by the same letter are not significantly different ($p < 0.05$).
*** high significant. *Change% = (Treated-Control) / Control) x 100. S.E: Standard error

Cholinesterase Activity:

Data of cholinesterase activity post 48 hrs of treatments the 4th instars larvae of *A. ipsilon* at LC_{50} and LC_{25} of methylene blue, bromophenol blue and safranin illustrated in Table (5). All the tested compounds showed a significant reduction in cholinesterase activity at LC_{50} and not significant between each other while not significant reduction was recorded at LC_{25} as regards with control. The most effective compounds were safranin (189.64 U/L) followed by methylene blue (195.43 U/L) then bromophenol blue (238.51 U/L) at LC_{50} values in spite of control (480.93 U/L) and the reduction % were -60.57, -59.36 and -50.41%, respectively. At LC_{25} the most reduced compound in cholinesterase activity was safranin (252.2 U/L) and the least was methylene blue (402.73 U/L) while bromophenol blue (304.59 U/L) showed moderate effect regards to (480.93 U/L) in control. In addition, the reduction % in cholinesterase with safranin, bromophenol blue and methylene blue treatments, -47.56, -36.67 and -16.26%, respectively regards to control. These results agree with Ben Amor *et al.,* (1998) who recorded a highly significant reduction in the activity of AchE in the photosensitized flies who suggests that the nervous system injure act a valuable role in the phototoxic proceeding of porphyrins. The significant reduction in the ChE activity of the photosensitized larvae supposed that the damage in the nervous system reflects an essential role in the phototoxic effect of safranin, methylene blue and bromophenol blue on *A. ipsilon.* This indicated that the photopesticidal action of these compounds can utilize for controlling the harmful pest's population in the lab as well as in the field conditions. These results agree with that recorded by Ben Amor, *et al.,* (2000) on *C. capitata* by Porphyrins where their photoinsecticidal activity improved by increasing hydrophobicity of its molecule. Photosensitizers gave a significant increase of the porphyrin by the pests and subsequent progress of photosensitivity on introduction to visible light. Also, ChE is mainly a neural enzyme affected in control by hydrolyzing the neurotransmitter acetylcholine in cholinergic synapses which could be used as an indicator for the cholinergic role (Glavan, 2020). Our data in harmony with Abd -El-Aziz and Thabit (2021), showed antifeedant effect and larval paralyzed post-feeding with safranin which was more toxic than methylene blue and bromophenol blue against *A. ipsilon*. The sensitivity of AChE didn't essentially indicate that insects could be sensitive to the distinct chemical when it was used as a pesticide, the toxicity effect of a distinct compound depends on several factors as well as their chemical nature, metabolic processes and cuticle penetration (Price, 1988). Abd El-Naby, (2019) the effect of the two tested concentrations of magnesium and copper chlorophyllin on *S. littoralis* larvae showed low various activity in acetylcholinesterase.

Table 5: The amounts and the changes % in the activity of cholinesterase of *A. ipsilon* larvae post-treatment with LC_{25} and LC_{50} values of methylene blue, bromophenol blue and safranin.

Treatments	Cholinesterase activity (U/L)			
	LC_{50}		LC_{25}	
	Mean ± S. E	*Change%	Mean ± S. E	*Change%
Methylene blue	195.43 ^{b} ±37.34	-59.36	402.73 ^{a} ±32.08	-16.26
Bromophenol blue	238.51 ^{b} ±30.54	-50.41	304.59 ^{a} ±40.97	-36.67
Safranin	189.64 ^{b} ±25.86	-60.57	252.2 ^{a} ±67.72	-47.56
Control	480.93 ^{a} ±68.73			
F value	0.0045**		0.2965^{ns}	
L.S. $D_{0.05}$	143.266		274.956	

Mean in a column followed by the same letter are not significantly different ($p < 0.05$).
*** high significant *Change%* = (Treated-Control) / Control) x 100. S.E: Standard error

Acid and Alkaline Phosphatase Activities:
Acid Phosphatase Activity:

As summarized in Table (6), the data showed a highly significant reduction in acid phosphatase at LC_{50} and LC_{25} values of methylene blue, bromophenol blue and safranin treatment. The corresponding amounts of acid phosphatases at LC_{50} values were 23.24 ,13.49 and 12.16 (U/L) with reduction % -43.48, -67.20 and -70.43%, respectively compared with control larvae 41.12 (U/L). The most effective compound was safranin then bromophenol blue and the least methylene blue. On the other hand, in case of bromophenol blue treatment with LC_{25} recorded, not a significant increase in acid phosphatase with the amount to 41.83 (U/L) with a slight increase of 1.73% compared with the control larvae. However, there is no significant difference was showed between the safranin and methylene blue and highly significant with control, the amount 25.36 and 26.62 (U/L) with reduction -38.31 and -35.26%, respectively compared with control.

