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BIOCHEMICAL AND TOXICOLOGICAL STUDIES OF SOME PESTICIDES ON COTTON LEAFWORM (*Spodoptera littoralis*)

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ABSTRACT

The present study is to through light on role of some insecticides (chemical insecticides (a.i. chlorphyrifos) Dursban® 48% EC, spinosyns group (a.i. spinosad) Tracer® 24% SC and insect growth regulator (a.i. lufenuron) Match® 5% EC) against insect attacking cotton cultivation *Spodoptera littoralis* under Egyptian conditions. The toxicological effects and biochemical analysis carried out against laboratory strain of target insects after 24 and 72 hours. Toxicity of the three insecticides chlorphyrifos, spinosad and lufenuron were tested against the 2nd instar larvae of the cotton leafworm, *spodoptera littoralis*. The results showed that lufenuron was more effective on the 2nd instar larvae than chlorphyrifos and spinosad. The LC_{25} values for lufenuron, chlorphyrifos and spinosad were 0.0005, 2.21 and 8.1 ppm, respectively. The biochemical study used for tested insecticides at LC_{25} on some biocomponents namely acetylcholinesterase (AchE), glutathione-s-transferase (GST), chitinase, phenoloxidase, aspartate (AST) and alanine (ALT) aminotransferases, α-esterase (α-EST), total protein and protein gel electrophoresis for laboratory strain were investigated. The tested chlorphyrifos and lufenuron were significantly increased (AchE). Spinosad was significantly decreased (GST) and phenoloxidase. Spinosad and lufenuron significantly increased (α-EST) and (chitinase) (ALT). Chlorphyrifos caused significantly decreased on α-esterase and total protein and (AST). Lufenuron significantly decreased total protein and phenoloxidase. Spinosad showed insignificant increase in (AchE) and total protein (levels). Chlorphyrifos decreased both of phenoloxidase and (ALT) levels insignificantly and increased the levels of (GST) and chitinase, while lufenuron, recorded insignificant decrease in GST levels. The total body proteins of 2nd instar larval of *S. littoralis* treated with LC_{25} of insecticides performed by SDS-PAGE. Control and different treatments were separated into 56 different bands according to their relative frequencies (R_f values), and molecular weights (MW). Samples of electrophoresis were carried out for three different insecticides namely chlorphyrifos, spinosad and lufenuron which used to treat insects. The treatment with insecticides on protein gel electrophoresis led to detection of new bands, and disappeared some bands in comparison to control. It is concluded that treatment with insecticides have strong efficacy on the soluble protein in the body of insects.

Keywords: Chlorpyrifos, Spinosad, Lufenuron, Biochemical study, Protein gel electrophoresis, *S. littoralis*.

INTRODUCTION

The cotton leafworm, *S. littoralis* is one of the most important insect. It is considered one of the most destructive polyphagous agricultural pest attacking different field crops, cotton, vegetables and lead to severe damage to them. As it is known, cotton plant is a crop of great economic importance in Egypt. The attack of pests to cotton lead to low yield which is related to damage in bolls as a result of attacking cotton leafworm to the crop. The control of cotton leafworm is done by using the chemical insecticides and this way is considered complicate due to the resistance of pests to these insecticides. The most economically and ecologically suitable insecticides are chlorpyrifos, spinosad and lufenuron.

Chlorpyrifos belongs to organophosphorothionates insecticides. It has been used in wide variety against agricultural crops. It is a non-systemic anticholinesterase activity with contact, stomach and respiratory action.

Spinosad is a neurotoxic insecticide effect on the nervous system of insects by contact or ingestion stomach. It is have a unique mechanism of action (MOA) involving disruption of nicotinic acetylcholine receptors. It causes their muscles to flex uncontrollably. This leads to paralysis and ultimately their death **(Kirst 2010)**.

The activators of enzyme may be components of the enzyme which have been lost during purification, or they may be metabolites that act as control signals in vivo, binding to specific sites on an enzyme lead it to active conformation and some enzymes require specific ion because the enzyme acts on a complex by substrate and cation. The increase in activity of enzyme is due to detoxification of enzyme against foreign compounds.

