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Biological and Physiological Disturbances of *Lucilia silvarum* Meigen (Diptera: Calliphoridae) Treated with Certain Insecticides

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ABSTRACT

Background: *Lucilia silvarum* Meigen, 1826 (Diptera: Calliphoridae) is one of the medically important blowflies.

Objective: This study aimed to estimate the toxicity of abamectin, deltamethrin and diazinon to eggs, larvae, and adult stages of *L. silvarum*. Moreover, the disturbances of some biological and physiological aspects were investigated.

Materials and methods: Newly hatched eggs, early third instar larvae and one-day-old adult flies of *L. silvarum* were exposed to different concentrations of the selected insecticides. To follow some biological aspects of the tested concentration survivors such as egg hatchability, larval mortality, pupation %, adult emergence % and sex ratio were studied. Spectrophotometric estimation of total proteins, carbohydrates, lipids and certain enzymes activity were determined in the 3rd larval instar that survived the tested concentrations.

Results: The results showed that abamectin provoked the highest toxicity value against the third larval instar, while deltamethrin elicited the most toxic effect against egg and adult stages. The mortality of eggs, larvae and adults increased with the increase of concentration. High significant larval and adult mortalities were observed after 48 h treatment. The percentage of pupation is reduced after treatment of the third larval instar with the three selected insecticides. A high significant reduction in the mean number of emerged males was observed after treatments with all concentrations of the three tested insecticides. The treatment with different concentrations of the investigated insecticides leads to physiological disturbances in total protein, carbohydrates and lipid contents of the treatment survived larvae. Moreover, the change in enzyme activities as MFOs, AChE, ALP, ACP, GST, α and β -estereases following insecticides application were measured.

Conclusion: The three tested insecticides induced some biological and physiological disturbances in the life of survived insect pests which may be considered as control agents against the myiasis producing fly *L. silvarum*.

INTRODUCTION

Calliphoridae (blowflies) present a great ecological diversity, are distributed worldwide occupying several habitats- from organic matter to animal tissues in decomposition. They cause medical problems and important losses to the animal industry (Zumpt, 1965; Ghandour, 1988; David *et al.*, 2008). *Lucilia* is one of the most important blowfly genera in forensic entomology due to their occurrence on human bodies (Smith, 1986). Adults of the common toad fly, *L. silvarum* are found in different European countries, for the first time on three forensic human bodies that were close to lakes, wetlands, or riversides (Hanski, 1976; Rognes, 1991; Prado e Castro *et al.*, 2011; Fremdt *et al.* 2012). The adults of *L. silvarum* feed on nectar- as the other species of the genus *Lucilia* do. Groth and Reissmuller (1973) found that larvae of *L. silvarum* have a strong preference for dead frogs. Besides the carrion colonizing taxa, it is a parasite of frogs and toads as well as species causing myiasis (Zumpt, 1965). There are also European records of *L. silvarum* as a parasite of living toads so the name common toad fly (Bolek and Coggins, 2002; Bolek and Janovy, 2004). Furthermore, there are individual records of infestations of animal others than amphibians, including a live rat (Dodge, 1952) and a dead duck (Brothers, 1970).

For previous decades, insecticides have been extensively used for the management of various insect pests especially Diptera. The good insecticides are effective on the insect without negatively affecting the environment or causes build up resistance. Among the applied insecticides abamectin, deltamethrin and diazinon are commonly used.

Abamectin (Abamex, 1.8% EC), is known as avermectin B1 and is a natural product of *Streptomyces avermitilis*, like most other insecticides, as nerve poison. They quicken the gamma-aminobutyric acid

(GABA) system, a chemical neurotransmitter that inhibits nerve impulses from nerve to nerve and muscle. The pretentious insect becomes paralyzed, prevents feeding, and finally dies. Abamectin is an eco-friendly insecticide as broken down quickly through photodegradation in the soil and at the soil surface. Moreover, abamectin has fast broken down in the water. Abamectin is used to regulate insect pests such as cockroaches and mites (Batiha *et al.*, 2020).

Deltamethrin (Neotox, 5 % EC) is a member of synthetic pyrethroids with great potency of insecticidal activity. It kills insect pests through contact and digestion. Deltamethrin shows high efficacy in the management of a wide range of medically important insects like flies, cockroaches, fleas, and bed bugs. It causes an irreversible effect on the nervous systems of insects by changes in nerve membrane potential through disturbance in permeability to sodium and potassium ion leading to insect knockdown (Leahey, 1985).

Diazinon (60% EC) is a contact organophosphorus insecticide, used mainly in the control of agricultural pests. It kills insects through the dysfunction of nervous system neurotransmitters. Minor exposures to diazinon are not likely to cause severe symptoms to man and non-target organisms (Tinoco-Ojanguren and Halperin, 1998).

This study aims to investigate the effect of three commercial compounds belong to three different groups of insecticides on a myiasis producing fly, *L. silvarum* and insight their impact on some biological aspects such as hatchability, larval mortalities, pupation, adult emergence percentage, sex ratio as well as some physiological disturbances in the third instar larvae that survived 48 h feeding on the insecticidal treated diet.

MATERIALS AND METHODS

Insect Colony:

Insects of *L. silvarum* (common toad fly) were collected from Sharkia Governorate,

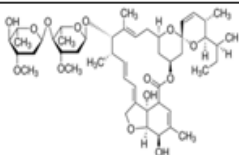
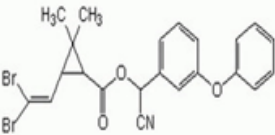
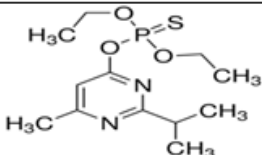
Egypt and reared in the laboratory of Zoology Department, Faculty of Science, Zagazig University. This insect was reared according to the method of El- Khateeb *et al.* (2003). To obtain the laboratory strain of *L. silvarum*: buffalo meat was used for oviposition. Hatched larvae were reared in plastic jars (10.5 x 7 cm) containing approximately 50 gm fresh buffalo meat. About 50 larvae were kept in each plastic jar to avoid competition. The pupae were transferred to new plastic jars containing sawdust as the medium for pupation. The pupae were obtained by sieving and transferred to rearing cages (30 x 30 x 30 cm) for adult emergence. Emerging adults were identified morphologically as described

by (Szpila, 2012), separated and reared for three generations under laboratory conditions (25±2 °C and 54-73% RH) using the aforementioned method. To maintain the colony flies were supplied with 10% sucrose solution, sugar crystals and offered fresh meat as a source of protein and medium for egg deposition (Khater, 2018).

Insecticides:

Three insecticides, namely abamectin, deltamethrin and diazinon were tested against eggs, larvae, and adult stages of *L. silvarum* in the laboratory. The list of tested insecticides with common name, chemical structure, trade name and source of availability is given in Table (1).

Table 1: The tested insecticides against *Lucilia silvarum*.

| Group | Avermectin | Pyrethroid | Organophosphate |
|--------------------|---|--|---|
| Common name | Abamectin | Deltamethrin | Diazinon |
| Chemical structure |  |  |  |
| Trade name | Abamax 1.8 % EC | Neotox 5 % EC | Diazinon 60 % EC |

All pesticide formulations were supplied by the Plant Protection Research Institute, Giza, Egypt.

Bioassay Experiments:

Egg Stage Treatment:

Newly hatched eggs of *L. silvarum* were used in this experiment. About 15 gm of meat dipped in 25ml of each concentration for 3min. Four concentrations (0.5, 0.25, 0.125 and 0.0625 ppm) from tested insecticides (abamectin, deltamethrin and diazinon) in the glass jar (6x9cm) were supplied as a hatching medium. In the control experiments, water was only added to the meat. The procedures

were replicated four times, 25 eggs were used for each replicate. The mean number of hatched eggs was counted after 24 hours of application. The eggs mortality was recorded and subjected to probit analysis to determine the lethal concentrations (LC₅₀ and LC₉₀).