Table 6: The amounts and the changes% in the activity of acid phosphatase of *A.ipsilon* larvae post-treatment with LC_{25} and LC_{50} values of methylene blue, bromophenol blue and safranin.

Treatments	Acid phosphatase (U/L)			
	LC_{50}		LC_{25}	
	Mean ± S. E	*Change%	Mean ± S. E	*Change%
Methylene blue	23.24 ^{b}±0.69	-43.48	26.62 ^{b}± 0.92	-35.26
Bromophenol blue	13.49 ^{c}± 0.17	-67.20	41.83 ^{a}±2.38	1.73
Safranin	12.16 ^{c}±0.56	-70.43	25.36 ^{b}±1.44	-38.31
Control	41.12^a±2.24			
F value	0.0000***		0.0002***	
L.S. $D_{0.05}$	3.933		6.01	

Mean in a column followed by the same letter are not significantly different ($p < 0.05$).
*** high significant. *Change% = (Treated-Control) / Control) x 100. S.E: Standard error

Alkaline Phosphatase Activity:

The observed data shown in Table (7), revealed that a significant reduction in alkaline phosphatase activity with LC_{50}s treatments of methylene blue, bromophenol blue and safranin relatively to control and not significant between each other with the amount 46.88, 49.99 and 62.60 (IU/L), respectively. The most effective photosensitizer compound was methylene blue in both treatments with LC_{50} and LC_{25}, by causing the highest reduction in the alkaline phosphatase (-46.25%) relatively to control, while safranin caused the lowest reduction (-28.23%) relatively to control, while, bromophenol blue caused reduction by (-42.70%) at LC_{50} values. On the other hand, no significant reduction detected between safranin and control, significant with methylene blue and bromophenol blue with the amount 80.56,56.42 and 58.53 (IU/L), respectively relatively to control 87.23 (IU/L). The highest reduction % as descending was -35.32, -32.90 and -7.65% at LC_{25} values of methylene blue, bromophenol blue and safranin, respectively.

The detoxifying enzymes include phosphatases act in response to pesticides showing their actions. Acid and alkaline phosphatases are considered essential enzymes and indicators for the digestive system cells. These enzymes active and soluble in the midgut tissues, transport membrane, microvillar membrane in the midgut of many species. These agree with Abd-El-Aziz, *et al.,* (2019) who reported the phosphatases to necessitate for all the surviving processes and their effect appeared post-treatment as destruction in the midgut structure of *A. ipsilon* with cell lysis that led to losing its function. That surely explained the disturbance that occurred during the development of larvae post-treatment with the three photosensitizers, safranin, bromophenol blue and methylene blue and debility of larvae to produce normal moths (Abd-El-Aziz and Thabit, 2021) and the disturbance in these enzymes reveals the toxicity effect of the treated compounds especially the reduction through the disability of larvae to absorb nutrients due to damage the midgut structure which may lead to an intensive release of enzymes or interruption in the ingestion and absorption process and may be indicated for antifeeding action. The reduction in enzyme may associate with the nature of treated compounds which produces alkaline media toward the enzyme and reduce it. However, reflect on pH of the environment and the reaction rates via fluctuating the charge status of the active set of substrate or enzyme (Li and Liu, 2007, Zibaee and Fazeli-Dinan, 2012 and Abd-El-Aziz, *et al.,* (2020).

Table 7: The amounts and the changes % in the activity of alkaline phosphatase of *A. ipsilon* larvae post-treatment with LC_{25} and LC_{50} values of methylene blue, bromophenol blue and safranin.