The substrate-binding cleft contains catalytic groups provided by the enzyme, and these participate in the reaction. The catalytic groups maybe donate protons to, or accept protons from. The catalytic groups maybe amino acid side chains, such as -COOH, $-NH_2$, -OH and –SH or maybe provided by non protein components of the enzyme called prosthetic groups. Some enzymes show specific ion requirements such as Mg^{+2}, Mn^{+2}, Cu^{+2} or Ca^{+2}.

The insect esterases can either cause broad-spectrum resistance to various insecticides through rapid binding and slow turnover of insecticide molecules (i.e. sequestration) or cause narrow-spectrum resistance to a very restricted range of insecticides containing a common ester linkage, such as malathion through rapid metabolism of the insecticides **(Karunaratne et al 1995).**

The tested IGRs induced a reduction in the activities of acid phosphatase and alpha and beta esterases. Therefore the tested IGRs may be not detoxify by these enzymes **(Bakr et al 2013)**

Detoxification enzymes in insects are generally demonstrated as the enzymatic defens against foreign compounds and play significant roles in maintaining their normal physiological function **(Li and Liu, 2007).**

A member of the esterase cluster probably plays a role in the detoxification of xenobiotic esters **(Gacar and Tasksn, 2009)** increased esterase activity is am ajar mechanism of insecticide insensitivity or even resistance is many insect species **(Zhou et al 2002).**

This investigation aimed to study

1- Evaluate the efficacy of chemical insecticides (chlorphyrifos), spinosyns group (spinosad) and insect growth regulatorin (lufenuron) in comparison with control against the laboratory strain of *Spodoptera littoralis*.
2- Study the effects of the tested insecticides at LC_{25} values on some biochemical aspects (i.e.acetylcholinesterase, glutathione-s-transferase (GST), chitinase, phenoloxidase, aspartate (AST) and alanine (ALT) aminotransferases, α-Esterase, total protein and Gel electrophorsis) in the insects homogenate of insect laboratory.

MATERIALS AND METHODS

Test insect

Laboratory strain: The laboratory strain of cotton leaf-worm *Spodoptera littoralis* (Boisd.) was provided by Central Agriculture Pesticides Laboratory (CAPL), Dokki, Giza. The culture was reared using the technique described by **El-Defrawi et al (1964)**. The obtained strain has been kept under laboratory conditions at 25±2°C and 65 ± 5 % R.H.
Pesticide: The formulation of **spinosad** used in the bioassay wastracer® 24%sc obtained from Dow Agro Sciences Co.,UK. **chlorphyrifos** (Dursban® 48% EC) from Dow Agro Sciences Co.,UK. and **lufenuron** (Match® 5% EC.) from Syngenta Co.

Bioassay test: Five different concentrations of each tested insecticide were prepared from the stock solutions by diluting with water (v/v) in volumetric flasks to give the necessary concentrations which produce mortality between 20-80% of each insecticide. The mortality was corrected by Abbott formula **(Abbott, 1925)**.

Determination of the LC values for tested insecticides groups

The toxicity of the tested insecticides against the 2nd instar larvae of susceptible strain and 2ndinstar larvae of laboratory strain were evaluated using leaf dipping technique according to **(Shepard 1958)**.Ten healthy 2thinstar larvae with five replications were subjected to each of the treated leaves. For insecticides, the larvae were left to feed on treated leaves for 24h in case chlorphyrifos, 72 h incase spinosad and then the mortality counts were recorded. In case of chitin inhibitor,

the larvae were exposed and fed on treated leaves for 48 hours. Then after the alive, larvae were transferred onto untreated leaves in clean jars and left to feed for 24 hours after that mortality counts were recorded. The LC_{25} values for each insecticide were calculated by probit analysis using Ldp-line software according to **Finney (1971)**, and the Toxicity index (Ti) was calculated using the following equation **(Sun, 1950).**

