### CONTENTS

	Page
	No.
INTRODUCTION	1
Aim of the work	4
II-REVIEW OF LITERATURE	5
2.1. Effect of pesticides on target and non-target organisms	5
2.1.1. Pyrethroid insecticides	5
2.1.2. Avermectin insecticides	6
2.1.3. Neonicotinoid insecticides	7
2.2. Toxicity of pesticides against target and non-target organisms	8
2.2.1. Toxicity of pesticides against target organisms (Spodoptera	9
littoralis)	
2.2.2. Toxicity of pesticides against non-target organisms (Apis	17
2.2 Effect of posticides on A ChE estivity	27
2.5. Effect of pesticides of ACHE activity	27
2.4. Antioxidant enzymes in insects	30
111-MATERIALS AND METHODS	<u> </u>
3.1. Tested Insecucides	27
3.2. Chemicals	27
3.3. Test organisms	31
3.3.1. Cotton lealworm ( <i>Spoaoptera littoralis</i> )	37
3.3.2. Honeybee ( <i>Apis mellifera</i> )	39
3.4. Bloassay Studies.	40
3.4.1. Bloassay studies for cotton learworm, <i>Spodoptera littoralis</i>	40
3.4.2. Bloassay studies for honeybees, <i>Apis mellifera</i>	40
3.5. Toxicity index of <i>Spodoptera littoralis</i> and <i>Apis mellifera</i>	42
3.6. Biochemical parameters.	42
3.6.1. Determination of acetylcholinesterase (AChE)	42
3.6.1.1. Reagents for acetylecholinesterase activity	42
3.6.1.2. Acetylecholinesterase of <i>Spodoptera littoralis</i>	42
3.6.1.3. Acetylecholinesterase of <i>Apis mellifera</i>	43
3.6.2. Protein determination	44
3.6.3. Determination of antioxidant enzymes	45
3.6.3.1. Determination of catalase (CAT) in both <i>S. littoralis</i> and <i>A. mallifera</i>	45
2.6.2.2 Determination of alutathiona a transforaça in both littaralia	
and Apis mellifera	47
3.6.3.3. Determination of glutathione peroxidase (GP <sub>x</sub> ) in both	
Spodoptera littoralis and Apis mellifera	49
. 3.6.4. Determination of lipid peroxidation (LPO) in both Spodoptera	51
littoralis and Apis mellifera	51

IV- RESULTS & DISCUSION		
4.1. Acute toxicity bioassay	53	
4.1.1. Insecticidal activity of $\alpha$ -cypermethrin, emamectin benzoate,	52	
and imidacloprid against Spodoptera littoralis	55	
4.1.2. Insecticidal activity of $\alpha$ -cypermethrin, emamectin benzoate,	67	
and imidacloprid against Apis mellifera	07	
4.2. Comparative toxicity of tested insecticides against cotton		
leafworm (Spodoptera littoralis) and honeybee workers (Apis	77	
mellifera)		
4.3. Biochemical studies	82	
4.3.1. Effect of tested insecticides on acetylcholinesterase (AChE)		
activity of Spodoptera littoralis and Apis mellifera	02	
4.3.2. Effect of tested insecticides on antioxidant enzymes activities		
and Malondialdehyde levels in Spodoptera littoralis and Apis	89	
mellifera		
V- CONCLUSION & RECOMMENDATION	110	
VI- SUMMARY	111	
VII-REFERENCES	114	
ARABIC SUMMARY		