Treatments	Alkaline phosphatase (IU/L)			
	LC_{50}		LC_{25}	
	Mean ± S. E	*Change%	Mean ± S. E	*Change%
Methylene blue	46.88 $^{b}\underline{+}$ 6.74	-46.25	56.42 ^{b} $\underline{+}$ 5.42	-35.32
Bromophenol blue	49.99 ^{b} $\underline{+}$ 5.36	-42.70	58.53 ^{b} $\underline{+}$ 5.33	-32.90
Safranin	62.60 $^{b}\underline{+}$ 8.67	-28.23	80.56 $^{a}\underline{+}$ 6.96	-7.65
Control	87.23 ^{a} $\underline{+}$3.89			
F value	0.0080**		0.0073**	
L.S. $D_{0.05}$	20.91		17.40	

Mean in a column followed by the same letter are not significantly different (p <0.05).
*** high significant. *Change% = (Treated-Control) / Control) x 100. S.E: Standard error.

Conclusion

The use of photosensitized compounds is considered an important choice for insect control due to their environmental safety properties which are used as biological staining. From our current results of the study, could conclude that the tested compounds (methylene blue, bromophenol blue and safranin) possess a toxic effect on *A. ipsilon* larvae, exhibited starvation, antifeedant with contact and stomach poisonous effect. The most efficient compound among all the tested photosensitizers was safranin followed by methylene blue then bromophenol blue. The tested concentrations induce highest reduction in *A. ipsilon* larvae population post 3 days from application in the field. So, it can recommend using these concentrations for controlling the black cutworm in the potato fields. Also, on the basis of the median and quarter values of lethal effects of the three tested photosensitizer compounds on the total protein, total lipids cholinesterase, acid and alkaline phosphatases in the 4th larval instars of the black cutworm, observed that the three photosensitizer compounds decreased all biochemical parameter except methylene blue slightly increased total lipids and acid phosphatase with bromophenol blue at LC_{25}. So could recommend the usage of the mentioned compounds for control the black cutworm on their host plants as toxic baits alternatives to the chemical insecticides to decrease insect-resistant build-up as a method during the integrated pest management program for this serious lepidopterous pest with further investigations in different points are needed.

REFERENCES

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18: 265-267.

Abd El-Naby, S. M. (2019). Toxicity of chlorophylin compound on field and susceptible strains of Spodoptera littoralis, and its biochemical impact on A, B and acetylcholine-esterases *Egyptian Journal of Agriculture Research*, 97 (1), 89-100.

Abd-El- Aziz, S. Hanan, Abd El Mageed, N. I. El Shaimaa and Salama, A. S. Mohamed (2019). Field evaluation of some insecticides for controlling black cutworm *Agrotis ypsilon* and their effect on some histological aspects. *Egyptian Academic Journal of Biological Sciences. D. Histology & Histochemistry*, 11(2): 57- 69.

Abd-El-Aziz, S. Hanan and Thabit, Th. Amal (2021). The Toxicity Effect of Certain Photosensitizing Compounds on Some Biological Aspects of Field Strain of *Agrotis ipsilon* (Hufnagel) Larvae. *Egyptian Academic Journal of Biological Sciences. F. Toxicology & Pest Control*,.13 (1):195-208.

Abd-El-Aziz, S. Hanan, Salama, A. S. M. and Hassan, A. T. (2020). Physiological Studies of Some Insecticides on *Spodoptera littoralis* Larvae. *Egyptian Academic Journal of Biological Sciences A. Entomology*,.13(2):333-344.

Abdin, M. I.,(1979). Standard technique for mass rearing of the black cutworm, *Agrotis ipsilon.* M.Sc. Thesis, Faculty of Agriculture Al Azhar Univ., Egypt.

Ahmed, S. Sayeda, Abd El-Rahman F. Soheir and Abdel Kader H. Mahmoud (2018). Field evaluation of some photosensitizers and nanocomposites against cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *Middle East Journal of Applied Sciences*, 08 :(4) p: 1471-1479.

Attia, Radwa, G. M. (2016): Effect of some photosensitizing compounds on the house fly, Musca domestica (Muscidae:Diptera) as a control approach. M.Sc. Thesis, Faculty of Science, Ain Shams University. pp 125.

Bailey, E., Horne, J. A., Izatt, M. E. G., and Hill, L. (1975). The effect of allatectomy on the lipid composition of the fat body and haemolymph of adult Locusta. *Copm. Biochemistry and Physiology*,52B: 525- 528.

Balevski, A., Genchev, N., Markov, A. and Georgiev, G. (1974). Chemical control of Noctuid larvae. *Rastitelna Zashchita*,, 22 (10):26-30.

Belfield, A. and Goldberg, D. M. (1971). Colorimetric determination of alkaline phosphatase activity. *Enzyme.* (12): 5 pp 561-568.

