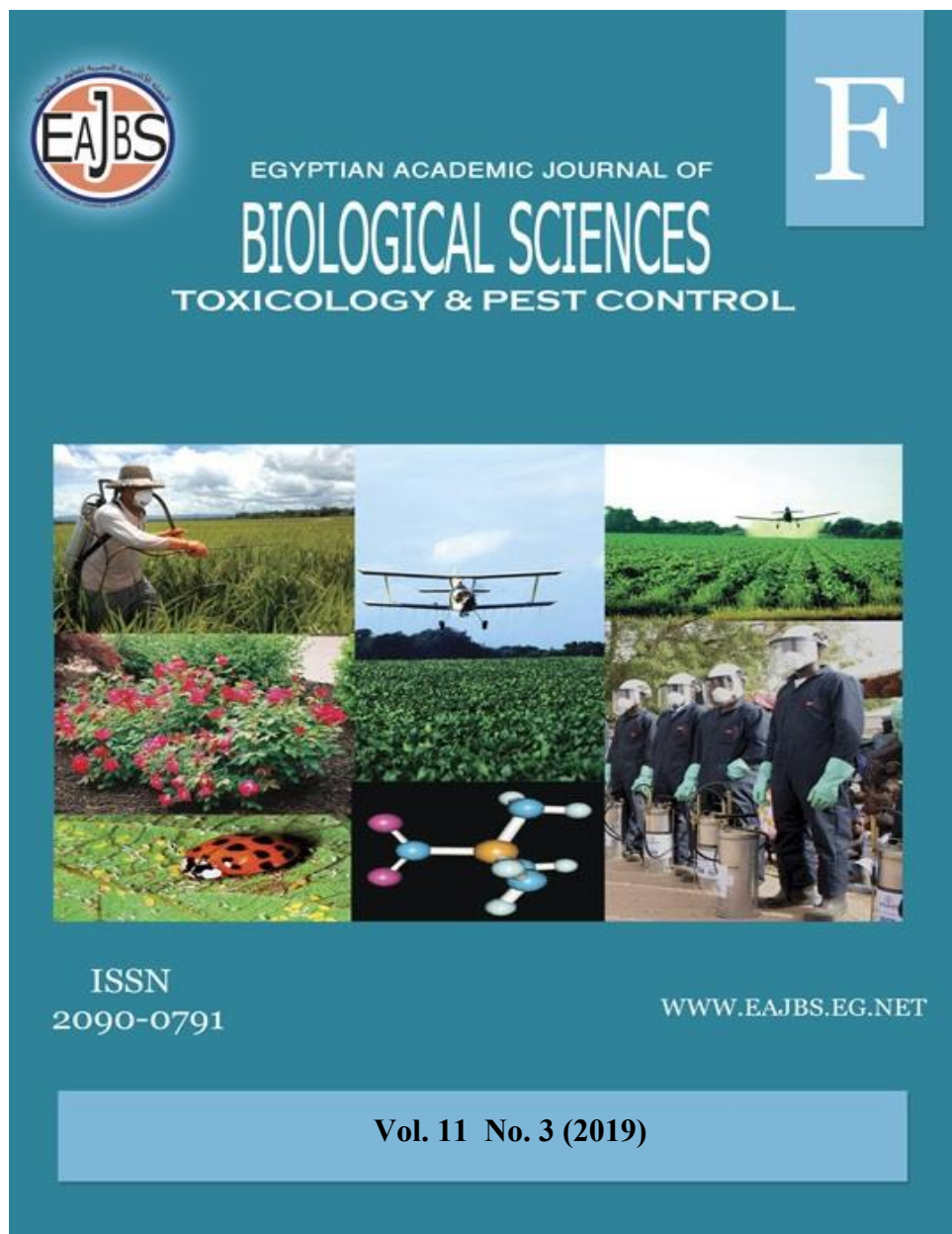


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Effects of some Insecticide Mixtures on Toxicity and Some Biochemical Parameters of Cotton Leafworm, *Spodoptera littoralis* (Boisd.)

