

## **TOXICITY OF CHLOROPHYLLIN COMPOUND ON FIELD AND SUSCEPTIBLE STRAINS OF *SPODOPTERA LITTORALIS*, AND ITS BIOCHEMICAL IMPACT ON A, B AND ACETYLCHOLIN-ESTERASES**

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### **Abstract**

Chlorophyllin (CHL) is water-soluble analogue of chlorophyll pigment everywhere. This is due to the presence of many useful properties and its use as food additives and in alternative medicine. The study field was investigational at station of the faculty of Agricultural, Cairo University, at Giza governorates, Egypt. Result indicated that sub-lethal concentrations of sodium copper chlorophyllin and magnesium copper chlorophyllin on field and susceptible strains *Spodoptera littoralis*. The concentration ( $10^{-2}$  M) for both sodium copper chlorophyllin and magnesium copper chlorophyllin was more effective than concentration ( $10^{-3}$  M) on field strains and susceptible strains of *Spodoptera littoralis*. The determination activity level  $\alpha$ -esterases,  $\beta$ -esterases and acetylcholine were determined in the total body homogenates of *Spodoptera littoralis*. The results show that CHL was affected both ( $\alpha - \beta$ ) esterase enzyme.  $\beta$  -esterase activity showed significant activity increase, and  $\alpha$ -esterase showed significant activity reduction in most treatment. on the other hand the acetylcholinesterase activity show low various.

**Key words:** *Spodoptera littoralis*, photoinsecticide, Photosensitizer, Copper chlorophyllin, Magnesium Chlorophyllin, Profenofos, Organophosphorous (OP),  $\alpha$  and  $\beta$  esterase enzyme.

### **INTRODUCTION**

Chlorophyll is extracted from the silkworm stools used as a medicine for stomach disorders such as hepatitis ulcers. It is also used to treat liver and blood diseases (Koulet *et al.*, 1994). Sodium chlorophyll and copper extracted from the activity of chlorophyll how's antibacterial and has medical applications. Used in the treatment of hepatitis, acute pancreatitis, chronic nephritis, gastric disorders and leukocyte deficiency (Majumder, 1997). Xanthene derivatives and other light-sensitive materials, after exposed to visible light producing superoxide anions and singlet oxygen and (Ben Amor and Jori, 2000). Photosensitization involves activation of light-sensitive compounds, producing chemical reactions that harm or destroy cells; in some cases the excited Photosensitizer was changed into a toxic photoproduct (Spikes, 1985). In many cases, the effect of the phototoxicity of the chemical compound is entirely developed only in the presence of oxygen; hence the photoinsecticide actions appear to be of the photodynamic style (Heitz, 1987). The light and many of dyes and related

compounds can operate as photosensitizers; Photosensitization is a phenomenon happen in biological systems (Black, 1987).

The three broad categories of enzymes involved in the detoxification of pesticides are the mixed function oxidases (MFO), esterases and glutathione S-transferases (WHO 1981, 1998). The major mechanism of Insecticide resistant to organophosphate in *Cx. quinquefasciatus* Say is through highly inactive non-specific esterase isoenzymes, which seem to metabolize pesticides to harmless products (Villani *et al.*, 1983).

The basic methods for insecticide resistance in all insects are changes in the insecticide target site or changes in the rate at which the insecticide is detoxified. So far esterases, are known to be involved in the detoxification of the major groups of insecticides (Herron, *et al.*, 2004; Pethuan, *et al.*, 2007). insecticide contain carboxylester and phosphotriester bonds, can be attack by esterase enzymes (Brattsten, 1992).The nerve synapses of insects contain a chemical mediator known as acetylcholine (AChE), through which nerve impulses transmitted from one nerve axon to another. Acetylcholinesterases hydrolyze acetylcholine to prevent its accumulation at the nerve synapses since its accumulation leading to death due to the disruption of nerve transmission. Acetylcholinesterase responsible to transport of cholinesterase in the nervous system and is the target site of inhibition by organophosphorus and carbamate insecticides (El-Defrawi, *et al.*, 1964). The changes in ChE activity of *S. littoralis* larvae during thecourse of poisoning with IGR's/insecticides mixtures was studied (Abdel Hafez *et al.*, 1993). All tested compounds caused variable reduction in the activity of acetylcholinesterase than in control. The highest level of reduction was obtained for the IGR diflubenzuron alone or in its mixtures. Data obtained from field experiments were the same as those on laboratory experiments. The non-specific esterases ( $\alpha$ - and  $\beta$ -esterases), which are considered as aromatic ester hydrolases. Evaluated the esterases hydrolyzing  $\alpha$ -naphthyl acetate and  $\beta$ -naphthyl acetate in larvae of *S. littoralis* was evaluated colourimetrically in two susceptible and four resistant strains (Riskallah, *et al.*, 1979). Their results indicated that fenitrothion resistant strain showed activity of esterases 2-4 times greater than the S-strain.