Biochemical study

A. Determination of enzymes activities

1- Determination of acetylcholinesterase (AChE) was done according to **Ellman et al (1961).**
2- Determination of glutathion s-transferase (GST) was carried out according to **Habig et al (1974).**
3- Determination of chitinase was carried out according to **Ishaaya and Casida (1974)**
4- Determination of phenoloxidase was made according to **Ishaaya (1971).**
5- Aspartate (AST) and alanine (ALT) aminotransferases was determined using the method of **Reitman and Frankle (1957).**
6- The method of **Van Asperen (1962)** was used to determine α-esterases.
7- Determination of total soluble proteins was carried out according to **Bradford (1976).**

B. Fractionation of protein patterns using SDS-PAGE

Ten percent of acrylamide gel was used and was prepared according to **Hames (1987)**. Equal protein amounts from the samples were mixed with the sample buffer at aratio 1:1 and incubated for 5 min in a (water bath) at 100°C. Each sample was loaded in separate well of the gel and the electrophoresis run was carried out at 100V for 1.5h,

After the running finished the gel was stained in coomassie blue solution overnight. The gel was destaine using acetic acid: ethanol (8:25) until clearing the background. The bands on gel were determined using the software TotalLab 1D.

Data analysis: The obtained data subjected to analysis of variance using proc. ANOVA in SAS, **(SAS Institute, 1998)**. Mean separation was conducted using LSD in the same program at a significant level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Laboratory strain

1. Toxicological and biochemical studies on the laboratory strains

Data in **Table (1)** represent the LC_{25} values of chlorphyrifos, spinosad and lufenuron for the 2nd larval instar of *S.littoralis*. The LC_{25} values were 2.21, 8.1 and 0.0005 ppm for the compounds chlorphyrifos, spinosad and lufenuron, respectively.

From these data the more effect of the tested compounds was lufenuron followed by chlorphyrifos and spinosad**.** The results show a significant difference in the toxicity to *S. littoralis* and the differences in LC_{25}& LC_{50}&LC_{90} values may be different modes of action of the tested insecticides. **EL-Khayat et al (2012)** reported that, the treatment with chlorpyrifos against cotton leaf worm *S. littoralis* 2nd and 4th instar larvae recorded high mortality for insect.) **El-Naggar (2013)** study the toxicity of two bio-insecticides under group spinosyns which was spinosad and spinetoram against 4th larval instar of *S. littoralis* (Boisd). It was shown that the spinetoram was more toxic than spinosad. **Megahed et al (2013)** investigated the effects of three bio-insecticides namely emamectin benzoate, abamectin, and spinosad on 4th larval instar of *S. littoralis* using leaf dipping technique .The data recorded that the effect of LC_{50} after 24hwere emamectin benzoate> abamectin > spinosad**. Osman and Mahmoud (2008)** mentioned effect that the insecticide lufenuron had a strong efficacy against eggs mass and 3rd& 5th larval instars of *S.littoralis*, but **Sabri et al (2016)** showed that lufenuron had low toxicity against *S. littoralis* larvae.

2. Effect of the tested compounds on the activities of some enzymes

2.1. Effect on acetylcholinesterase (AchE), Glutathione S-transferase (GST) and α-esterases (α-EST) activity

2.1.1. Effect on acetylcholinesterase (AchE)

The data in **Table (2)** shows that all tested insecticides increased the acetylcholinesterase in homogenates of the 2nd instar larvae. It was noticed that LC_{25} of chlorphyrifos and lufenuron reported significant difference comparing with control (50%) and (200%), respectively, while spinosad caused insignificant difference. This result agree with that obtained by **Fetoh and Asiry (2013)**.

Table 1. The toxicity insecticides and chitin synthesis inhibitor on 2nd larval instar of susceptibility *S. littoralis*

Insecticides	LC_{25}(ppm) (95%FL)	LC_{50} (ppm) (95% FL)	Slope ± S.E.	LC_{90} (ppm) (95% FL)	Ti %
Chlorphyrifos	2.21 (0.99-3.60)	6.25 (3.89-9.32)	1.49±0.24	44.96 (26.35-111.59)	0.24
Spinosad	8.1 (2.57-14.19)	29.86 (17.94-49.62)	1.19±0.26	355.98 (153.93-2506.29)	0.05
Lufenuron	0.0005 (- -0.0027)	0.015 (0.0031-0.051)	0.46±0.10	9.38 (1.28-995.41)	100