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ABSTRACT

This study was evaluated the toxicity of three insecticides contains two effective substances, chlorpyrifos & lufenuron (Tempo XL), lufenuron & emamectin benzoate (Heater) and thiamethoxam & chlorantraniliprole (Folliam Felixi) against the 2nd and 4th larval instars of *S. littoralis* under semi-field condition in tomato field Ismailia Governorate during 2018 & 2019 seasons and assessing their biochemical effect against treated larvae. In laboratory results indicated that Heater had the highest efficacy against 2nd and 4th larval instars, with LC₅₀ of 0.041 and 0.135 ppm for 2nd and 4th larval instars, respectively, followed by Folliam Felixi with LC₅₀ of 0.076 and 0.233 ppm and finally Tempo XL with LC₅₀ of 0.097 and 0.411 ppm for 2nd and 4th larval instars, respectively. Under the semi-field condition observed that the initial effect during 2018 & 2019 seasons, Heater proved to be the most effective and had the highest corrected larval mortality, while the general mean of residual activity was more than 90% in all treatments. In biochemical studies, alpha and beta esterases were the highest activities in *S. littoralis* larvae treated with tested compounds compared to untreated. The highest enzyme activity of acetyl- cholinesterase, acid and alkaline phosphatase was observed in Tempo XL, while in the case of Heater and Folliam Felixi there was no significant difference between them. The results also showed that with all treatments there was a significant decrease in the total protein.

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd.); is one of the most destructive agricultural lepidopterous pests. It can attack numerous economically important crops all the year-round, which causes extensive damage to a wide range of fiber and forage crops in agro-ecosystems of North Africa and the Middle East (Sadek *et al.* 2010). Furthermore, its broad host range also includes many vegetable crops such as tomato, *Lycopersicon esculentum* Mill. (Solanaceae), which are often, consumed fresh. Given the short interval between insecticide application and consumption, *S. littoralis* management on tomato with conventional insecticides is indeed very difficult, costly and unsustainable. Therefore, alternative methods are urgently needed for managing *S. littoralis*. Although the importance of the insecticide used for agriculture to prevent insect associated losses cannot be overlooked, there is a greater need to develop alternative or additional

techniques, which would allow rational use of pesticides and provides adequate crop protection for sustainable food, feed, and fiber production. Many farmers are using the organophosphorus (chlorpyrifos-methyl & profenofos) and the carbamate methomyl to control this serious pest (Tomlin, 2000).

Chlorantraniliprole is a new anthranilic diamide insecticide, which effectively controls pest insects belonging to Lepidoptera, Coleoptera, Diptera, and Hemiptera, and has been shown to be effective against insects that have developed resistance to older classes of chemistry (Bentley *et al.*, 2010). Anthranilic diamides selectively bind to ryanodine receptors in insect muscles resulting in an uncontrolled release of calcium from internal stores in the sarcoplasmic reticulum (Lahm *et al.*, 2005 and Cordova *et al.* 2006), causing impaired regulation of muscle contraction leading to feeding cessation, lethargy, paralysis, and death of target organisms. Anthranilic diamides have very low vertebrate toxicity due to a >500-fold differential selectivity toward insect over mammalian ryanodine receptors (Cordova *et al.* 2006).

Emamectin benzoate is a novel semi-synthetic derivative of natural product abamectin in Avermectin family. Avermectins including emamectin benzoate have been shown to be effective against a broad spectrum of arthropod pests (Putter *et al.*, 1981). These materials act by interfering with the action of gamma-aminobutyric acid (GABA) (Fritz *et al.*, 1979). It blocks post-synaptic potentials of the neuromuscular junction, leading to paralysis.

The uses of IGR's compounds in insect control are known as insect development inhibitions, which inhibit or prevent normal metamorphosis of immature stages to the adult's stage. Herein, many IGRs have shown potentiality against lepidopterous insects (Abdel- Aal, 2003 and Seth *et al.*, 2004). lufenuron (match) is an insect growth regulator that interferes with chitin synthesis, disrupt the hormonal balance with exchanging in the molting process, and inhibit the insect's growth (Oberlander and Silhacek, 1998).

Thiamethoxam is a broad spectrum neonicotenoid contact insecticide. The insecticidal activity of neonicotenoid is primarily attributed to its action on nicotinic acetylcholine receptors (nAChRs) (Karlin, 2002, Tomizawa and Casida, 2005). Neonicotenoid acute toxicity is ascribed primarily to their action as nicotinic agonists, acting on insect and mammal nAChRs (Tomizawa and Casida, 2003). On the other hand, several pieces of evidence in the literature show that neonicotenoid insecticides present a higher selectivity for the insect nAChRs than for the mammalian ones. Because of this selective activity, thiamethoxam has been evaluated as a seed treatment for several major field crops, including cotton (Arthur *et al.*, 2004).