#### **Aim of the work**

The present study was to investigate the Toxicity of chlorophyllin compound on field and susceptible of strains *Spodoptera littoralis*, and its biochemical impact on  $\alpha$ ,  $\beta$  and acetylcholin- esterases

## MATERIALS AND METHODS

### Expeimental field

The study field was investigational at station of the faculty of Agricultural, Cairo University, Giza, Egypt during the summer season 2015/2016.

### Toxicity expeimental design

The experimental area of about 0.5 feddan was divided into 61plots,each plots equal (20 m<sup>2</sup>). Each plot consisted of 3 rows, 6 meter long and onemeter wide. Tomato seedlings were planted on rows separated by 90 cm distance. One-month-old tomato seedlings were planted. All experimental plots received regular agricultural practices except insecticide application. The treatments were performed two-month-day post planting of tomato seedlings and they were distributed as follows:

- Three experimental plots were sprayed with Photosensitizer (Copper chlorophyllin Cu 10<sup>-2</sup> M ).
- Three experimental plots were sprayed with Photosensitizer (Copper chlorophyllin Cu 10<sup>-3</sup> M ).
- Threexperimental plots were sprayed with Photosensitizer ( Magnesium Chlorophyllin Mg 10<sup>-2</sup> M ).
- Three experimental plots were sprayed with Photosensitizer (Magnesium Chlorophyllin Mg 10<sup>-3</sup> M ).
- Three experimental plots were sprayed with insecticide (Profenofos72% E.C) using recommended rate by the manufacturer (1 cm / liter).
- Three experimental plots were sprayed with water as a control group.

Plant samples were taken before spraying and after spraying on 1<sup>st</sup>, 3<sup>rd</sup>, 7<sup>th</sup>, 10<sup>th</sup> and 15 day. The sample included 20 plants which were randomly selected from each plot and investigated for alive insects. The third leave of each seedling was carefully investigated, observations and recorded the number of cotton leaf worm *Spodoptera littoralis* larvae. The reduction in the number was calculated by using Henderson and Tillton equation(Henderson and Tilton, 1955).

### Data analysis

Data were analyzed by completely randomized ANOVA, and compared by Duncan's multiple range test at P <0.05.

### Biochemical study:

#### Insects:

A laboratory strain of the cotton leafworm *Spodoptera littoralis* was obtained from the Central Agricultural Pesticide Laboratory that established under constant conditions of 25°C ± 1 and 70 ± 5 % R.H. and out of any contamination with chemicals till the time of study. The strain was reared in the laboratory as described

by El-Defrawi *et al.*, (1964) under the previous optimum condition during the experiment. Serial concentration of Copper chlorophyllin and different concentration of Magnesium chlorophyllin, a Castor-bean leaves were dipped for 60 seconds in each aqueous concentration of the tested compound then left for one hour to dry. Newly molted 2<sup>nd</sup> larval instar was fed on the treated leaves in glass jars (1 lb.) covered with muslin for 24 hrs. After feeding, the treated larvae were transferred onto untreated leaves and exposed to sunlight. three replicates (each one had 20 larvae).

#### **Direct Sunlight :**

The treated larvae were exposed to the sunlight for 30 min., 60 and 120 min. The fluency rate measured by the dosimeter taken as the average of intensities during exposure time.


#### **Dark experiment :**

The larvae treated with Copper chlorophyllin, Magnesium Chlorophyllin left in the dark until the end of larval life.

#### **Enzyme assays :**

The activity enzyme (alpha Esterases, beta esterase and acetylcholine esterase) were determined in the total body homogenates for enzyme assays after exposure for different interval to sunlight.

#### **-Determination of acetylcholine esterase (AChE) activity:**

 Transaminases were determined according to Simpson *et al.* (1964) using acetylcholine bromide (AChBr) as substrate.

#### **Determination of non-specific esterases activities :**

$\alpha$  and  $\beta$  esterases were determined according to Van Asperen (1962) using  $\alpha$ - and  $\beta$ -naphthyl acetate as substrates respectively.