Larval Stage Treatment:

Early third instar larvae of *L. silvarum* were fed on minced meat treated with five different concentrations of the three selected insecticides (2.5, 5, 10, 20, 40 ppm). One and

a half ml of each concentration was added to 15 gm of minced meat in a glass jar (6 x 9 cm). Water only was added to the minced meat of the control. Three replicates were used for each concentration. Thirty larvae were used for each replicate. Experiments were carried out according to (Smith *et al.*, 2000) with some modifications, under laboratory conditions of 27 ± 2 °C, 80 ± 5 % RH and 12: 12 light: dark. Larval mortality was recorded after 24 and 48 h of treatments (WHO, 2005). Some biological parameters (pupation percentage, adult emergence percentage and sex ratio) were investigated for the insecticidal survivors.

Adult Stage Treatment:

Adult flies of *L. silvarum* were subjected to three concentrations (0.50, 0.25, 0.125 ppm) of the three abovementioned insecticides. Three pairs of one day- old adults were introduced to cages (30x30x30cm) and cotton wool pieces (2gm) soaked in 5ml of each concentration in a Petri dish (3cm) were supplied to each cage. The procedures were replicated three times for each concentration. In the control experiments, water only was used. The mortality was recorded after 24 h and 48 h of treatments.

Physiological Disturbances:

Spectrophotometric estimation of total proteins, total carbohydrates, total lipids and certain enzymes activity in the 3rd larval instar of *L. silvarum* that survived 48 h fed on the treated diet (minced meat) with the tested insecticides were pursued. Larval homogenate intended for biochemical constituents were prepared as described by Amin (1998) to determine:

1. Total protein was evaluated by the method of Bradford (1976).
2. The total carbohydrate content was determined according to Singh and Sinha (1977)
3. Total lipids were estimated according to the method described by Frings *et al.* (1972)
4. Mixed function oxidase enzyme (MFO) was measured using P-Nitroanisole O-demethylation to determine MFOs activity according to the method described by Hansen and Hodgson (1971).
5. Acetylcholinesterase enzyme (AChE) activity was estimated by the method of Simpson *et al.* (1964).
6. Acid and alkaline phosphatases activities were determined according to the method of Laufer and Schin (1971).
7. Glutathione-S-transferase enzyme (GST) activity was measured by Habig *et al.* (1974) method.
8. α - & β - esterase enzyme activity was determined according to Asperen (1962)

Statistical Analysis of Data:

Average larval mortality rates were subjected to probit model analysis for calculating LC₅₀ and LC₉₀. The results were analyzed by one-way analysis of variance (ANOVA) using the Statistical Software SPSS, 14.00 software (SPSS Inc., 2009). Significance levels were calculated using the Chi-square test for lethal concentrations, least significance differences (LSD) and Duncan test for quantitative data (Means \pm SE). The level of significance was stated to be highly significant (P<0.01), significant (P<0.05) and non-significant (P>0.05).

RESULTS

Toxicity of Insecticides:

The first part of the present study was carried out to evaluate the toxicity of abamectin, deltamethrin, and diazinon to egg, 3rd larval instar and adult stage under laboratory conditions.

Toxicity to the Egg Stage:

Data presented in Table (2) show that deltamethrin was the most potent insecticide to the egg stage since the calculated LC₅₀

value was 0.002 ppm. While diazinon came in the second position of toxicity which recorded the value of 0.011 ppm as LC₅₀. Abamectin

was the least toxic insecticide recording the LC₅₀ value of 0.020 ppm.

Table 2: Toxicity data of abamectin, deltamethrin and diazinon tested against eggs, larvae and adults of *L. silvarum*.

| Calculated value | Tested insecticide | Egg (after 24 h) | Larva | Adult |
|--|--------------------|-----------------------|-------------------------|-----------------------|
| | | | After 48 h | |
| LC ₅₀ (in ppm) (95% CLs) | Abamectin | 0.020 (0.006- 0.034) | 9.47 (7.55 – 11.83) | 0.64 (0.31 – 0.71) |
| | Deltamethrin | 0.002 (0.000- 0.017) | 12.46 (9.48 – 16.94) | 0.43 (0.20-0.45) |
| | Diazinon | 0.011 (0.001-0.025) | 37.95 (28.29 – 58.53) | 1.18 (0.42-6.73) |
| LC ₉₀ (in ppm) (95% CLs) | Abamectin | 0.092 (0.068-0.119) | 93.19 (58.50-191.33) | 5.43 (1.07-7.13) |
| | Deltamethrin | 0.651 (0.286-3412.16) | 228.00 (110.21-813.53) | 4.81 (0.71-5.94) |
| | Diazinon | 0.113 (0.073-0.164) | 332.04 (169.01-1006.51) | 45.52 (2.74– 5230.74) |
| Slope ± SE | Abamectin | 1.963±0.44 | 1.29±0.15 | 1.38±0.76 |
| | Deltamethrin | 0.52±0.23 | 1.01±0.14 | 1.22±0.72 |
| | Diazinon | 1.24±0.32 | 1.36±0.17 | 0.80±0.74 |
| Chi-Square | Abamectin | 2.340 | 5.53 | 0.01 |
| | Deltamethrin | 0.080 | 1.33 | 0.30 |
| | Diazinon | 0.740 | 6.28 | 0.02 |

LC: lethal concentration; CLs: confidence limits.

Toxicity to the Larval Stage:

The LC₅₀ and LC₉₀ values from the treatment of the third larval instar of *L. silvarum* with the three tested insecticides were presented in Table (2). The LC₅₀ values were 9.47, 12.46 and 37.95 ppm for abamectin, deltamethrin and diazinon, respectively. It was clear that abamectin is considered the most toxic than deltamethrin and diazinon compared with control.

Toxicity to the Adult Stage:

Results obtained (Table, 2) reveal that The LC₅₀ and LC₉₀ values of the adult stage of *L. silvarum* were 0.43, 0.64 & 1.18 and 4.81, 5.43 & 45.52 ppm for deltamethrin, abamectin and diazinon, respectively. It clear that deltamethrin was the most toxic to the adult stage.

Some Biological Disturbances of The Insecticidal Survived Larvae:

Larval Mortality:

The results showed that the percentage of larval mortality increased as the insecticide concentration incremented (Table 3). Abamectin gave the highest larval mortality percentage than deltamethrin and diazinon following 24 h and 48 h of

treatments in comparison with control. After 24 h of insecticides application, the highest significant larval mortalities were recorded for the highest concentration 40 ppm and scored 4.00±0.57, 4.33±0.33 and 2.33±0.58 for deltamethrin, abamectin and diazinon, respectively. After 48 h of treatment, the larval mortality was increased with highly significant effect for all tested concentrations recording the highest mortality for the highest concentration (40 ppm) being 22.66±1.45, 20.66±0.88 and 17.33±0.66 for abamectin, deltamethrin and diazinon, respectively.

Pupation Percentage:

The mean number of pupated larvae was reduced after treatment of third larval instar with the three selected insecticides; it decreased to 24.44±2.93, 10.00±1.92 and 31.11±2.22 after treatment with the concentration, 40 ppm of deltamethrin, abamectin and diazinon, respectively compared with the control (Table 3).

Adult Emergence Percentage:

Data in Table (3) show that the reverse relationship between adult emergence and different concentrations. It decreased with increased concentrations until reach to very low emergence (50.00±5.77%, 54.23±2.16%

and $55.55\pm 5.55\%$) at the highest concentration (40 ppm) for diazinon, deltamethrin and abamectin, respectively compared with the untreated counterparts (control) which were $96.66\pm 3.33\%$. This decrease was highly significant concerning the tested concentrations within each treatment and control.