Resistance to pesticides is probably the biggest challenge facing pesticide research today. Consequently, insecticides from different chemical groups with a different mode of action and also some of their combination should be tested against *S. littoralis* to help to develop a sound control program in the future (Ghoneim, 2002). The combination of such bioactive agents with insecticides was investigated as an attempt to increase their efficiency on *Spodoptera littoralis* and reduce the amounts of insecticides release in the environment which is appreciable from an environmental safety point of view (Aly and Eldahan, 1987).

The objective of the present study was to assess the toxicity of three insecticides contains two effective substances chlorpyrifos & lufenuron (Tempo XL), lufenuron & emamectin benzoate (Heater) and thiamethoxam & chlorantraniliprole (Folliam Felixi) against the 2nd and 4th larval instars of *S. littoralis* under semi-field condition in tomato field and their biochemical effect against treated larvae.

MATERIALS AND METHODS

Rearing Technique:

A laboratory strain of *S. littoralis* larvae was obtained from Cotton Leafworm Department, Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt. It was maintained under constant conditions at 27 ± 2 °C and 65 ± 2 % RH and kept away from any contamination with chemicals to obtain a susceptible strain (El-Defrawi *et al.*, 1964).

The Tested Compound:

1- Common name: chlorpyrifos 25% + lufenuron 5% (30% EC), the trade name is Tempo XL, the application rate is 500 ml/feddan and it is the product of Starchem Industrial Chemicals - Egypt.

2- Common name: lufenuron 2% + emamectin benzoate 1% (3% SC), the trade name is Heater, the application rate is 100 ml/100L water and it is the product of Starchem Industrial Chemicals - Egypt.

3- Common name: thiamethoxam 20% + chlorantraniliprole 20% (40% WG), the trade name is Folliam Felixi, the application rate is 80g/feddan and it is the product of Syngenta Agro - Egypt.

Bioassay Test:

Bioassays were performed on 2nd & 4th instar larvae using the leaf dipping technique was adopted according to Abo El-Ghar *et al.* (1994). Castor-bean leaves were dipped for 5 sec in an aqueous solution of the prepared concentrations (4, 2, 1, 0.5, 0.25, 0.125 and 0.0625 ppm) of Tempo XL, Heater, and Folliam Felixi then left to dry for 1 h in room temperature before being offered to the 2nd & 4th instar larvae; in addition, larvae treated with water served as control. Larvae were fed for 24 h on the treated leaves and then transferred to fresh untreated leaves for three days. Three replicates (twenty newly molted larvae / replicate) were used for each tested concentration as well as control. The corrected mortality was calculated then the lethal concentration (LC₅₀, LC₉₀) and slope values of the mentioned compounds were investigated.

Semi-Field Studies:

Field experiments carried out at Al-Fardan village, Ismailia Governorate during seasons of 2018 & 2019, which planted Tomato plants, *Lycopersicon esculentum* (Var. Alisa). The field area was consists of 262.5 m² (1/16 feddan) for each treatment which divided into 4 replicates and involved an untreated check. The treatment was arranged in randomized complete blocks design (RCBD) with four replicates each. Spraying of insecticide was done with the recommended rate by using a knapsack sprayer on April 18 and March 29 on 2018 and 2019 seasons, respectively. Tomato leaves collected on for ten sequential days. Treated and untreated leaves transfer directly to the laboratory to feeding separate sets of second or fourth instar larvae of the cotton leafworm. Mortalities were recorded after 24 hrs, 7 days and 10 days treated leaves of tomato with insecticide were offered to larvae.

Biochemical Studies:

For biochemical studies, the treated castor bean leaves with LC₅₀ were fed the 4th instar larvae for 48 hrs and the total body samples of the 6th instar for biochemical studies were collected from the surviving treated as well as untreated larvae.

Preparation of Haemogenate Samples for Biochemical Analysis:

Haemogenate was collected from 3 pooled larval samples By haemogenizing in insect physiological saline. Haemogenate was collected in cold tubes (on ice). The samples were centrifugated at 4500 rpm for 5 minutes under cooling (4°C) to remove the remnant of tissues. After centrifugation, the supernatant fluid was collected and divided

into small aliquots (0.5 ml) and stored at -20°C until analysis.