FL: Fiducial limits SE: Standard error Ti: Toxicity index

whofound the AChE activity for 4th larval instar of *S.littoralis* treated with chlorpyrifos was increased. **Megahed et al (2013)** found that spinosad caused maximum level of the change percentage of AChE activity at 48 h, while the minimum level was noticed after 72 h. The insensitivity of the target site to organophosphates and pyrethroids is predominantly due to the activity of the acetylcholine esterase enzyme (AChE) **Baek et al (2005)**.

2.1.2. Effect on glutathione s-transferase (GST)

The data in **Table (2)** show that spinosad significantly decrease the glutathione s-transferase (GST) levels (-35.88%) comparing to the control, whereas both of chlorphyrifos and lufenuron showed insignificant increase and decrease in GST levels, respectively. The obtained data in harmony with **Abou-Taleb et al (2015)** the effect of lufenuron on GST activity in *S. littoralis* which was decreased.

2.1.3. Effect on α-esterases (α-EST) activity

The data in **Table (2)** show the affect of all tested insecticides on α-esterases (α-EST) the data indicated that spinosad and lufenuron caused significant increase in comparion with control (8.68%) and (91.88%), respectively. Whereas the LC_{25} of chlorphyrifos recorded significant decrease (-40.19%). This result disagree with that obtained by **Dahi et al (2017)** who noticed that the treatments with spinetoram and chlorpyrifos against *S.littoralis* 4th larval instar led to increase the level of α-esterase activity in the insect. The insect esterases can cause resistance to some insecticides through rapid binding and slow turnover of insecticide molecule **(Karunaratne et al 1995)**. **Zhou et al (2002) and El-Sheikh (2012)** showed that the insecticide resistance maybe due to some resistance mechanisms such as increased detoxification against some of insecticides. The resistance in cotton leafworm is attributed to increase detoxification by increasing activity of esterases, oxides and/or glutathione s-transferases. On the contrary some insecticides lead to inhibition of some enzyme, e.g. spinosad and lufenuron led to decrease activity of GST. Chlorphyrifos, spinosad and lufenuron led to increase acetyl cholinesterase activity owing to the detoxification of this enzyme. Moreover chlorphyrifos induce enhancement in glutathione s-transferase (GST). Through complexing in many reactions catalysis apart (R) of the foreign compound RX that is transferred to the thiol group of glutathione, since its content cysteine, glycerin, glutamic acid. The hydrogen atom of cysteine thiol group is transferred to the residue X to give a substance HX. The latter is often a dealkylated or dearylated derivative of RX with low toxicity, but spinosad and lufenuron decreased the activity of this enzyme. As it is known glutathione is tripeptide and it conjugates with foreign compounds to give complex of compounds. **Walker (1975)** mentioned that glutamic acid is hydrolytically removed, a cysteine desivatives of the foreign compounds remains, but in insects glutathione conjugates tend to either excreted unchanged or consented to cysteine rather than acetyl cysteine derivatives, for this reason GST can conjugate with insecticides via (-SH) group, moreover the GST is a 26 KDa protein which occurs as a dimer, each monomer has two domains one that bind GSH and

Table 2. Changes of acetylcholinesterase (AchE), Glutathione S-transferase (GST) and α-esterases (α-EST) activity in the body homogenate of tested insects treated with some insecticide at LC_{25} after 24,72h of treatment

Insecticides treatments	AChE (µmol / per min / mg total protein)		GST (nm / min / mg total protein)		α-EST (ug α-naphthol/min/ml/mg total protein)	
	*Mean ± SE	Change %	*Mean ± SE	Change %	*Mean ± SE	Change %
Chlorphyrifos	0.009 ± 0.00003*	50	0.291 ± 0.012	22.78	68.54 ± 1.54*	-40.19
Spinosad	0.0096 ± 0.0005	6.67	0.243 ± 0.019*	-35.88	123.27 ± 1.60*	8.68
Lufenuron	0.027 ± 0.0004*	200	0.352 ± 0.067	-7.12	217.63 ± 3.56*	91.88
Control 24h	0.006 ± 0.0001	----	0.237 ± 0.009	----	114.60 ± 1.53	----
Control 72h	0.009 ± 0.0003	----	0.379 ± 0.015	----	113.42 ± 1.00	----