A high significant reduction in the mean number of emerged males was observed after treatments with all concentrations of the three tested compounds (Table 3). Abamectin insecticide was the most toxic following treatment with 20 and 40 ppm which the mean number was 1.33 ± 0.66 and 0.66 ± 0.66 . On contrary, diazinon was the least toxic

(2.33 ± 0.88 and 2.00 ± 0.57) with highly significant differences.

A high significant reduction in the mean number of emerged females was observed after treatments with all concentrations of the three tested compounds (Table 3). Treatment with the highest concentration, 40 ppm caused reduction was 0.66 ± 0.33 , 2.33 ± 0.33 and 2.66 ± 0.33 for abamectin, deltamethrin and diazinon, respectively compared with 17 ± 0.57 in the control. There was a predominance of males over females (ratio, 1.04: 0.96 and 1.0: 0.67), respectively after treatment with 2.5 and 40 ppm of abamectin compared with the control 1.03: 1.5 (♂:♀).

Table 3: Larval mortalities and some biological disturbances of *L. silvarum* larvae that survived deltamethrin, abamectin and diazinon treatments.

| Insecticide | Concentration (ppm) | Immature stage | | | Adult stage | | | |
|--------------|---------------------|-----------------------------|--------------------------|--------------------------|-----------------------------|-------------------------|---------------------------|------------------|
| | | Larval mortality (Mean± SE) | | Pupation % (Mean± SE) | % Adult emergence (Mean±SE) | Emergent male (Mean±SE) | Emergent female (Mean±SE) | Sex ratio (♀: ♂) |
| | | After 24 h | After 48 h | | | | | |
| Deltamethrin | 2.5 | 0.33±0.33 ^c | 7.66±0.88 ^c | 48.89±4.00 ^b | 79.70±3.00 ^{ab} | 5.0±0.57 ^b | 6.66±0.33 ^b | 1.4:1.1 |
| | 5 | 1.66±0.33 ^{bc} | 10.00±0.57 ^{bc} | 40.00±3.85 ^{bc} | 75.47±2.48 ^{bc} | 3.66±0.66 ^b | 5.33±0.33 ^{bc} | 1.5:1.1 |
| | 10 | 2.00±0.57 ^{abc} | 12.66±0.88 ^b | 32.22±1.11 ^d | 68.52±7.09 ^{bc} | 3.00±1.00 ^b | 3.66±0.33 ^{cd} | 1.4:1.2 |
| | 20 | 2.66±0.66 ^{ab} | 18.66±0.88 ^a | 27.77±2.22 ^{cd} | 55.02±6.88 ^c | 2.00±1.00 ^b | 2.66±0.66 ^d | 1.4:1.1 |
| | 40 | 4.00±0.57 ^a | 20.66±0.88 ^a | 24.44±2.93 ^d | 54.23±2.16 ^c | 1.66±0.66 ^b | 2.33±0.33 ^d | 1.5:1.04 |
| F. test | | 9.93** | 99.59** | 103.58** | 12.07** | 19.41** | 148.67** | - |
| Abamectin | 2.5 | 0.66±0.33 ^{bc} | 7.00±0.57 ^c | 36.66±3.33 ^b | 75.64±2.95 ^b | 4.33±0.66 ^b | 4.00±0.57 ^{bc} | 0.96:1.04 |
| | 5 | 1.00±0.57 ^{bc} | 8.66±1.20 ^c | 35.55±4.00 ^b | 69.77±4.68 ^{bc} | 2.33±0.66 ^{bc} | 5.00±0.00 ^b | 2.3:1.1 |
| | 10 | 2.33±0.88 ^{abc} | 18.00±0.57 ^b | 30.00±1.92 ^{bc} | 63.05±1.94 ^{bc} | 2.33±0.33 ^{bc} | 3.33±0.33 ^{bc} | 1.5:1.03 |
| | 20 | 3.00±0.57 ^{ab} | 20.33±0.33 ^b | 21.11±2.93 ^{cd} | 57.50±3.81 ^c | 1.33±0.66 ^{bc} | 2.33±0.66 ^{cd} | 2.1:1.2 |
| | 40 | 4.33±0.33 ^a | 22.66±1.45 ^a | 10.00±1.92 ^d | 55.55±5.55 ^c | 0.66±0.66 ^d | 0.66±0.33 ^d | 0.67:1.0 |
| F. test | | 9.54** | 109.57** | 138.37** | 15.22** | 32.66** | 155.48** | - |
| Diazinon | 2.5 | 0.33±0.33 ^{bc} | 2.66±0.66 ^c | 51.11±2.93 ^b | 80.27±1.07 ^b | 5.66±0.66 ^b | 6.66±0.33 ^b | 1.4:1.1 |
| | 5 | 0.66±0.33 ^{bc} | 3.33±0.33 ^c | 43.33±3.84 ^{bc} | 71.76±1.27 ^{bc} | 4.33±0.88 ^b | 5.00±0.00 ^c | 1.3:1.2 |
| | 10 | 1.33±0.35 ^{abc} | 4.66±0.66 ^c | 41.11±4.00 ^{bc} | 61.94±1.25 ^{cd} | 3.66±0.66 ^b | 4.00±0.57 ^{cd} | 1.4:1.2 |
| | 20 | 1.66±0.50 ^{ab} | 9.33±0.33 ^b | 33.33±1.92 ^c | 52.69±1.70 ^d | 2.33±0.88 ^b | 3.00±0.00 ^d | 1.4:1.1 |
| | 40 | 2.33±0.58 ^a | 17.33±0.66 ^a | 31.11±2.22 ^c | 50.00±5.77 ^d | 2.00±0.57 ^b | 2.66±0.33 ^d | 1.4:1.1 |
| F. test | | 8.36** | 150.62** | 81.23** | 23.26** | 19.78** | 196.62** | - |
| Control | - | 0.00±0.00 ^c | 0.00±0.00 ^d | 100.00±0.00 ^a | 96.66±3.33 ^a | 12.0±1.15 ^a | 17±0.57 ^a | 1.5:1.03 |

Each datum represents the mean of three replicates; means within the same column followed by the same superscript are not significantly different (Duncan test, $p < 0.01$ **); the total number of treated larvae for each concentration = 90 larvae.

Adult Mortality:

Data recorded in Table (4) provoke that the adult stage of *L. silvarum* was more susceptible to the three tested insecticides at all concentrations. The mortality increased as

the concentration increased for all tested periods of exposure. Deltamethrin seemed to be more effective at all concentrations especially at the highest concentration (0.50 ppm) which scored 3.00 ± 0.57 and 3.33 ± 0.33

after 24 h and 48 h, successively. Deltamethrin followed by abamectin, gains 2.33 ± 0.88 and 2.66 ± 0.66 after 24 h and 48 h post-treatment at the highest concentration, while diazinon was the least toxic against the adult stage (1.66 ± 0.033 & 2.33 ± 0.35) after the same post-treatment periods and concentrations. The results in Table (4)

insight that the male individuals were more susceptible to the chosen insecticides than the females. Sex ratio (♀: ♂) was 1.25:3.75, 1.00:4.00 and 0.71:4.30 after treatment with the highest concentration (0.50 ppm) for abamectin, deltamethrin and diazinon, respectively.