1. Determination of Non-Specific Esterases Activities:

Alpha- and beta-esterases (α -esterases) and beta-esterases (β -esterases) activities were determined according to the method of Van Asperen (1962) using α -naphthyl acetate and β -naphthyl acetate as substrates, respectively.

2. Determination of Acetylcholine Esterase Activity:

The activity of acetylcholine esterase (AChE) was measured according to the method described by Simpson *et al.* (1964).

3. Determination of Phosphatases Activity:

Acid and alkaline phosphatase activities were determined by the method described by Laufer and Schin (1971).

4. Determination of Total Protein Content:

The protein content of the Haemogenate samples was determined using folic phenol reagent according to the method of Lowry *et al.* (1951). Biochemical tests were determined in the Physiology Department, Plant Protection Research Institute, Dokki, Egypt.

Statistical Analysis:

The statistical analysis of data on mortality was subjected to the Abbott formula (Abbott, 1925) for correction wherever required. Probit analysis was determined to calculate LC_{50} (Finney, 1971), through a software computer program. Statistical significant differences between individual means were determined by one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Toxicity of Tested Compounds against Second and Fourth Larval Instars of *Spodoptera littoralis*:

Data presented in Tables (1 & 2), show the efficacies of tested compounds represented as LC_{50} 's against the 2nd and 4th larval instars of *S. littoralis*. From the demonstrated results, it could be shown that Heater achieved superior toxic efficacy compared to other tested compounds, where, it had the highest efficacy against 2nd and 4th larval instars, with LC_{50} of 0.041 and 0.135 ppm for 2nd and 4th larval instars, respectively, followed by Folliam Felixi with LC_{50} of 0.076 and 0.233 ppm and finally Tempo XL with LC_{50} of 0.097 and 0.411 ppm for 2nd and 4th larval instars, respectively (Figs. 1 & 2). The present results are confirmed with the results of Ezz El-Din *et al.*, (2009) and Abdu-Allah (2010) reported that emamectin-benzoate was the most effective compound against 4th instar larvae of *S. littoralis*.

Table (1): Efficacy of the tested compounds against the 2nd larval instar of *S. littoralis*

Treatments	LC_{50}	Lower limit	Upper limit	Slope	LC_{90}	Toxicity index
Heater	0.041	0.02	0.058	2.262± 0.453	0.149	100
Folliam Felixi	0.076	0.033	0.112	2.146± 0.453	0.302	53.95
Tempo XL	0.097	0.035	0.151	1.639± 0.387	0.585	42.27

Table (2): Efficacy of the tested compounds against the 4th larval instar of *S. littoralis*

Treatments	LC_{50}	Lower limit	Upper limit	Slope	LC_{90}	Toxicity index
Heater	0.135	0.074	0.203	1.723± 0.311	0.750	100
Folliam Felixi	0.233	0.166	0.325	2.256± 0.320	0.862	57.94
Tempo XL	0.411	0.276	0.595	1.596± 0.181	2.609	32.85

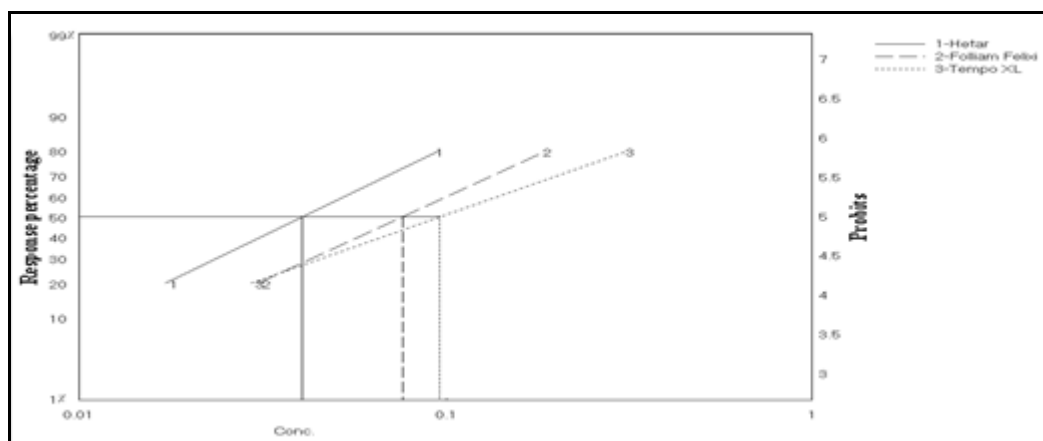


Fig. (1): Efficacy of the tested compounds against the 2nd larval instar of *S. littoralis*.