*Mean with the same letter (s) are not significantly different (P <0.05).
Change % = (Test-Control) /Control x 100

the other all helical that binds the substrate. The residue is serine. Scrine -OH can conjugate with lufenuron or spinosad insecticides via nitrogen atom or via oxygen atom, and correspondingly induced inhibition of enzyme. Spinosad and lufenuron insecticides increased the activities of α-esterase and this result is due to the detoxification of α-esterase. On the contrary chlorphyrifos decreased the activity of α-esterase.The active side of α-esterase contains a catalytic tied formed by Ser-Asp-His residues. These face it may be conjugate with chlorphyrifos via –OH (serine) or with carboxylic group (Asp) or with nitrogen in histidine, which lead to inhibition of enzyme.

2.2. Effect of insecticides on total protein, aspartate amino transferase (AST) and alanine amino transferases (ALT) activities

2.2.1. Effect of insecticides on total protein

Table (3) show the total protein in homogenates of the 2nd instar larvae. The data indicated that LC_{25} of chlorphyris and lufenuron caused significant decrease in total protein (-12.95%) and (-38.69%) compared with control, respectively. The LC_{25} of spinosad caused nonsignificant increase (1.31%). This results are in agreement with that obtained by **Fetoh and Asiry (2013)** who noticed that the content of total protein was reduced in the 4th larval instar of *S. littoralis* treated with chlorpyrifos. **Awadalla et al (2017)** indicated that the treatment with insecticide teflubenzuron belong to IGRs group and insecticide chlorpyrifos belong to organophosphate against 4th larval instar of *S. littoralis* caused decrease the total protein content.This results disagree with that obtained by **Megahed et al (2013)** who found that spinosad significantly lowered total protein and caused the reduction of protein content and it may be due to inhibition of DNA and RNA synthesis.

2.2.2. Effect of insecticides on aspartate (AST) and alanine (ALT) amino transferases activities

Table (3) show the aspartate amino transferases (AST) in homogenates of the 2nd instar larvae. The data indicated that LC_{25} of spinosad and lufenuron increased significantly the enzyme activity (35%) and (57.22%), respectively. Whereas the LC_{25} of chlorphyrifos caused significant decrease (-84.18%), but for alanine amino transferases (ALT) the data show that LC_{25} of spinosad and lufenuron led to significant increase (38.09%) and (388.19%) compared with control, respectively. The LC_{25} of chlorphyrifos caused nonsignificant decrease (-5.45%). **Biddinger et al (1996) and Li and Liu (2007)** noticed that the increase in activity of enzyme against insecticides may be due to the detoxification of these enzyme in insects which is considered defense against foreign compounds

Table 3. Changes of total protein, aspartate amino transferase (AST) and alanine amino transferases (ALT) activity in the body homogenate of tested insects treated with some insecticide at LC_{25} after 24,72 hr of treatment

Insecticides treatments	Total protein (mg/ml)		AST (units / ml)		ALT (u x 10^3 / ml)	
	*Mean ± SE	Change %	*Mean ± SE	Change %	*Mean ± SE	Change %
Chlorphyrifos	6.142 ± 0.063*	-12.95	0.875 ± 0.072*	-84.18	737.5 ± 7.22	-5.45
Spinosad	6.279 ± 0.043	1.31	6.075 ± 0.043*	35	1450 ± 11.26*	38.09
Lufenuron	3.800 ± 0.130*	-38.69	7.075 ± 0.032*	57.22	5126 ± 42.72*	388.19
Control 24h	7.056 ± 0.068	----	5.530 ± 0.075	----	780 ± 11.55	----
Control 72h	6.198 ± 0.193	----	4.500 ± 0.058	----	1050 ± 28.87	----