Ben Amor, T. and Jori, G. (2000). Sunlight-activated insecticides: historical background and mechanisms of phototoxic activity. *Insect Biochemistry and Molecular Biology*, 30:915–925

Ben Amor, T., Bortolotto, L. and Jori, G. (2000). Porphyrins and Related Compounds as

Photoactivatable Insecticides. Laboratory and Field Studies. *Photochemistry and Photobiology*,71(2): 124–128 Symposium-in-Print

Ben Amor, T., Trochin M., Bortolotto L., Verdiglione R., Jori G. (1998). Porphyrins and related compounds as photoactivatable insecticides. 1. Phototoxic activity of hematoporphyrin toward *Ceratitis capitata* and *Bactroceraoleae*. *Photochemistry and Photobiology*, 67: 206–211.

El–Ghobary, M. A., Asmaa Khafagy, I. F. and Ibrahim, Sh. M. Amira (2018). Potency of some Photosensitizing Compounds against the Cotton Leaf Worm, *Spodoptera littoralis* (Boisduval) in Relation to some Biochemical Aspects. *Journal of Plant Protection and Pathology, Mansoura Univeristy*,9 (3): 187 – 193.

Finney, D.J. (1971): Probit Analysis 3rd Ed., Cambridge Univ., Press, London U. K. 318pp.

Gilbert, L.I. (1967). Lipid metabolism and function in insects. *Advance of Insect Physiology*,4:69-211

Glavan, G. (2020). Histochemical staining of acetylcholinesterase in carnelian honey bee (*Apis mellifera carnica*) brain after chronic exposure to organophosphate diazinon. *Journal of Apicultural Science*, 64 (1):123-130.

Gornal, A. G., Bardawill, G. J. and David, M. M. (1949). Determination of serum protein by means of the biuret reaction. *Journal of Biological Chemistry*,177(2):751-766.

Heitz, J. R. (1987). Development of photoactivated compounds as pesticides. In: Heitz, J.R., Downum, K.R. (Eds.), Light Activated Pesticides. *ACS, Washington DC,* pp. 1–21. ACS Symposium Series 339.

Henderson, C. F. and Tilton, E. W. (1955). Tested with acaricides against the brow wheat mite. *Journal of Economic Entomology*. 48: 157-161.

Hodgson, E. (1983): The significance of cytochrome P450 in insects. *Insect Biochemistry*,13:237-246.

Khalaf, A. A. (1998). Biochemical and physiological impact of two volatile plant oils *on Muscina stabulans* (Diptera: Muscidae). *Egyptian German Society of Zoology and Entomology (E)*, 27: 315- 329.

Khater, H., Hendawy N., Govindarajan, M., Murugan, K., Benelli, G. (2016). Photosensitizers in the fight against ticks: safranin as a novel photodynamic fluorescent acaricide to control the camel tick *Hyalomma dromedarii* (Ixodidae). *Parasitology Research,* 115(10):3747–3758.

Knedel, M., and Bottger, R. (1967). Klin. Wschr. 45:325.

Laufer, H. and Schin, K. S. (1971). Quantitative studies of hydrolytic enzymes activity in the salivary gland of *Chironomus tentans* (Diptera: Chironomidae) during metamorphosis. *Canadian Journal of Entomology*, 103: 454-457.

Lenke, L.A., Koehler, P.G., Patterson, R.S., Feger, M.B., Eickhoff, A., 1987. Field development of photooxidative dyes as insecticides. In: Heitz, J.R., Downum, K.R. (Eds.), Light Activated Pesticides. *ACS, Washington DC*, pp. 156–167. ACS Symposium Serves 339.

Li, X.-Z. and Liu, Y. H. (2007). Diet influences the detoxification enzyme activity of *Bactrocera tau* (Walker) (Diptera: Tephritidae). *Acta Entomologica Sinica,* 50(10): 989-995.

Lukšienė, Ž., N. Kurilčik, S. Juršėnas, S. Radžiutė and V. Būda, (2007). Towards environmentally and human friendly insect pest control technologies: Photosensitization of leaf miner flies *Liriomyza bryoniae*. *Journal of Photochemistry and Photobiology B: Biology,* 89(1): 15-21.

Paillous, N., Fery- Forgues, S. (1994). Interest of photochemical methods for induction of lipid peroxidation. *Biochemistry,* 76:355-368.