Table 4: Adult mortality (female mortality, male mortality, and sex ratio) of *L. silvarum* treated with deltamethrin, abamectin and diazinon.

| Insecticide | Concentration (ppm) | Adult mortality (Mean± SE) | | Female mortality (Mean± SE) | Male mortality (Mean± SE) | Sex ratio (mortality) (♀ : ♂) |
|----------------|---------------------|----------------------------|----------------------|-----------------------------|---------------------------|-------------------------------|
| | | After 24 h | After 48 h | | | |
| Deltamethrin | 0.50 | 3.00 ± 0.57^a | 3.33 ± 0.33^a | 0.66 ± 0.34^a | 2.66 ± 0.34^a | 1.00: 4.00 |
| | 0.25 | 1.33 ± 0.88^{ab} | 2.00 ± 0.57^a | 0.33 ± 0.33^a | 1.66 ± 0.33^a | 0.83:4.20 |
| | 0.125 | 0.66 ± 0.33^{ab} | 1.66 ± 0.33^{ab} | 0.33 ± 0.33^a | 1.33 ± 0.33^{ab} | 1.00:4.00 |
| F. test | | 5.42* | 13.53** | 0.88^{NS} | 14.55** | - |
| Abamectin | 0.50 | 2.33 ± 0.88^a | 2.66 ± 0.66^a | 0.66 ± 0.34^a | 2.00 ± 0.57^a | 1.25:3.75 |
| | 0.25 | 1.33 ± 0.33^a | 1.66 ± 0.33^{ab} | 0.33 ± 0.33^a | 1.33 ± 0.33^{ab} | 1.00:4.00 |
| | 0.125 | 0.66 ± 0.33^a | 1.00 ± 0.00^{ab} | 0.33 ± 0.33^a | 0.66 ± 0.33^{ab} | 1.10:2.20 |
| F. test | | 3.96^{NS} | 9.06** | 0.88^{NS} | 5.33* | - |
| Diazinon | 0.50 | 1.66 ± 0.033^a | 2.33 ± 0.35^a | 0.33 ± 0.33^a | 2.00 ± 0.00^a | 0.71:4.30 |
| | 0.25 | 1.33 ± 0.033^a | 1.66 ± 0.33^a | 0.33 ± 0.33^a | 1.33 ± 0.33^{ab} | 1.00:4.00 |
| | 0.125 | 1.00 ± 0.57^a | 1.33 ± 0.33^{ab} | 0.33 ± 0.33^a | 1.00 ± 0.00^b | 1.25:3.75 |
| F. test | | 3.73^{NS} | 11.55** | 0.33^{NS} | 25.00** | - |
| Control | - | 0.00 ± 0.00^a | 0.00 ± 0.00^b | 0.00 ± 0.00^a | 0.00 ± 0.00^b | 0.00:0.00 |

Each datum represents the mean of three replicates; means within the same column followed by the same superscript are not significantly different (Duncan test, NS, non-significant; significant, $P < 0.05^*$); highly significant, ($p < 0.01^{**}$); Total number of treated adults for each concentration = 9 pairs.

Physiological disturbances in *L. silvarum* larvae the survived insecticidal treatment: Total protein:

The total protein content showed increased significantly after diazinon treatments in *L. silvarum* larvae comparing with control. The most promoting effect recorded as 88.67 ± 1.22 mg/g b.wt at 40 ppm with 288.73% elevation percentage compared to 22.81 ± 0.87 mg/g b.wt. In contrast, abamectin application showed a highly significant reduction in total protein content, the most inhibiting effect was 15.67 ± 0.22 mg/g b.wt at 10 ppm concentration (-31.30%) and the least effect was 22.64 ± 1.38 mg/g b.wt. at 40 ppm with a -74% reduction

percentage. Meanwhile, a fluctuated effect was recorded following deltamethrin, a significant decrease measured by 2.5 and 5 ppm with -22.18 % and -16.52% while, total protein was increased after treatment with concentrations of 10, 20 and 40 ppm with increasing percentage (0.70, 27.35 and 41.38%) respectively (Table 5).

Total Carbohydrate:

Data in Table (5) show a highly significant reduction in total carbohydrate content of the third larval instar of *L. silvarum* after treatment with the three selected insecticides at different concentration levels. While non-significant reduction (114.60 ± 7.22 mg/g. b. wt) with change

percentage (-2.21%) was recorded following treatment with 2.5 ppm abamectin compared with 117.20 ± 7.06 mg/g. b. wt. in the control experiment. From the obtained results, it was cleared that the application of 40 ppm diazinon causes the most reducing effect on total carbohydrate content (18.47 ± 4.37 mg/g. b. wt) with a change percentage (-84.24%).

Total Lipids:

The observed results demonstrate that all treatments lead to a highly significant decrease in total lipid content. Moreover, high significant the highest reduction in total lipid content was 1.21 ± 0.21 (-86.87%) and 1.44 ± 0.12 mg/g. b. wt. (-84.38%) measured at 40 ppm concentration of both abamectin and diazinon, respectively in comparison with 9.22 ± 0.11 mg/g. b. wt. in control (Table 5).

Table 5: Total proteins, total carbohydrates and total lipids content in 3rd instar larvae of *L. silvarum* after feeding on the treated diet with the tested insecticides.

| Treatment | Concentration (ppm) | Concentration (mg /g.b.wt)±SE | | |
|----------------|---------------------|--------------------------------|--------------------------------|-------------------------------|
| | | Total protein | Total carbohydrate | Total lipid |
| Deltamethrin | 2.5 | 17.75 ± 2.42^b (-22.18) | 39.34 ± 0.72^b (-66.43) | 8.33 ± 0.33^{ab} (-9.65) |
| | 5 | 19.04 ± 2.15^b (-16.52) | 32.74 ± 1.37^b (-72.06) | 7.54 ± 0.53^{bc} (-18.22) |
| | 10 | 22.97 ± 1.89^{ab} (0.70) | 30.33 ± 0.37^b (-74.12) | 7.06 ± 0.03^{cd} (-23.42) |
| | 20 | 29.05 ± 4.51^{ab} (27.35) | 29.59 ± 0.32^b (-74.75) | 6.27 ± 0.09^a (-31.99) |
| | 40 | 32.25 ± 2.90^a (41.38) | 24.22 ± 2.08^b (-79.33) | 5.03 ± 0.03^a (-45.44) |
| F. test | | 4.38* | 25.25** | 31.42** |
| Abamectin | 2.5 | 17.42 ± 0.40^{bc} (-23.62) | 114.60 ± 7.22^a (-2.21) | 4.07 ± 0.57^b (-55.85) |
| | 5 | 19.91 ± 0.43^{ab} (-12.71) | 93.64 ± 6.64^{ab} (-20.10) | 3.80 ± 1.19^{bc} (-58.78) |
| | 10 | 15.67 ± 0.22^c (-31.30) | 73.89 ± 4.27^{ab} (-36.95) | 2.94 ± 0.34^{bc} (-68.11) |
| | 20 | 19.94 ± 0.67^{ab} (-12.58) | 36.59 ± 3.21^b (-68.77) | 2.06 ± 0.07^{bc} (-77.65) |
| | 40 | 22.64 ± 1.38^a (-0.74) | 46.90 ± 3.29^b (-59.98) | 1.21 ± 0.21^c (-86.87) |
| F. test | | 13.46** | 6.87** | 24.68** |
| Diazinon | 2.5 | 24.89 ± 0.57^c (9.11) | 32.00 ± 6.10^b (-72.69) | 4.85 ± 0.91^b (-47.39) |
| | 5 | 28.83 ± 3.33^c (26.39) | 30.39 ± 5.31^b (-74.06) | 2.97 ± 0.18^b (-67.78) |
| | 10 | 39.62 ± 2.33^b (73.69) | 25.17 ± 5.03^b (-78.52) | 3.06 ± 1.39^b (-66.81) |
| | 20 | 45.97 ± 0.96^b (101.53) | 23.03 ± 4.18^b (-80.34) | 2.82 ± 0.74^b (-69.41) |
| | 40 | 88.67 ± 1.22^a (288.73) | 18.47 ± 4.37^b (-84.24) | 1.44 ± 0.12^b (-84.38) |
| F. test | | 181.47** | 20.29** | 13.41** |
| Control | - | 22.81 ± 0.87^a | 117.20 ± 7.06^a | 9.22 ± 0.11^a |