### **LIST OF TABLES**

<u>Table</u>	<u>Title</u>	<u>Page</u> <u>No.</u>
1	Scientific classification of Spodoptera littoralis	38
2	Scientific classification of Apis. mellifera	39
3	Reagents of determination of catalase (CAT) enzyme in both <i>S. littoralis</i> and <i>A. mellifera</i>	46
4	Procedure of determination of catalase (CAT) enzyme in both <i>S. littoralis</i> and <i>A. mellifera</i>	46
5	Procedure of determination of glutathione- <i>s</i> -transterase (GST) in both <i>S. littoralis</i> and <i>A. mellifera</i>	48
6	Reagents of determination of glutathione peroxidase (GP <sub>x</sub> ) in both <i>S. littoralis</i> and <i>A. mellifera</i>	50
7	Procedure of determination of glutathione peroxidase $(GP_x)$ on both <i>S. littoralis</i> and <i>A. mellifera</i>	50
8	Reagents of determination of lipid peroxidation (LPO) in both <i>S. littoralis</i> and <i>A. mellifera</i>	51
9	Procedure of determination of lipid peroxidation (LPO) on both <i>S. littoralis</i> and <i>A. mellifera</i>	52
10	Comparative toxicity as a speed of action (initial effect) of tested insecticides against 2 <sup>nd</sup> instar larvae of <i>S. littoralis</i> after 6, 12 and 24 hr of treatment	54
11	Comparative toxicity as a speed of action (initial effect) of tested insecticides against 4 <sup>th</sup> instar larvae of <i>S. littoralis</i> after 6, 12 and 24 hr of treatment	57
12	Comparative toxicity as residual (long acting effect) of tested insecticides against 2 <sup>nd</sup> instar larvae of <i>S. littoralis</i> after 48, 72 and 96 hr of treatment.	60
13	Comparative toxicity as residual (long acting effect) of tested insecticides against 4 <sup>th</sup> instar larvae of <i>S. littoralis</i> after 48, 72 and 96hr of treatment.	63
14	Comparative toxicity as a speed of action (initial effect) of tested insecticides against <i>A. mellifera</i> after 6, 12 and 24 hr of treatment.	69
15	Comparative toxicity as residual (long acting effect) of tested insecticides against <i>A. mellifera</i> after 48, 72 and 96 hr of	72

	treatment	
16	Relative potency (Toxicity Index) $(T.I^*)$ of tested insecticides to $2^{nd}$ and $4^{th}$ instars larvae of <i>S. littoralis</i> and <i>A. mellifera</i> at 6, 12 and 48 hr of treatment	78
17	Relative potency (Toxicity Index) $(T.I^*)$ of tested insecticides to $2^{nd}$ and $4^{th}$ instars larvae of <i>S. littoralis</i> and <i>A. mellifera</i> at 48, 72 and 96 hr of treatment	79
18	Comparison between mean $LC_{50}$ values of tested insecticides at first three times, as an initial effect on $2^{nd}$ and $4^{th}$ instars larvae of <i>S. littoralis</i> and <i>A. mellifera</i>	80
19	Comparison between mean $LC_{50}$ values of tested insecticides at last three times, as long acting effect on $2^{nd}$ and $4^{th}$ instars larvae of <i>S. littoralis</i> and <i>A. mellifera</i>	81
20	In vivo effects of $\alpha$ -cypermethrin on acetylcholinesterase activity (AChE) in both 4 <sup>th</sup> instar larvae of <i>S. littoralis</i> and <i>A. mellifera</i>	84
21	<i>In vivo</i> effects of emamectin benzoate on acetylecholinesterase activity (AChE) in both 4 <sup>th</sup> instar larvae of <i>S.littoralis</i> and <i>A. mellifera</i>	85
22	<i>In vivo</i> effects of imidacloprid on acetylecholinesterase activity (AChE) in both 4 <sup>th</sup> instar larvae of <i>S.littoralis</i> and <i>A. mellifera</i>	86
23	<i>In vivo</i> effects of α-cypermethrin on catalase (CAT) activity in both 4 <sup>th</sup> instar larvae of <i>S.littoralis</i> and <i>A. mellifera</i>	92
24	<i>In vivo</i> effects of emamectin benzoate on catalase (CAT) activity in both 4 <sup>th</sup> instar larvae of <i>S.littoralis</i> and <i>A. mellifera</i>	93
25	<i>In vivo</i> effects of imidacloprid on catalase (CAT) activity in both 4 <sup>th</sup> instars larvae of <i>S.littoralis</i> and <i>A. mellifera</i>	94
26	In vivo effects of $\alpha$ -cypermethrin on glutathione- <i>s</i> -transferase (GST) activity in both 4 <sup>th</sup> instar larvae of <i>S</i> . <i>littoralis</i> and <i>A</i> . <i>mellifera</i> .	96
27	<i>In vivo</i> effects of emamectin benzoate on glutathione- <i>s</i> -transferase (GST) activity in both 4 <sup>th</sup> instar larvae of <i>S.littoralis</i> and <i>A. mellifera</i>	97
28	<i>In vivo</i> effects of imidacloprid on glutathione- <i>s</i> -transferase (GST) activity in both 4 <sup>th</sup> instar larvae of <i>S.littoralis</i> and <i>A. mellifera</i>	98
29	In vivo effects of $\alpha$ -cypermethrin on glutathione peroxidase (GP <sub>X</sub> ) activity in both 4 <sup>th</sup> instar larvae of <i>S.littoralis</i> and <i>A. mellifera</i>	100
30	<i>In vivo</i> effects emamectin benzoate on glutathione peroxidase $(GP_X)$ activity in both 4 <sup>th</sup> instar larvae of <i>S.littoralis</i> and <i>A</i> .	101