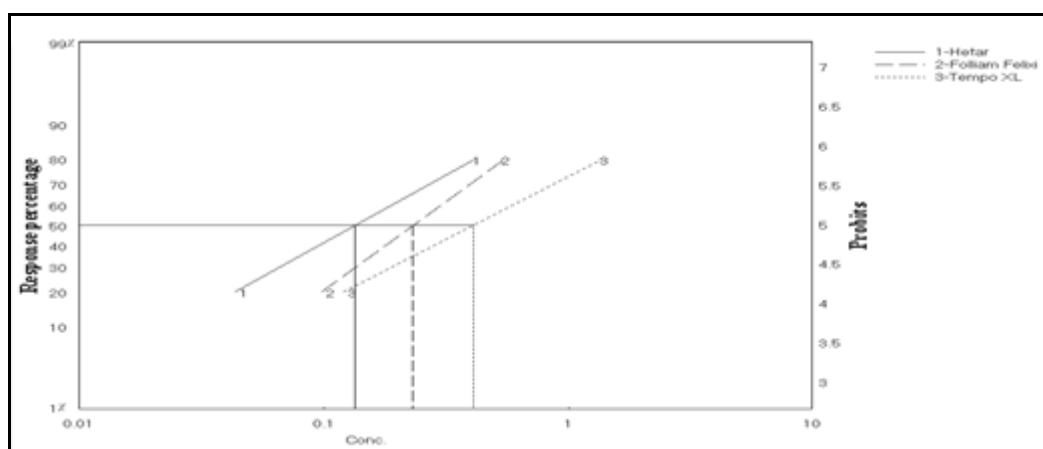


Fig. (2): Efficacy of the tested compounds against the 4th larval instar of *S. littoralis*.

Effect of Treated Tomato Leaves with the Tested Compounds on Cotton Leafworm, *S. littoralis* in the Semifield:

Semi-field application was determined to study initial (at 24 hrs. after spray) and residual effect (at 7 and 10 days after spray) of Heater, Folliam Felix and Tempo XL against second and fourth larval instars of *S. littoralis*, when the larvae were fed on treated tomato leaves and percentage corrected larval mortality calculated after (24hrs., 7 and 10 days) from spray of these insecticides.

With regard to the initial effect during season 2018, Heater proved to be the most effective where they caused 79.8 and 66.7% mortalities (for 2nd and 4th larval instars, respectively) when the larvae were fed on treated tomato leaves for one day, while Tempo XL was the least effective (65.4 and 56.7% for 2nd and 4th larval instars, respectively) (Table 3). Based on the general mean of residual activity of the tested compounds, Folliam Felix was superior compound giving 99.1 and 99.1% for 2nd and 4th larval instars, respectively, followed by Heater and Tempo XL (99.2 & 96.5% and 96.5 & 93.9% for 2nd & 4th larval instars, respectively).

As observed during season 2019, the initial effect manifested higher efficacies on *S. littoralis* 2nd instar larvae when fed larvae on tomato leave treated with Heater resulting in 79.2% mortality. While the 4th instar larvae the insecticide, Folliam Felix caused the highest mortality rate (71.3%).

In the case of the general mean of residual activity of the tested compounds, Heater was the highest effective against 2nd and 4th larval instars, resulting 97.5 and 95.4% mortalities, respectively followed by Tempo XL (96.7 and 94.8%) then Folliam

Felixa (96.0 and 94.1%). With all treatments against second instar larvae, it is clear from data in Table (4) that the percentage corrected mortality after 10 days were recorded 100% mortality

Table 3: Percentage corrected larval mortality of 2nd & 4th larval instars of cotton leafworm after treatment with the tested compounds during 2018 season in Ismailia governorate

Tested compounds	Instar	% Corrected mortality after			
		Initial	Residual effect		
		1 days	7 days	10 days	Mean
Tempo XL	Second	65.4	94.7	98.3	96.5
	Fourth	56.7	91.2	96.7	93.9
Heater	Second	79.8	98.3	100.0	99.2
	Fourth	66.7	94.7	98.3	96.5
Folliam Felixa	Second	79.2	98.2	100.0	99.1
	Fourth	58.3	98.2	100.0	99.1

Table 4: Percentage corrected larval mortality of 2nd & 4th larval instars of cotton leafworm after treatment with the tested compounds during 2019 season in Ismailia governorate.