Each datum represents the mean and standard error (M±SE) of three replicates; All concentrations showed non-significant results (P>0.05); and significant results (P<0.05); while means of other concentrations with different symbols within the same column are high significant (P<0.01); numbers between parentheses refer to "Change percentage (%)"

Enzymes Activity:

-Mixed Function Oxidases (MFOs):

Mixed function oxidase activity (MFOs) provoked high significant increase with diazinon application after treatment with all selected concentration except after treatment with 2.5 ppm a decrease in enzyme activity was recorded (0.53 ± 0.09 m mol sub./min./ g.bwt) with -8.62% reduction. Meanwhile significant decrease in MFOs activity measured following treatment with concentrations of 2.5, 5, 10, 20 ppm abamectin and scored 0.31 ± 0.05 , 0.41 ± 0.05 ,

0.48 ± 0.06 , 0.52 ± 0.01 m mol sub./min./ g.b.wt., respectively. On contrary, at 40 ppm concentration of abamectin cause significant increase in MFOs activity by 0.73 ± 0.09 m mol sub./min./ g.b.wt. with increasing 25.86%. Deltamethrin treatment showed different effects on MFOs activity, highly significant increase by 0.76 ± 0.02 , 0.96 ± 0.03 , 1.00 ± 0.00 m mol sub./min./ g.b.wt. following treatment with 10, 20, 40 ppm, respectively. While high significant decrease in 2.5 and 5 ppm concentrations reported (0.46 ± 0.02 and 0.56 ± 0.02 b m mol sub./min./ g.b.wt.)

compared with control (0.58 ± 0.14 m mol sub./min./ g. bwt.) (Table 6).

-AChE Enzyme Activity:

The AChE activity showed a highly significant reduction after treatment with the different concentrations of the three insecticides. Data in Table (6) showed that treatment with diazinon provoked the most inhibitory effect by 0.83 ± 0.40 μ g AchBr/min./g.b.wt. at the highest concentration, 40 ppm compared with 8.27 ± 0.40 μ g AchBr/min./g.b.wt. in the control experiment. Treatment with the same concentration of deltamethrin and abamectin caused the same effect by 4.21 ± 0.57 and 4.68 ± 0.15 μ g AchBr/min./g.b.wt., respectively.

-ALP Enzyme Activity:

Treatment of third larval instar of *L. silvarum* with the three selected insecticides elicited a clear inhibitory effect on ALP activity after 48 h. Deltamethrin has the most pronounced effect in this respect. After treatment with a 40-ppm concentration of deltamethrin the ALP activity provoked 7.13 ± 0.12 μ g phenol/g.b.wt. compared with 47.00 ± 1.28 μ g phenol/g.b.wt. in control. Also, treatment with the highest concentration 40 ppm of abamectin and diazinon scored 19.25 ± 0.58 and 15.17 ± 0.51 μ g phenol/g.b.wt. In contrast, a significant increase in enzyme activity was recorded after application with 2.5 ppm abamectin by 50.00 ± 1.53 μ g phenol/g.b.wt. (6.38%) compared with the control experiment (Table 6).

-ACP Enzyme Activity:

The ACP activity showed high significant reduction after treatment with all different concentrations of the three insecticides under investigation. The results showed that treatment with abamectin provoked the most inhibitory effect. Application with 40 ppm reveal 2.47 ± 0.13 (-78.96%), 2.60 ± 0.22 (-77.85%) and 3.13 ± 0.09 μ g phenol/g.b.wt. (-73.33%) in abamectin, deltamethrin and diazinon, respectively in

comparison with control (11.74 ± 0.64 μ g phenol/g.b.wt.) (Table 6).

-GST Enzyme Activity:

GST enzyme activity showed a unique trend represented in a highly significant decrease after treatment with 2.5 ppm of the three investigated compounds, 0.63 ± 0.04 , 0.78 ± 0.10 m.mol.Sub. conjugated/min/g b.wt. and 0.79 ± 0.02 m.mol.Sub. conjugated/min/g b.wt. by deltamethrin, abamectin and diazinon, respectively. While the highest increase was measured following the application of 40 ppm concentrations of all tested insecticides, diazinon elicited the most enhanced effect 1.70 ± 0.02 m.mol.Sub. conjugated/min/g b.wt. with an increasing percentage of 91.01% followed by 1.24 ± 0.12 m.mol.Sub. conjugated/min/g b.wt. in abamectin and scored 1.08 ± 0.06 m.mol.Sub. conjugated/min/g b.wt. in deltamethrin treatment (Table 6).

- α - EST Enzyme Activity:

Alpha esterase activity showed high significant elevation after application with the three tested insecticides. The most promoting effect reported at 40 ppm in diazinon treatment was 86.58 ± 1.84 μ g α naphthol/min./g. b.wt. (495.05%) followed by 42.90 ± 1.18 μ g α naphthol/min./g. b.wt. (194.84%) with 20 ppm of the same compound. Fluctuation in α - EST enzyme activity after treatment with both deltamethrin and abamectin. High significant reduction in enzyme activity recorded 9.93 ± 0.17 μ g α naphthol/min./g. b.wt. with 10 ppm abamectin followed by 10.87 ± 0.33 μ g α naphthol/min./g. b.wt. at 2.5 ppm of the same insecticide. A high significant increase of 17.48 ± 1.52 was reported after application with 40 ppm abamectin. α - EST enzyme activity showed high significant increase 22.93 ± 4.44 and 27.46 ± 3.18 μ g α naphthol/min./g. b.wt. after treatment with 20 and 40 ppm deltamethrin compared with 14.55 ± 0.41 μ g α naphthol/min./g. b.wt. in the control experiment (Table 6).

-β- EST Enzyme Activity:

The measured activity of β- EST demonstrates that diazinon high significantly increased the activity of β- EST at all applied concentrations. The highest value was 24.12±0.80 µg β naphthol/ min./g. b.wt. (212.84%) detected post 40 ppm concentration. Deltamethrin has an inhibitory

effect at different concentrations. The highest inhibitory action reported by 2.5 ppm was 3.41±0.48 µg β naphthol/ min./g.b.wt. Abamectin application leads to a non-significant reduction in enzyme activity at all concentration levels while 40 ppm concentration was increased which recorded 8.80±3.51 in comparison with 7.71±0.37 µg β naphthol/ min./g. b.wt. in control (Table 6).

Table 6: Activity of some enzymes in 3rd instar larvae of *L. silvarum* after feeding on the treated diet with the tested insecticides.