## RESULTS AND DISCUSSION

### Toxicological study:

Table 1. Toxicity data and resistance ratios of Copper chlorophyllin and Magnesium Chlorophyllin against 2<sup>nd</sup> instar larvae of *S. littoralis* susceptible strain.

No	Line name	LC <sub>50</sub>	LC <sub>90</sub>	Index	RR	Slope	Lower limit	Upper limit
1	Mg-Ch 2 hr	0.0008	725.647	100	1	0.216	----	----
2	Mg-Ch 30min	0.0023	17.45	34.783	2.875	0.33	----	----
3	Mg-Ch 1 hr	0.0051	1.02E+05	15.686	6.375	0.176	----	----
4	CU-Ch 2 hr	0.013	21485.29	6.154	16.25	0.206	----	----
5	CU-Ch 1 hr	0.063	2.20E+05	1.27	78.75	0.196	----	----
6	CU-Ch 30min	62021.64	4.39E+20	1.29E-06	7.75E+07	0.081	----	----

Index compared with Mg-Ch 2 hr Resistance Ratio (RR) compared with Mg-Ch 2 hr

\*Mg-CH 30min : (*S. littoralis* treated by magnesium chlorophyllin, exposure for half hour of sunlight )

\*Mg-CH 1h : (*S. littoralis* treated by magnesium chlorophyllin, exposure for one hour of sunlight )

\*Mg-CH 2h : (*S. littoralis* treated by magnesium chlorophyllin, exposure for two hour of sunlight )

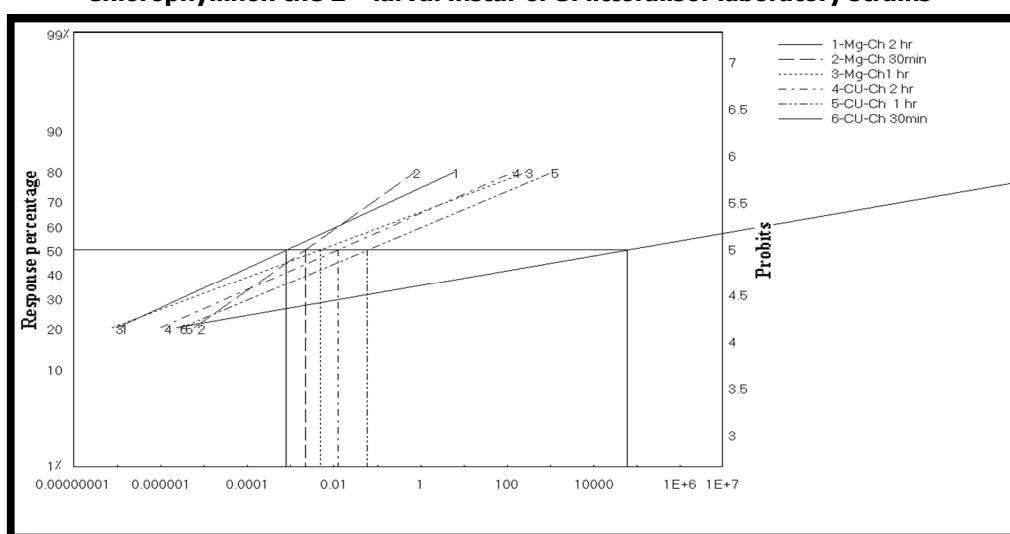
\*CU-CH 30min: (*S. littoralis* treated by Sodium copper chlorophyllin, exposure for half hour of sunlight )

\*CU-CH 1h : (*S. littoralis* treated by Sodium copper chlorophyllin, exposure for one hour of sunlight )

\*CU-CH 2h : (*S. littoralis* treated by Sodium copper chlorophyllin, exposure for two hour of sunlight )