Price, N. R. (1988). Insecticide-insensitive acetylcholinesterase from a laboratory selected and field strain of housefly (*Musca domestica*). *Comp. Biochemistry and Physiology*, 90: 221-224

Reasxys. Bromophenol blue (115-39-9) Chemical Names and Synonyms.

Rosenberg, L. (1971). Chemical basis for the histological use of safranin o in the study of articular cartilage. *Journal of Bon Join Surgery*,.53 (1): 69-82.

Sharaby, Aziza and EL-Dosary, Mona (2016). Toxic and Biochemical Effects of *Juniperus communis* Essential Oil on the Red Palm Weevil *Rhynchophorus ferrugineus* (Olivier.) (Coleoptera: Curculionidae). *Egyptian Journal of Biological Pest Control*, 26:(2)339-344.

Shoukry, I. F., Khalaf, A. A., Hussein, K.T. and Khater, K. S. (2003). Toxicological evaluation of some botanical oils on biochemical aspects in the Indian meal moth *Plodia interpunctella* HB.

Spikes, I. D. (1985). The historical development of ideas on applications of photosensitized reactions in the health science, R.V. Benssason. E.J. L and, G. Jori and T.G. Truscott (eds.) pp. 124-144 In Primary photoprocesses in biology and medicine. Plenum Press New York.

Wainwright, M. (2009). Photosensitizers in biomedicine, 1st edn. Wiley, Oxford

Zibaee, A. and Fazeli-Dinan, M. (2012). Purification and characterization of a digestive lipiase in *Naranga aenescens moore* (Lepidoptera: Noctuidae). *SOAJ of Entomological Studies*, 1: 38-54.

ARABIC SUMMARY

تطبيقات حقليه ودراسات بيوكيميائيه لبعض المستحسات الضوئيه المختاره ضد الدوده القارضه السوداء

حنان صديق عبدالعزيز[1]- امال ثابت ثابت[2]

1- مركز البحوث الزراعيه-معهد بحوث وقاية النباتات الدقى- جيزه- مصر[1]-

2- معهد الدراسات والبحوث البيئية – جامعه عين شمس[2]

يرقات الاجروتس ابسيلون المعروفه باسم الدوده القارضه السوداء هى واحدة من الافات الحشريه الاكثر تدميرا وخطورة تحت الارض فهى تهاجم المحاصيل الحقليه المختلفه وتسبب ضررا كبيرا خاصه انها تفضل مهاجمة النبته الصغيره فى مراحل نموها الاولى وقد ادى الاستخدام المكثف للمبيدات الحشريه لمكافحتها لحمايه المحصول من الانحفاض الى زيادة مقاومة الحشره لهذه المبيدات لذلك اجرينا تجارب حقليه لتقيم بعض المركبات المستحسه للضوء , ميثيلين الازرق و الصفرانين و بروموفينول الازرق وهى تنتمى الى مجموعات كيميائيه مختلفة ضد يرقات الاجروتس ابسيلون فى محصول البطاطس فى محافظه الجيزه بمصر خلال موسيمين متتالين فبراير 2020 و 2021 تحت الظروف الحقليه باستخدام ضعف قيم التركيز النصف مميت LC_{50} و المميت ل LC_{90} لهذه المركبات وقد وجد انخفاض شديد المعنويه فى تعداد الدوده القارضه بعد 3 ايام من المعاملة مع جميع المستحسات الضوئية المختبره باستخدام الطعم السام واختلاف طفيف بين الموسمين. وعلى ضوء دراسة تأثير المعاملة بالجرعات النصف والربع مميتهLC_{25} وLC_{50} للمركبات الثلاثه المستحسة للضوء ميثيلين الازرق و بروموفينول الازرق و الصفرانين على البروتينات الكليه و الدهون الكلية والكولين استيريز والفوسفاتيز الحامضى والقاعدى فى الطور اليرقى الرابع للدوده القارضه السوداء وقد اثبتت النتائج ان جميع المركبات المختبره تسببت فى انخفاض هذه المحتويات البيوكيميائية باستثناء معاملات بروموفينول الازرق سببت زياده طفيفه فى الفوسفاتيز الحامضى عند معاملة الربع مميتة LC_{25} و ميثيلين الازرق سبب زيادة فى الدهون الكليه مع معاملات النصف والربع مميتهLC_{25} وLC_{50} مقارنة بالكنترول