| Treatment | Concentration (ppm) | Activity (Mean ±SE) | | | | | | |
|--------------|---------------------|-------------------------------------|-------------------------------------|--------------------------------------|------------------------------------|---|--------------------------------------|-------------------------------------|
| | | MFO (m mol sub./min/g b.wt.) | AChE (µg AchBr/min/g b.wt.) | ALP (µg phenol/g b.wt.) | ACP (µg phenol/g b.wt.) | GST (m.mol.Sub. conjugated/min/g b.wt.) | α - EST (µg α-naphthol/min/g.b.wt.) | β- EST (µg β-naphthol/min/g.b.wt.) |
| Deltamethrin | 2.5 | 0.46±0.02 ^a (-20.68) | 7.21±0.26 ^{ab} (-12.81) | 12.89±0.09 ^a (-72.57) | 6.66±0.08 ^b (-43.27) | 0.63±0.04 ^a (-29.21) | 10.94±2.19 ^a (-24.81) | 3.41±0.48 ^a (-55.77) |
| | 5 | 0.56±0.02 ^{bc} (-3.44) | 6.70±0.30 ^{ab} (-18.98) | 23.43±1.28 ^b (-50.14) | 5.74±0.12 ^b (-51.10) | 0.77±0.03 ^{bc} (-13.48) | 12.50±1.85 ^b (-14.08) | 3.63±0.36 ^{bc} (-52.91) |
| | 10 | 0.76±0.02 ^{cd} (31.03) | 6.32±0.39 ^b (+23.57) | 12.90±0.21 ^c (-72.55) | 6.64±0.28 ^b (-43.44) | 0.90±0.05 ^{cd} (1.12) | 16.47±2.32 ^{cd} (13.19) | 4.29±0.46 ^{cd} (-44.35) |
| | 20 | 0.96±0.03 ^d (65.51) | 5.66±0.33 ^{bc} (-31.55) | 9.96±0.20 ^{cd} (-78.80) | 3.33±0.08 ^c (-71.63) | 1.00±0.00 ^d (12.35) | 22.93±4.44 ^{cd} (57.59) | 4.03±0.58 ^{cd} (-47.73) |
| | 40 | 1.00±0.00 ^e (72.41) | 4.21±0.57 ^b (-49.09) | 7.13±0.12 ^d (-84.82) | 2.60±0.22 ^c (-77.85) | 1.08±0.06 ^e (21.34) | 27.46±3.18 ^e (88.72) | 5.50±0.30 ^e (-28.66) |
| F. test | | 12.70** | 12.45** | 387.62** | 111.14** | 14.36** | 5.66** | 13.60** |
| Abamectin | 2.5 | 0.31±0.05 ^a (-46.55) | 6.95±0.04 ^b (-15.96) | 50.00±1.53 ^a (6.38) | 8.46±0.20 ^b (-27.93) | 0.78±0.10 ^b (-12.35) | 10.87±0.33 ^{cd} (-25.29) | 3.70±0.25 ^a (-25.01) |
| | 5 | 0.41±0.05 ^{ab} (-29.31) | 6.51±0.08 ^{bc} (-21.28) | 34.83±1.52 ^b (-25.89) | 8.29±0.06 ^b (-29.38) | 0.86±0.08 ^{cd} (-3.37) | 13.63±0.47 ^{cd} (-6.32) | 4.51±0.52 ^a (-41.50) |
| | 10 | 0.48±0.06 ^{ab} (-17.24) | 5.69±0.11 ^{cd} (-31.19) | 29.17±2.30 ^{bc} (-37.93) | 5.64±0.22 ^b (-72.99) | 1.06±0.07 ^{cd} (19.10) | 9.93±0.17 ^d (-31.75) | 4.43±0.89 ^b (-42.54) |
| | 20 | 0.52±0.01 ^{ab} (-10.34) | 5.47±0.26 ^{cd} (-33.85) | 22.44±4.42 ^{cd} (-52.25) | 3.17±0.08 ^c (-72.99) | 1.24±0.03 ^d (39.32) | 14.20±0.54 ^d (-2.40) | 6.71±1.20 ^b (-12.97) |
| | 40 | 0.73±0.09 ^a (25.86) | 4.68±0.15 ^c (-43.40) | 19.25±0.58 ^d (-59.04) | 2.47±0.13 ^b (-78.96) | 1.24±0.12 ^d (39.32) | 17.48±1.52 ^d (20.13) | 8.80±3.51 ^a (14.13) |
| F. test | | 3.27 * | 34.47** | 53.69** | 142.03** | 5.70** | 14.05** | 1.68 ^{ns} |
| Diazinon | 2.5 | 0.53±0.09 ^a (-8.62) | 5.31±0.15 ^b (-35.79) | 35.00±1.15 ^{ab} (-25.53) | 3.60±0.22 ^b (-69.33) | 0.79±0.02 ^c (-11.23) | 19.74±0.46 ^{cd} (35.67) | 8.37±0.79 ^a (8.56) |
| | 5 | 0.64±0.06 ^{bc} (10.34) | 4.60±0.20 ^{bc} (-44.37) | 32.17±1.12 ^{ab} (-31.55) | 3.09±0.05 ^c (-73.67) | 0.93±0.02 ^c (4.49) | 24.14±3.10 ^c (65.91) | 8.37±0.23 ^a (8.56) |
| | 10 | 1.03±0.03 ^d (77.58) | 3.79±0.06 ^c (-54.17) | 33.10±8.88 ^b (-29.57) | 8.49±0.31 ^b (-27.68) | 0.93±0.03 ^c (4.49) | 35.37±2.36 ^c (143.09) | 14.27±0.54 ^b (85.08) |
| | 20 | 0.96±0.03 ^{cd} (65.51) | 2.43±0.23 ^d (-70.61) | 19.13±0.57 ^{bc} (-59.29) | 3.57±0.17 ^b (-69.59) | 1.17±0.09 ^b (31.46) | 42.90±1.18 ^b (194.84) | 15.44±0.38 ^b (100.25) |
| | 40 | 1.04±0.02 ^d (79.31) | 0.83±0.40 ^c (-89.96) | 15.17±0.51 ^c (-67.72) | 3.13±0.09 ^b (-73.33) | 1.70±0.02 ^d (91.01) | 86.58±1.48 ^b (495.05) | 24.12±0.80 ^b (212.84) |
| F. test | | 9.29** | 86.55** | 9.53** | 133.68** | 53.58** | 216.42** | 125.88** |
| Control | - | 0.58±0.14 ^{ab} | 8.27±0.40 ^a | 47.00±1.28 ^a | 11.74±0.63 ^a | 0.89±0.01 ^{bc} | 14.55±0.41 ^{ab} | 7.71±0.37 ^{ab} |

Each datum represents the mean and standard error (M±SE) of three replicates; All concentrations showed non-significant results (P>0.05); and significant results (P<0.05); while means of other concentrations with different symbols within the same column are high significant (P<0.01); numbers between parentheses refer to "Change percentage (%)"

DISCUSSION

The present work demonstrates the strong impact of deltamethrin, abamectin and diazinon on some biological and physiological parameters of the 3rd larval instar of *L. silvarum*. A significant effect was induced on larval mortality after treatment

with as the most effective larvicidal compounds. It was cleared that, abamectin was considered to be more effective against the third larval stage of *L. silvarum* while, deltamethrin was considered to be more effective against eggs and adult flies.

Biological Disturbances:

A high significant reduction in the number of pupae after treatment of third larval instar of *L. silvarum* with the three selected insecticides was observed. The number of emerged adults is greatly reduced following treatments with all concentrations. There was a predominance of males over females (ratio, 1.04:0.96 and 1.00:0.67), respectively after treatment with 2.5 and 40 ppm abamectin compared with ratio 1.5:1.03 (♀:♂) in control and all treatments.

Previous studies investigated the effect of organophosphorus and other compounds on different insect species. Dahm (1971) reported greater toxicity to house fly by a diisopropyl homologue of parathion than the honeybee. Abd Rashid *et al.* (2008) reported that the larvae of *Chrysomya megacephala* show retarded development after treatment with malathion. Liu *et al.* (2009) studied the effect of malathion on *C. megacephala* fed on meat treated with malathion. They found that treatment causes delaying effects on development compared with control. Yan-Wei *et al.* (2010) indicated that treatment of *C. megacephala* with malathion prolongs larval and pupal duration. Ahmed and Vogel (2015) investigated the effect of some insecticides against *Aedes aegypti*. They added that diafenthiuron, diflubenzuron, novaluron, and Lufenuron were the least toxic while Chlorfenapyr insecticide shows the highest toxicity against fourth instar larvae. Askari- Saryazdi *et al.* (2015) observed high toxicity on second instar larvae of *Liriomyza sativa* after treatment with chlorpyrifos. Asid *et al.* (2017) suggested that diazinon and pirimiphos-methyl when mixed showed higher toxicity to house fly than when applied separately. Khater, 2018 reported that chlorpyrifos is more effective against *Chrysomya albiceps* larvae than phenthoate, a highly significant reduction in the number of pupae after treatment of third larval instar of *c. albiceps*

with the two selected compounds were observed. The number of emerged adults is greatly reduced following treatments with all concentrations and completely ceased following treatments of larvae with 200 and 250 ppm of phenthoate. Zhao *et al.* (2018) said that treatment of *Bradysia odoriphaga* by the median lethal concentration of chlorfenapyr reduces the population parameters. They added that consumption of food by larvae and pupal weight were significantly decreased compared with control. Khater (2021) recording LC₅₀, values for lufenuron and chlorfluazuron (146 and 194 ppm, respectively), lufenuron showed more toxic effects on the third larval instar of *C. albiceps* than chlorfluazuron, and at a lower concentration. Reduction in pupation percentage, complete cessation of adult emergence from pupae investigated.