*Mean with the same letter (s) are not significantly different (P <0.05).
Change % = (Test-Control) /Control x 100

maintaining their normal physiological functions. Spinosad and lufenuron insecticides increased significantly AST enzyme activity and may be due to detoxification of this enzyme against the two insecticides. Chlorphyrifos decreased significantly the value of AST enzyme. AST consists of two identical subunits the large domain binds the PLP cofactor via an aldmine linkage to the E-amino group of Lys. Other residues in the domain are Asp and Tyr the functional group in aspartic acid i.e. carboxylic group can combine with pyridine in this insecticides and (-OH) from tyrosine can conjugate with oxygen in the structure of chlorphyrifos.

This results agree with that obtained by **Megahed et al (2013)**who found that the treatment with spinosad against 4th larval instar of *S.littoralis* increase the activity of (AST)causing the changes in GOT activities .**Abou-Taleb et al (2015)** noticed increase in (AST) activity in *S.littoralis*larvae after 48 and 72h of treatment with lufenuron. While **Mead et al (2008)** recorded reduction in the activity of (AST) at 4th larval instar of *S.littoralis*treated with spinosad.

2.3. Effect of insecticides on chitinase and phenoloxidase activities

2.3.1. Effect of insecticides on chitinase activity

Table (4) show the activity of chitinase in homogenates of the 2nd instar larvae since LC_{25} of spinosad and lufenuron led to significant increase in its activity (8.50%) and (14.96%), respectively. The LC_{25} of chlorphyrifos caused nonsignificant increase (9.16%).

The obtained results were in not harmony with **Gelbic et al (2011)** who elucidated that the insecticides tebufenozide and lufenuron inhibited the synthesis of chitin at *S.littoralis*. The data confirmed that lufenuron is more active than tebufenozide. **Fetoh and Asiry (2013)** found reduction in the chitinase activity in the 4th larval instar of *S.littoralis* treated by chlorpyrifos. Lufenuron caused inhibition to the production of chitin; therefore, the larvae are unable to successfully moult into the next stage **(Kassem et al 1986)**.

Verloop (1977) reported increase in chitinase activity when treating pupae with Teflubenzuron due to the secondary effect of chitin synthesis inhibitor. The primary effect involves blocking of incorporation of uridine 5'-diphospho-N-acetylglucose-amine into chitin. Chitin synthetase carried out this reaction through the polymerization step.

2.3.2. Effect of insecticides on phenoloxidase activity

Data in **Table (4)** show the activity of phenoloxidase. LC_{25} of spinosad and lufenuron caused significant decrease (-7.02%) and (-33.27%), respectively in comparision with control. The LC_{25} of chlorphyrifos caused nonsignificant decrease in the activity of phenoloxidase (-0.44%).

Table 4. Changes of Chitinase and Phenoloxidase activity in the body homogenate of tested insects treated with some insecticide at LC_{25} after 24, 72 hrs. of treatment

Insecticides treatments	Chitinase (ng NAGA/min/mg total protein)		Phenoloxidase (O.D. units/min/mg total protein)	
	*Mean ± SE	Change %	*Mean ± SE	Change %
Chlorphyrifos	572 ± 10.59	9.16	13.57 ± 0.19	-0.44
Spinosad	1059 ± 21.94*	8.50	15.37 ± 0.15*	-7.02
Lufenuron	1122 ± 27.06*	14.96	11.03 ± 0.26*	-33.27
Control 24h	524 ± 19.97	----	13.63 ± 0.19	----
Control 72h	976 ± 33.83	----	16.53 ± 0.32	----

*Mean with the same letter (s) are not significantly different (P <0.05).
Change % = (Test-Control) /Control x 100

This results disagree with that obtained by **Fetoh and Asiry (2013)** who evaluated the activity of phenoloxidase against 4th larval instar of S.littoralis treated with chlorpyrifos.

The three insecticides decreased the activity of phenoloxidase enzyme. Phenoloxidase enzyme (PO) is a tetramer containing four atoms of copper per molecule and two binding sites. Copper may be acts as a catalyst between enzyme and foreign compounds. The (PO) enzyme is oxidative agent and can reduce oxygen in the three insecticides producing (-OH) group. These factors lead to inhibition and decrease the activity of phenoloxidase enzyme.