Table (1) and fig. (1), indicated that the tested susceptible strain to the photosensitizer (magnesium chlorophyllin) exposure to sunlight for two hour was the most susceptible  $LC_{50} = 0.0008$  M compared to the other treated and has resistance ratio (RR = 1), on the other hand, the treated *S.littoralis* after exposure to sunlight for 30 min. showed  $LC_{50} = 0.0023$  M and resistance ratio (RR = 2.875) and treated *S.littoralis* exposure to sunlight for one hour show  $LC_{50} = 0.0051$  M and resistance ratio (RR = 6.375). From this information it could be conclude that there is a direct correlation with the exposure time, and concentration with mortality, where the increase of exposure time and concentration led to increase the proportion of death. the photosensitizer magnesium chlorophyllin more effective than copper chlorophyllin, where exposure to sunlight for two hour give  $LC_{50} = 0.013$  M compared to the other treated and resistance ratio (RR =16.25).on the other hand, treated *Spodoptera littoralis* exposure to sunlight for one hour show  $LC_{50} = 0.063$  M and resistance ratio (RR = 78.75) and the treated *Spodoptera littoralis* exposure to sunlight for 30 min. showed  $LC_{50} = 62021.64$  M and resistance ratio (RR =  $7.75E+07$ ). In this treatment by chlorophyllin under darkness, no significant mortality occurs. Photosensitization involving light, photosensitizer and oxygen is potentially damaging, Singlet oxygen one of the main responsible for caused harmful in biological systems (Weishaput, *et al.*, 1976). UV/Visible light can penetrate in tissues about 1 cm depth, deepened on wavelength (Svaasand *et al.*, 1990).

**Fig. 1. Log-probit concentration lines of Copper chlorophyllin and Magnesium Chlorophyllin on the 2<sup>nd</sup> larval instar of *S. littoralis* of laboratory strains**



### Field study

Two photosensitizers with two concentrations for each, were applied against *S.littoralis* in tomato field during summer at season 2015/2016. At the same time profenofos insecticide was sprayed with recommended dose and water was sprayed as a control group.

The number live larvae of *S. littoralis* was counted on the 1<sup>st</sup>, 7<sup>th</sup>, 10<sup>th</sup> and 15<sup>th</sup> day in each treatment. Statistical analysis were conducted using one way ANOVA between all treatments through investigated days and also between days for each treatment. Reduction percentages were also calculated using means of a live larvae for each treatment in comparing with those alive in control group.

From table (2), the tested compound, Photosensitizer Magnesium Chlorophyllin( $10^{-2}$  M and  $10^{-3}$  M ), Photosensitizer Copper chlorophyllin,(  $10^{-2}$  M and  $10^{-3}$  M) and profenofos insecticide after one day sprayed showed high reduction against second instars *S. littoralis*( 74.60%, 61.83%, 56.31%, 46.33% and 55.24%), respectively. After 7<sup>th</sup>days sprayed the data showed that, All the tested compound, Photosensitizer Magnesium Chlorophyllin( $10^{-2}$  M and  $10^{-3}$  M), Photosensitizer Copper Chlorophyllin( $10^{-2}$  M and  $10^{-3}$  M) and profenofos insecticide after one day sprayed showed high reduction against second instars *S. littoralis*( 85.20%, 73.63%, 75.99%, 70.56% and 73.88%), respectively. After 10<sup>th</sup> days sprayed the data showed that, All the tested compound, Photosensitizer Magnesium Chlorophyllin ( $10^{-2}$  M and  $10^{-3}$  M), Photosensitizer Copper Chlorophyllin ( $10^{-2}$  and  $10^{-3}$ ) and profenofos insecticide after one day sprayed showed high

Reduction against second instars *S. littoralis* (88.54%, 80.93%, 80.53%, 74.31% and 80.33%), respectively. After 15 days sprayed the data showed that, All the tested compound, Photosensitizer Magnesium Chlorophyllin ( $10^{-2}$  and  $10^{-3}$ ), Photosensitizer Copper Chlorophyllin( $10^{-2}$  and  $10^{-3}$ ) and profenofos insecticide after one day sprayed showed high reduction against second instars *Spodoptera littoralis*( 86.59%, 72.05%, 74.32%, 71.34% and 83.53%), respectively.

These results showed that Magnesium Chlorophyllin  $10^{-2}$  M was more effective from first day against second instars *Spodoptera littoralis*. The results showed that, Means within a column by same letter are no significantly different by Duncan's Multiple Range Test. Small letters indicate to the significant differences between days while capital letters indicate to significant differences between treatments. IS= insignificant

The data showed gradual increase in the mortality with increase time interval after treatment. The level of mortality was also increased generally with concentration of the tested compounds. The same results were found by McNeill and Goldson (2001). The Current results show that the efficiency of photosensitizers as pesticides is influenced by a variety of factors.

Concentration of photosensitizers and exposure period. The rate of the photosensitized killing of insects appeared to rise with prolongation of post treatment exposure to light.

Table 2. Efficiency of Magnesium Chlorophyllin, Copper chlorophyllin and Profenofos against cotton leaf worm (*Spodoptera littoralis*) larvae on Tomato field.