Measurements of the biochemical aspects in organisms exposed to certain poisons are considered as an index to check turbulences that occurred in the organism. Biochemical parameters are valuable analytic tools to evaluate the influence of insecticides (Radwan *et al.*, 2008).

Physiological Disturbances:**Total Protein:**

No literature has been carried out to determine the biochemical aspects in *L. silvarum* following treatment with deltamethrin, abamectin and diazinon. In the current investigation, the total protein content increased significantly in *L. silvarum* larvae after treatment with all concentrations of diazinon. The increase in protein content with different diazinon concentrations may be attributed to the enhancement of protein biosynthesis by amino acids. This signified that both diazinon and 10, 20, 40 ppm deltamethrin promoted an increase in the incorporation of amino acids. Many workers obtained similar findings on other species of insects (Shakoori and Saleem 1991), attributing the greater protein synthesis in

Tribolium castaneum with insecticidal treatment to the synthesis of the proteinases needed for insecticide detoxification. This may be due to the conversion of carbohydrates and lipids to proteins. Kinnear *et al.* (1971) suggested that increased protein levels were due to increased synthesis of new proteins by the fat body, haemolymph and other tissues of the larvae. The increase in the total haemolymph protein may be a kind of detoxification mechanism (Wilkinson (1976) Abamectin application showed a highly significant reduction in total protein content. This may be due to the enhanced utilization of some amino acids as a source of energy. Similar literature are in harmony with these results. Total protein levels in *Culex fatigans* were decreased by λ -cyhalothrin (Yousuf *et al.*, 2014); Halawa *et al.* (2013) showed that the level of total proteins was decreased in *Bacterocera zonata* pupae resulted of larvae treated with Beticol, Biosad, Elsan, Lufox, Mani, Match and Radiant during all tested periods as compared to control. Total protein content in *Culex pipiens* declined while total lipids increased gradually with proceeded the generations (Gharib *et al.*, 2020).

Total Carbohydrate:

Carbohydrates play a major role in insect development like metabolism, metamorphosis, development of flight muscles, reproduction and embryonic development (Chapman, 1998). Significant reduction in total carbohydrate content of third larval instar of *L. silvarum* after treatment with the three selected insecticides at different concentration levels investigated compared with control experiment Except for 2.5 ppm abamectin. The result of this study is in line with findings of (Sak *et al.*, 2006) on females of *Pimpla turionellae*, an endoparasitoid fed on *Galleria mellonella* treated to various sublethal doses of cypermethrin presented to the host larvae's meal showed the most striking decrease in glycogen content whereas larvae were more

susceptible to cypermethrin than pupae and adults in terms of decrease in protein and lipid contents. On contrary, many studies recorded an increase in carbohydrate content, application of emamectin, spinetoram, hexaflumuron and teflubenzuron led to an increase in total carbohydrates in the 4th instar larvae of *S. littoralis* compared with control (Assar *et al.*, 2016).

Total lipid:

The observed results demonstrate that all treatments lead significant decrease in total lipid content. These observations are in harmony with (Assar *et al.*, 2016) on the 4th larval instar of *S. littoralis* with emamectin benzoate, spinetoram, hexaflumuron and teflubenzuron. Hill and Izatt (1974) attributed this reduction refer to the buildup of lipid is related to a lack of juvenile hormones. The utilization of tested insecticide does not act on corpora allata, the site of juvenile hormone secretion.

Enzymes Activity:

Enzymes are the biological catalysts that accelerate cellular chemical and metabolic processes (Bar-Even *et al.*, 2011). Detoxification enzymes in insects are important tools in defense mechanisms against toxins and xenobiotics. They play valuable roles in upholding the normal physiological status (Li and Liu, 2007). Four types of detoxifying enzymes have been found to respond against insecticides, or compounds exhibiting insecticidal activities. The detoxifying enzymes like general esterases, glutathione S-transferase and phosphatases were found to counter against insecticides activities (Zibae *et al.*, 2011). Induction of detoxification metabolic system plays an important role in insect's detoxification mechanism (Terriere, 1984).

-MFOs:

Mixed function oxidase activity (MFOs) provoked a highly significant increase with diazinon application after treatment with all concentrations except with

2.5 ppm. Meanwhile, the significant decrease in MFOs activity was measured following treatment with 2.5, 5, 10, 20 ppm abamectin. Last instar larvae of *Heliothis virescens* had the highest mixed-function oxidase activity at the post-feeding period (60–80 hr) with Methyl parathion, which was 28 times that of last instar larvae assayed during the first period (0–19 hr) (Hodgson, 1980). *Anopheles darlingi* mosquitoes from Amé-Beté in Colombia presented significantly increased levels of both mixed-function oxidase and nonspecific esterase to lambda-cyhalothrin and DDT. Increased metabolism through mixed-function oxidase and nonspecific esterase may be involved in cross-resistance between lambda-cyhalothrin and DDT, (Fonseca-González, *et al.*, 2009)

-AChE:

AChE is critical in the propagation of nerve impulses, which terminates nerve impulses in the synaptic cleft by catalyzing the hydrolysis of the major neurotransmitter, acetylcholine to acetate and choline. The AChE activity in this study showed a significant reduction in the third larval instar of *L. silvarum* after treatment with the different concentrations of the abamectin, deltamethrin and diazinon insecticides. The obtained results agree with those of Halawa *et al.* (2013) who reported a decrease in AChE enzyme of 2-day pupae of *B. zonata* post-treatment with biosad, elsan, lufox, mani, and match; Farag *et al.* (2017) illustrated that the activity of acetylcholine esterase decrease in treated *B. zonata* with biomectin and tracer; and (Assar *et al.*, 2016) in with *S. littoralis* larvae treated hexaflumuron and spinetoram; Acetylcholinesterase (AChE) activity promoted in *Culex fatigans* treated with lambda-cyhalothrin (Yousuf *et al.*, 2014). The maximum AChE activity in treated larvae of *H. armigera* was recorded in lambda-cyhalothrin and bifenthrin compared with control (Bilal *et al.*, 2018). Glavan *et al.* (2018) elucidated that the toxic effect of diazinon on the

honeybee is related to the effect on acetylcholine esterase.