3. Fractionation of protein patterns

SDS – PAGE was used to the fractionation of the total body proteins of 2nd instar larval of *S. littoralis* treated with LC_{25}. Control and different treatments were separated into 56 different bands according to their relative frequencies R_f values, and molecular weights (MW). Samples of electrophoresis were carried out for three different insecticides chlorphyrifos, spinosad and lufenuron that used to treat insects.

3.1. Treatment with chlorphyrifos

The results in **Fig. (1)** revealed that, treatment of laboratory strain with chlorphyrifos at LC_{25} caused the appearance of several specific protein bands. The bands (1, 3, 7, 8, 10, 11 and 12 with R_f 0.022, 0.238, 0.361, 0.388, 0.436, 0.555 and 0.648 and MW 192.243, 54.113, 39.623, 36.461, 32.538, 28.636 and 25.401 KDa) were characteristic bands compared with sample lane 1.

3.2. Treatment with Spinosad

Fig. (1) shows that the present results revealed that, treatment of laboratory strain with spinosad at LC_{25} caused the appearance of several specific protein bands No. (2, 6, 9, 10, 12, 15, 17 and 19 with R_f 0.035, 0.225, 0.344, 0.396, 0.436, 0.608, 0.775 and 0.859 and MW 169.434, 54.826, 41.978, 35.562, 32.538, 26.902, 20.719 and 17.75 KDa),were characteristic bands in comparison with control sample lane 1.

3.3. Treatment with lufenuron

Fig. (1) shows that the present results revealed that, treatment of laboratory strain with lufenuron at LC_{25} caused the appearance of several specific protein bands No. (2, 5, 7, 10, 13, 15, 17 and 20 with R_f 0.062, 0.142, 0.235, 0.393, 0.442, 0.633, 0.761 and 0.956 and MW 130.062, 73.531, 54.041, 36, 32.239, 25.901, 21.188 and 14.313 KDa) were characteristic bands in comparison with control sample lane 1.

Generally, the treatment with insecticides led to detection of new bands, and disappeared some bands in comparison to control. It is concluded that treatment with insecticide has strong efficacy on the soluble protein in the body of insects.