Formulation	Rate ml/L	Mean of alive larvae $\pm$ SE and its reduction percentage									F value	P value
		Before spray	Day post spray									
			1		7		10		15			
			Mean $\pm$ SE	Reduction (%)	Mean $\pm$ SE	Reduction (%)	Mean $\pm$ SE	Reduction (%)	Mean $\pm$ SE	Reduction (%)		
Magnesium Chlorophyllin	10 <sup>-2</sup>	9.73 $\pm$ 0.55Aa	2.30 $\pm$ 0.15Bd	74.60	1.27 $\pm$ 0.11Cc	85.2	0.90 $\pm$ 0.11CDc	88.54	0.43 $\pm$ 0.09Cc	86.59	211.087	0.000
	10 <sup>-3</sup>	7.60 $\pm$ 0.44Ab	2.70 $\pm$ 0.12Bd	61.83	1.77 $\pm$ 0.12Cc	73.63	1.17 $\pm$ 0.14CDc	80.93	0.70 $\pm$ 0.13Dbc	72.05	151.899	0.000
Copper chlorophyllin	10 <sup>-2</sup>	9.10 $\pm$ 0.67Aa	3.70 $\pm$ 0.20Bc	56.31	1.93 $\pm$ 0.14Cc	75.99	1.43 $\pm$ 0.18CDbc	80.53	0.77 $\pm$ 0.10Dbc	74.32	101.970	0.000
	10 <sup>-3</sup>	10.27 $\pm$ 0.56Aa	5.13 $\pm$ 0.21Bb	46.33	2.67 $\pm$ 0.25Cb	70.56	2.13 $\pm$ 0.29Cb	74.31	0.97 $\pm$ 0.14Dbc	71.34	131.014	0.000
Profenofos	cm/L	7.37 $\pm$ 0.44Ab	3.07 $\pm$ 0.20Bcd	55.24	1.70 $\pm$ 0.23 Cc	73.88	1.17 $\pm$ 0.20 Cc	80.33	0.40 $\pm$ 0.09 Dc	83.53	113.566	0.000
Control	0	10.53 $\pm$ 0.52Aa	9.80 $\pm$ 0.53 Ba		9.30 $\pm$ 0.40BCa		8.50 $\pm$ 0.40 Ca		3.47 $\pm$ 0.23 Da		41.777	0.000
F value	-	6.356	106.982		177.878		134.219		70.745			
P value	-	0.000	0.000		0.000		0.000		0.000			

Means within a column followed by the same letter are no significantly different using Duncan's Multiple Range Test. Small letters indicate to the significant differences between days while capital letters indicate to significant differences between treatments. IS= insignificant

### Effect of photosensitizer on Enzyme:

Table 3.  $\alpha$ - Esterase enzyme activities in the homogenates of susceptible strain 2<sup>nd</sup> instar of *S. littoralis* after treatment with photosensitizer

$\alpha$ - Esterase activity ( $\mu\text{g } \alpha$ - naphtho/ min. / g.b.wt.)												
	Compound	2 <sup>nd</sup> Instar larvae			%	*	Compound	2 <sup>nd</sup> Instar larvae			%	*
1	Cu 1h	831.67	±	0.81	38.37	- 61.63	Mg 1h	393.67	±	0.61	18.16	- 81.84
2	Cu 2h	492.67	±	0.63	22.73	- 77.27	Mg 2h	595.00	±	0.80	27.45	- 72.55
3	Cu Dark	477.00	±	0.62	22.01	- 77.99	Mg Dark	519.67	±	0.97	23.97	- 76.03
4	Control	2167.67	±	0.58	100.00	00.00	Control	2167.67	±	0.58	100.00	00.00

\* = percentage decrease relative to control

Results in table (3) show a good correlation found between the results of bioassays and biochemical assays in our study. Photosensitizer; magnesium chlorophyllin and copper chlorophyllin affected  $\alpha$  - Esterase in the 2<sup>nd</sup> instar larvae. Photosensitizer caused reduction in the activity of  $\alpha$ - Esterase in all treated larvae relative to the controls of the 2<sup>nd</sup> instars *S.littoralis*.