-GST:

GST enzyme activity showed a unique trend represented in a significant decrease after treatment with 2.5 ppm of the three investigated compounds. While the highly significant increase was measured following the application of both 20 and 40 ppm of all tested insecticides, diazinon elicited the most enhanced effect. These results were following those reported by Muthusamy *et al.*, (2011) that GST activity was 2 times less in lambda-cyhalothrin treatment than dichlorvos. Results are in line with Gamil *et al.* (2011) findings, the GST activity increased on *S. littoralis* larvae after treatment with indoxacarb. Indoxacarb treatment leads to a reduction in GST activity in 4th instar larvae of *S. littoralis* and followed by pyridalyl and emamectin benzoate insecticides respectively compared with control (Abd-El-Aziz, 2014)

-ACP:

In insects, ACP and ALP are responsible for the cytolysis of tissues during insect development (Dadd, 1970; Spates and Wright, 1975). Also, they may act as hydrolases during the final stages of digestion (Cheung and Low, 1975). In general, these hydrolyzing enzymes are responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids in alkaline and acidic conditions, respectively under the name of dephosphorylation (Zibae *et al.*, 2011). The ACP activity in this investigation showed a very high significant reduction after treatment with all different concentrations of the three insecticides under investigation. The result showed that treatment with diazinon provoked the most inhibitory effect. Also, Nathan and Kalaivani (2005) investigated, a reduction in ACP activity led to a reduction in the liberation of phosphorus for energy metabolism and decrease metabolism. Also, decreasing ACP activity was recorded in *M.*

domestica by methoprene (Qureshi *et al.*, 1983), and by triflumuron (El- Hassanein *et al.*, 1996). The ACP enzyme activity in *Rhynchophorus ferrugineus* after 24-h treatment of early 5th instar larvae decreased in hemolymph and fat bodies after treatment with Spinetoram (Hamadah, 2019). Methoxyfenozide caused an insignificant decrease in the activity of acid phosphatase (ACP) and a significant reduction in the activity of alkaline phosphatase (ALP) in both ovaries and testes of *S. littoralis* adults as compared to adult controls (Sabry *et al.*, 2017).

-ALP:

Treatment of third larval instar of *L. silvarum* with the three selected insecticides elicited a clear inhibitory effect on ALP activity after 48 h. The results of this study are in line with the findings of Hamadah (2019) as ALP activity in the fat body of *R. ferrugineus* decreased after treatment with LC₅₀ of pyriproxyfen. On contrary, Pyriproxyfen (LC₅₀) stimulated both ACP and ALP in the fat body only. Also, ALP increased in *M. domestica* treated with some insect growth regulators (Assar *et al.*, 2010); *Schistocerca gregaria* by pyridalyl (Teleb *et al.*, 2012). Moreover, lufenuron and chlorfluazuron effect on enzyme activities in *S. littoralis* tissues (Abou-Taleb *et al.*, 2015) and biochemical components in the hemolymph and fat bodies of *Eurygaster integriceps* affected by pyriproxyfen (Zibae *et al.*, 2011). ALP activity decreased in haemolymph of 4th larval instar of *S. littoralis* treated with flufenoxuron (Abdel-Mageed *et al.*, 2018). ACP is more critical than ALP in some physiological processes in the larvae of *L. silvarum*, which may be due to the pH value. ACP and ALP play roles in the final steps of lipid digestion in insects (Klowden 2013). Therefore, the changes in the activity of these enzymes may be affecting the physiological process that depends on the

digestion and positive transportation of nutrients (Etebari *et al.*, 2007).

- α and β -esreases:

Alpha and beta-esterase activity showed high significant elevation after application with diazinon. The most promoting effect was reported at 40 ppm treatment. Increase and decrease in α - EST enzyme activity after treatment with both deltamethrin and abamectin. Deltamethrin has an inhibitory effect on β -esreases at different concentrations. The same reduction was obtained by (Bakr *et al.*, 2010) against 2nd and 4th larval instars of *S. littoralis* following flufenoxuron application. It is concluded that non-specific esterase plays an important role in the metabolites of insecticides.

It is interesting to note that the increased activity of several detoxification enzymes such as esterases has been shown to protect the insect from insecticide poisoning as a part of defence mechanism or added stress on enzyme expression system to synthesize new and higher amount of detoxification enzymes where could be the possible reasons for the arrested growth and mortality (Wheeler and Isman 2000). Increasing activity of esterase enzymes post-treatment and decreasing with a high dose may be due to the decrease of body weight defense against insecticide stress and indicating that esterases are likely involved in the metabolism of synthetic pyrethroids (Riskallah, 1983; Delorme *et al.*, 1988 and Muthusamy *et al.*, 2011). But the purified esterases hydrolyzed permethrin at a slow rate (Vontas *et al.*, 2001). In *S. littoralis* It is noticed that teflubenzuron and spinetoram significantly increased alpha esterases in contrast, decreased with hexaflumuron and emamectin benzoate. A highly significant decreased in beta esterases was induced by teflubenzuron and hexaflumuron and increased with spinetoram and emamectin benzoate (Assar *et al.*, 2016).

In conclusion: The repressive effects of the three tested insecticides induced some biological and physiological disturbances in the life of insect survived of treatments. These may be considered as control agents for the myiasis-producing fly *L. silvarum*. Also, they might be involved in integrated pest management (IPM) strategies.

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ARABIC SUMMARY

إضطرابات بيولوجية وفسيوولوجية في ذبابة اللوسيليا سيلفاروم, *Lucilia silvarum* Meigen, (ثنائية الأجنحة: كاليفوريدي) المعاملة ببعض المبيدات.

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1- قسم علم الحيوان كلية العلوم جامعة الزقازيق - مصر

2- قسم وقاية النبات كلية الزراعة جامعة الزقازيق - مصر

تعتبر ذبابة لوسيليا سيلفاروم التابعة لرتبة ثنائية الأجنحة، عائلة كاليفوريدي أحد الذباب الطبي الهام. تهدف الدراسة الي تقييم التأثير السام لكل من الألامكتين والدلتاميثرين والديازينون ضد طور البيضة واليرقات والطور البالغ لهذه الحشرة. علاوة على ذلك، تم دراسة الإضطرابات في بعض النواحي البيولوجية والفسيوولوجية. حيث عُرض البيض حديث الفقس، العمر اليرقي الثالث المبكر والطور البالغ عمر يوم لتركيزات مختلفة من المبيدات المختبرة، ثم تم متابعة بعض النواحي البيولوجية للأفراد الحية المتبقية من المعاملة بالتركيزات المختبرة مثل فقس البيض، الموت اليرقي، نسبة التعذر، نسبة خروج الطور البالغ والنسبة الجنسية، كما تم تقييم التقدير الطيفي للبروتينات الكلية، الكربوهيدرات، الدهون ونشاط بعض الأنزيمات في يرقات العمر اليرقي الثالث الحية المتبقية من المعاملة بالتركيزات المختبرة. أوضحت النتائج أن الألامكتين أكثر سمية ضد العمر اليرقي الثالث، بينما الدلتاميثرين سبب التأثير الأكثر سمية ضد طور البيضة والطور البالغ. كما وجد أن الموت في طور البيضة، اليرقات والطور البالغ يزداد بزيادة التركيز مع الوقت. ولوحظ أيضاً موت اليرقات والطور البالغ بصورة عالية المعنوية بعد 48 ساعة من المعاملة. كما انخفضت نسبة التعذر بعد معاملة الطور اليرقي الثالث بالمبيدات المختبرة. ولوحظ إنخفاض عالي المعنوية في متوسط عدد الذكور الناتجة بعد المعاملة بكل التركيزات المستخدمة من الثلاث مبيدات المختبرة. وقد أدت المعاملة بالتركيزات المختلفة إلى حدوث خلل فسيولوجي في كلا من محتوى البروتين الكلي، الكربوهيدرات والدهون في اليرقات الحية المتبقية من المعاملة. علاوة على ذلك حدث تغير في أنزيمات إزالة السمية مثل نشاط أنزيمات الأكسيديز متعدد الوظائف، الأسيتيل كولين إستريز، الفوسفاتيز الحامضي والقاعدي، الجلوتاثيون-أس-ترانسفيريز والألفا والبيتا إستريز. نستنتج من ذلك أن هذه المبيدات الثلاث المختبرة موضع الدراسة والتي تسببت في حدوث خلل بيولوجي وفسيوولوجي في حشرة اللوسيليا سيلفاروم المسببة للتدويد تعد أحد عناصر مكافحتها.