Tested compounds	Instar	% Corrected mortality after			
		Initial	Residual effect		
		1 days	7 days	10 days	Mean
Tempo XL	Second	64.9	93.3	100.0	96.7
	Fourth	56.3	90.8	98.8	94.8
Heater	Second	79.2	95.0	100.0	97.5
	Fourth	61.3	93.3	97.5	95.4
Folliam Felixa	Second	76.6	92.0	100.0	96.0
	Fourth	71.3	89.5	98.8	94.1

Biochemical Changes:

Results in Table (5) show the effect of tested compounds on α - esterase, β - esterase and Acetylcholinesterase enzymes activity in 4th instar larvae of *S. littoralis*.

Alpha-Estrases Enzymes:

The activity of α -esterases increased in all treatments than control (123.78 $\mu\text{g } \alpha$ - Naphthol/min/ml) and it observed significant differences between the tested compounds where it was reached in Tempo XL to the highest value (362.59 $\mu\text{g } \alpha$ - Naphthol/min/ml), followed by Folliam Felixa and Heater (220.75 and 169.38 $\mu\text{g } \alpha$ - Naphthol/min/ml, respectively).

Beta-Estrases Enzymes:

The activity of β -estrases enzymes had the similar behavior of α -estrases enzymes, where the activity is more in Heater (240.16 $\mu\text{g } \beta$ - Naphthol/min/ml) in comparison with control (93.20 $\mu\text{g } \beta$ - Naphthol/min/ml) with significant impact in Tempo XL and Folliam Felixa. Abdel-Mageed *et al.*, (2018) found that IGR's compounds increased the activity of both alpha & beta esterases in *S. littoralis* and can facilitate the development of truly selective insecticides that can be employed in integrated pest management strategies.

Acetyl- Cholinesterase:

The highest enzyme activity of acetyl- cholinesterase was observed in Tempo XL (806.36 μg Acetyl-cholinebromide/min/ml), while in the case of Heater and Folliam

Felixa there was no significant difference between them. These results are in agreement with Raslan (1994) indicated that the tested compounds synthetic pyrethroids, organophosphates, and carbamate compounds caused a high level of A.Ch.E, in the treated larvae of PBW compared with the untreated one.

Table 5: Effects of LC₅₀ of tested compounds on esterases and Acetyl- cholinestrace activity of *Spodoptera littoralis*.

Treatments	α -Estrase \pm SE ($\mu\text{g } \alpha$ -Naphthol/min/ml)	β - Estrase \pm SE ($\mu\text{g } \beta$ -Naphthol/min/ml)	Acetyl- cholinestrace \pm SE ($\mu\text{g Acetyl-}$ $\text{cholinebromide/min/ml}$)
Tempo XL	362.59 \pm 16.29a	205.34 \pm 5.73b	806.36 \pm 26.27a
Heater	169.38 \pm 4.33c	240.16 \pm 14.95a	613.21 \pm 26.76b
Folliam Felixi	220.75 \pm 4.38b	123.79 \pm 3.59c	550.28 \pm 13.53b
Control	123.78 \pm 6.47d	93.20 \pm 1.07d	418.31 \pm 14.03c

- SE = Standard Error

- Means in the same column followed by the same letter are not significantly different according to Duncan Multiple Range Test (1955)

Acid and Alkaline Phosphatase:

The activity of both acid and alkaline phosphatase was studied in *S. littoralis* larvae in Table (6). Results revealed that the activity of acid phosphatase was higher than of alkaline phosphatase in all treatments.

The least activity of acid and alkaline phosphatase occurred in control with (4.02 and 4.96 $\mu\text{g phosphate/min/ml}$, respectively). The highest enzyme activity of acid and alkaline phosphatase was observed in Tempo XL (11.69 and 10.34 $\mu\text{g phosphate/min/ml}$, respectively), while the activity of both acid and alkaline phosphatase was similar in the case of treated larvae by Heater and Folliam Felixi. Bassel and Ismail (1985) and Reda *et al.* (2007). Suggested that the most probable action of the IGRs is possible via strong inhibition of the ecdyson that followed by a subsequent decrease in the number of lysosomes which reflect a decrease in lysosomal ACP activity.