Table 4.  $\beta$  - Esterase enzyme activities in the homogenates susceptible strains 2<sup>nd</sup> instar of *S. littoralis* after treatment with photosensitizer

$\beta$ - Esterase activity ( $\mu\text{g } \beta$ -naphtho/ min. / g.b.wt.)												
	Compound	2 <sup>nd</sup> Instar larvae			%	*	Compound	2 <sup>nd</sup> Instar larvae			%	*
1	Cu 1h	349.7	±	0.97	242.34	142.34	Mg 1h	232.3	±	0.97	160.98	60.98
2	Cu 2h	341.3	±	0.61	236.52	136.52	Mg 2h	167.7	±	0.73	116.22	16.22
3	Cu Dark	223.7	±	0.82	155.02	55.02	Mg Dark	280.7	±	0.62	194.53	94.53
4	Control	144.3	±	0.63	100.00	00.00	Control	144.3	±	0.63	100.00	00.00

\* = percentage increase relative to control

Results in table (4) show that  $\beta$  - esterases had greater activity in all treated the 2<sup>nd</sup> instar *S.littoralis* by copper chlorophyllin more than all treated 2<sup>nd</sup> instar *S.littoralis* by magnesium chlorophyllin of relative to the controls of the 2<sup>nd</sup> instars *Spodoptera littoralis*. A member of the esterase cluster may play a role in detoxification of xenobiotic esters (Gacar and Tasksn, 2009). Increased esterase activity is a major mechanism of resistance in many insect species (Zhou *et al.*, 2002). The present study had shown that Acetylcholinesterase activity table (5) was inhibited after exposed of 2<sup>nd</sup> instar to both copper chlorophyllin, magnesium chlorophyllin. Inhibition of Acetylcholinesterase has been used as biomarker for insecticides (Payne *et al.*, 1996).



Table 5. Acetylcholinesterase activities in the homogenates susceptible strains 2<sup>nd</sup> instar of *S. littoralis* after treatment with photosensitizer

AChE activity ( $\mu\text{g AChBr}/\text{min/g body weight}$ )												
	Compound	2 <sup>nd</sup> Instar larvae			%	*	Compound	2 <sup>nd</sup> Instar larvae			%	*
1	Cu 1h	188.33	±	0.97	93.54	- 6.46	Mg 1h	216.00	±	0.61	107.29	7.29
2	Cu 2h	207.67	±	0.63	103.15	3.15	Mg 2h	182.00	±	0.62	90.40	- 9.60
3	Cu Dark	190.67	±	0.73	94.71	- 5.29	Mg Dark	207.33	±	0.97	102.98	2.98
4	Control	201.33	±	0.86	100.00	00.00	Control	201.33	±	0.86	100.00	00.00

\* = increase or decrease percentage relative to control

## DISCUSSION

The results showed that, efficiency of photosensitizers as pesticides is influenced by a variety of factors, concentration of photosensitizers and duration exposure light. The rate of the photosensitized killing of insects appeared to increase with prolongation of post treatment exposure to light (Ben Amor et al. 1998). In these experiments, the intake of chlorophyllin under a rule of constant darkness led to no serious mortality during the larval phase. Therefore, sunlight activated pesticides, based on photosensitizer compounds, which could be used as effective bio-insecticides; represent a viable to traditional chemical insecticides (Filiberti *et al.* 2009). Fetal photochemical reactions cause death of the target pest when ingested food, these compounds accumulate inside the pest; subsequently, exposure to visible light. Our results suggest that, Light, photosensitizer and oxygen is potentially damaging, Singlet oxygen one of the main responsible for caused harmful in biological systems (Weishaput *et al.*, 1976).

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تقييم سمية مركبات الكلوروفيلين على السلالة الحقلية و  
الحساسية لدودة ورق القطن والتأثير البيوكيميائي على  
نشاط ( الفا-بيتا) استيريزوالاستيل كولين استيريز

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كلوروفيلين ، ملح الصوديوم والنحاس ويذوب في الماء ، الكلوروفيل صبغه خضراء ولديها العديد من الخصائص المفيدة. أجريت التجارب الحقلية في محطة كلية الزراعة ، جامعة القاهرة ، محافظة- الجيزة . في التجربة لوحظ أن التركيز العالي ( $10^{-2}$ ) لكل من كلوروفيلين نحاس الصوديوم و كلوروفيلين النحاس المغنيسيوم أكثر فعالية من التركيز ( $10^{-3}$ ) في المعاملات الحقلية والسلالة الحساسة ، وتم تحديد مستوى نشاط إنزيمات الفا استيريز، بيتا استيريزوالاستيل كولينفي الجسم الكلي المطحون لدودة ورق القطن حيث أظهرت النتائج انخفاض كبير في نشاط إنزيم الفا استيريز، بينما بيتا استيريز أظهرت زيادة في النشاط ، وأظهرت النتائج في معظم المعاملات لانزيم أستيل كولين نشاط منخفض.