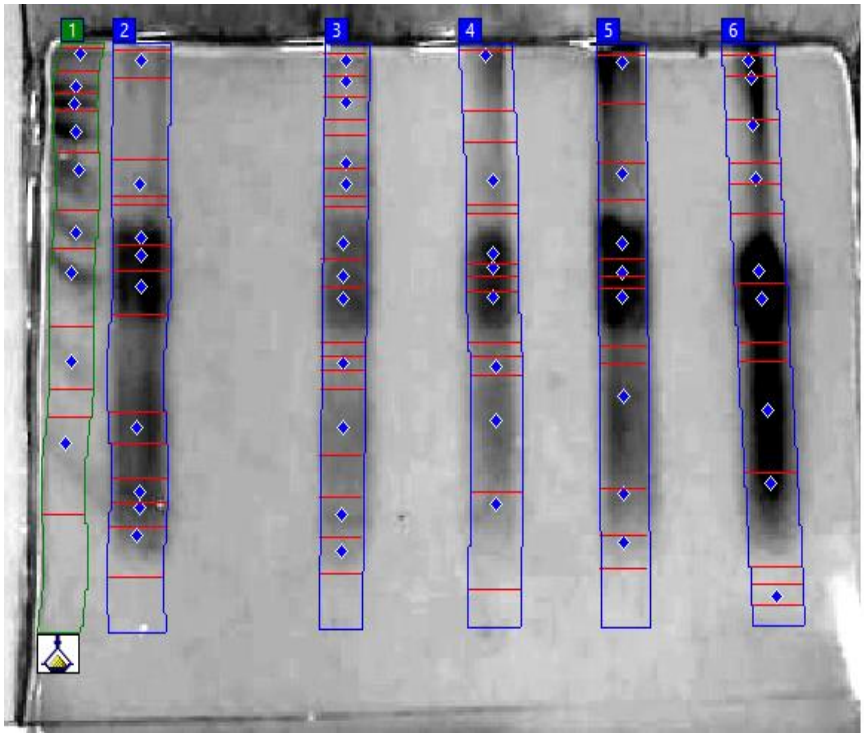

Fig. 1. Protein profile for larval Homogenate of 2nd instar larvae of *S. littoralis* treated with $LC_{25}$of chlorphyrifos, spinosad and lufenuron
L1 - Molecular weight standard
L2 – control 24h
L3 – control 72h
L4 – treatment with chlorphyrifos
L5 – treatment with spinosad
L6 – treatment with lufenuron

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دراسات بيوكيميائية و سمية لبعض المبيدات على دودة ورق القطن

[197]

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تهدف هذه الدراسة إلى تسليط الضوء على دور بعض المبيدات الحشرية (المبيدات الحشرية الكيميائية كلوربيرفوس (دروسبان اتش 48%) وسبينوساد (ترسير 24%) ومنظم نمو الحشرات لوفينورون (ماتش 5%) ضد دودة ورق القطن تحت الظروف المصرية. تم إجراء التأثيرات السمية والتحليل الكيميائي الحيوي ضد السلالة المختبرية للحشرات المستهدفة بعد 24 و 72 ساعة. تم اختبار سمية المبيدات الحشرية لثلاثة مبيدات هى كلوربيرفوس وسبينوساد ولوفينورون ضد يرقات الطور الثاني لدودة ورق القطن، أظهرت النتائج أن اللوفينورون كان أكثر فعالية على يرقات الطور الثانى من الكلوربيرفوس وسبينوساد. وكانت قيم التركيز المميت LC_{25} للوفينورون والكلوروفيروس وسبينوساد، 0.0005 و2.21 و8.1 جزء في المليون على التوالي. حيث تمت الدراسة الكيميائية الحيوية بهذه المبيـدات التي على بعض المركبات الحيوية وهى استيل كولين استيريز، جلوتاثيون –اس– ترانسفيريز، كيتينيـز، فينـــول اوكسيديـــز، اسبارتـــات والانيلين امينوترانسفيريز، والفا استيريز والمحتوى البروتين الكلى وتم فصل البروتين باستخدام التفريد الكهربائى لسلالة المختبر. ادى استخدم كلوربيرفوس ولوفينورون الى زياده معنوية على أستيل كولين استيراز. انخفاض معنوى لسبينوساد على الجلوتاثيون–اس– ترانسفيريز والفينول اوكسيديز. زاد سبينوساد ولوفينورون وزيادة معنوية للانزيم الفا استيريز والكيتينز والانيلين امينو ترانسفيريز. تسبب الكلوربيرفوس انخفاضًا معنوياً على الفا استيريز والبروتين الكلي واسبارتات امينو ترانسفيريز. ادى لوفينورون الى انخفاض معنوي على البروتين الكلي والفينول اوكسيديز. تسبب سبينوساد زيادة غير معنوية على أستيل كولين استيريز والبروتين الكلي. تسبب الكلوربيرفوس فى انخفاض غير معنوى على الفينول اوكسيديز والأنيلين أمينوترانسفيريز وزيادة غير معنوية على الجلوتاثيون–اس ترانسفيريز الكتينيز، بينما تسبب لوفينورون في انخفاض غير معنوى على جلوتاثيون–اس–ترانسفيريز. كما تم استخدام صوديوم دودسيل سلفـات بولـــى اكرميـــد جيـل الالكتروفوريس لتحليل البروتينات الكلية لعمر اليرقى الثانى لدودة ورق القطن باستخدام الثلاث مبيدات المختبرة وكانت النتيجة ظهور حزم جديدة مقارنة ويتضح من ذلك ان المعامله بالمبيدات الحشرية لها فعالية قوية على البروتين الذائب في جسم الحشرات.

الكلمات الدالة: كلوربيوفوس، سبينوساد، لوفينورون، دراسات البيوكيميائية، فصل بروتين بإستخدام التفريد الكهربائى، دودة ورق القطن

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