Total Protein:

The concentration of total proteins in haemolymph of *S. litorallis* larvae was studied. As clearly shown from the results compiled in Table (6), which increased in untreated was 359.15 $\mu\text{g/ml}$ as compared with other treatments. Folliam Felixi showed the highest activity (262.24 $\mu\text{g/ml}$) while the lowest one was shown in Tempo XL (233.38 $\mu\text{g/ml}$). The decrease of the total protein may reflect the decrease in the enzymatic activities of various enzymes. These results in accordance with the demonstrated by Abd El-Aziz *et al.*, (2007) and Elbarky *et al.*, (2008). However, our results disagree with results obtained by Raja *et al.*, (1986) and Lohar and Wright (1990).

In conclusion, the present study suggests the using some insecticide mixtures that contain two effective substances had high toxicity and reduce the population growth of *S. littoralis* by affecting the development and reproduction. Use these insecticides was considered useful tool in IPM of *S. littoralis*, however, the resistance risk of cotton leafworm on these insecticides should not be overlook.

Table 6: Effects of LC₅₀ of tested compounds on phosphatases activity and total protein of *Spodoptera littoralis*

Treatments	Acid phosphatase ±SE (µg phosphate/min/ml)	Alkaline phosphatase ±SE (µg phosphate/min/ml)	Total Protein ±SE (µg /ml)
Tempo XL	11.69± 0.21a	10.34± 0.37a	233.38± 5.69c
Heater	8.48± 0.96b	6.27± 0.28b	250.13± 2.88bc
Folliam Felixi	8.61± 0.56b	6.30± 0.35b	262.24± 7.66b
Control	4.96± 0.39c	4.02± 0.16c	359.15± 4.13a

- SE = Standard Error
- Means in the same column followed by the same letter are not significantly different according to Duncan Multiple Range Test (1955).

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ARABIC SAMMARY

تأثير بعض مخاليط المبيدات الحشرية على السمية وبعض الدلائل البيوكيميائية لدودة ورق القطن

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تم في هذه الدراسة تقييم سمية ثلاثة مبيدات حشرية تحتوي على مادتين فعالتين و هم كلوربيريفوس ولوفينورون (تمبو اكس ال) ، لوفينورون وإيمايكتين بنزوات (هيتير) ، وثياميثوكسام وكلورانترانيلبول (فوليام فليكسي) ضد الاعمار اليرقية الثاني والرابع لدودة ورق القطن في المعمل ، كما تم تقدير نسبة الموت المصححة لليرقات المعاملة تحت ظروف تجربة شبه حقلية في حقل الطماطم بمحافظة الإسماعيلية خلال موسمي 2018 و 2019 وأيضا تقييم تأثيرها الكيميائي الحيوي على اليرقات المعاملة. أشارت النتائج إلى أن مركب هيتير كان له أعلى فاعلية ضد الاعمار اليرقية الثاني والرابع ، حيث بلغ LC_{50} 0.041 و 0.135 جزء في المليون للعمر اليرقي الثاني والرابع على التوالي ، يليه فوليام فليكسي بـ LC_{50} 0.076 و 0.233 جزء في المليون وأخيراً تمبو اكس ال LC_{50} 0.097 و 0.411 جزء في المليون للعمر اليرقي الثاني والرابع على التوالي. و تحت الظروف شبه الحقل لاحظ أن اعلي نسبة موت مصححة للتأثير الفوري خلال موسمي 2018 و 2019 كان اعلي ما يكون للمركب هيتير ، في حين أن المتوسط العام للأثر المتبقي كان أكثر من 90 ٪ في جميع المعاملات في كلا موسمي الدراسة. في الدراسة الكيموحيوية لوحظ ان نشاط الانزيمات الفا وبيتا استيريز كانت أعلى في اليرقات المعاملة بالمركبات تحت الدراسة مقارنة بالغير معاملة. وقد لوحظت أعلى نشاط لإنزيمات الاستيل كولين استيريز، والفوسفات الحامضي و القلوي عند معاملة اليرقات بمركب تمبو اكس ال، بينما في حالة هيتير و فوليام فليكسي لم يكن هناك فرق معنوي بينهما. كما أظهرت النتائج ان مع كل المعاملات هناك تناقص معنوي في المحتوي الكلي للبروتين.