	mellifera	
31	In vivo effects imidacloprid on glutathione peroxidase $(GP_x)$ activity in both 4 <sup>th</sup> instars larvae of <i>S.littoralis</i> and <i>A. mellifera</i>	102
32	In vivo effects of $\alpha$ -cypermethrin on Malondialdehyde level (MDA) in both 4 <sup>th</sup> instar larvae of <i>S.littoralis</i> and <i>A. mellifera</i>	104
33	<i>In vivo</i> effects of emamectin benzoate on Malondialdehyde v level (MDA) in both 4 <sup>th</sup> instar larvae of <i>S.littoralis</i> and <i>A. mellifera</i>	105
34	<i>In vivo</i> effects of imidacloprid on Malondialdehyde level (MDA) in both 4 <sup>th</sup> instar larvae of <i>S.littoralis</i> and <i>A. mellifera</i>	106

### **VI- SUMMARY**

Pesticides are of great importance in the field of agriculture and will continue during the current period and in the future because they are still of the most important means of pest control. Cotton leafworm, *Spodoptera littoralis* is one of the most important pests which cause a lot of damage to the cotton crop.

When sprayed, pesticides can cause unintended harm by killing beneficial insects such as pollinators and the natural enemies of the crop's pests.

The poisoning of honeybees (*Apis mellifera*) and other beneficial insects by pesticides can be a serious problem. Honeybees provide a valuable service to agriculture because they are the most important pollinators of cultivated crops. They also produce honey, royal jelly, and bee wax. In addition, its morphological and ethological features of the honeybee can be considered an excellent bio-indicator for environmental contamination.

Therefore, toxicity of insecticides against the beneficial insects is important as well as toxicity of insecticides against harmful insects. Therefore, this investigation aims to:

- Show the effect of three insecticides {α-cypermethrin, emamectin benzoate, and imidacloprid} on the 2<sup>nd</sup> and 4<sup>th</sup> instars larvae of cotton leafworm (*S. lttoralis*) and honeybee (*A. mellifera*);
- 2. determine the effects of the tested insecticides on the activities of some *S. lttoralis* and *A. mellifera* enzymes these were: catalase (CAT), glutatione-*s*-transferase (GST) and glutathione peroxidase (GP<sub>x</sub>); and
- 3. estimate the accumulated lipid peroxidation in the tissue by evaluating the level of Malondialdehyde (MDA).

#### • The obtained data could be summarized as follows:

# (1) Insecticidal activity of α-cypermethrin, emamectin benzoate and imidacloprid against *S. littoralis* and *A. mellifera*:-

#### a. In case of S. littoralis:

It is evident from LC<sub>50</sub> values of the tested compounds that emamectin benzoate was the most toxic to 2<sup>nd</sup> and 4<sup>th</sup> instars larvae of *S. lttoralis* with the LC<sub>50</sub> values of (1.163, 5.788ppm); (0.465, 1.678ppm); (0.311, 0.381ppm); (0.052, 0.161ppm); (0.0057, 0.0310ppm); and (0.0013, 0.0044ppm) after 6, 12, 24, 48, 72, 96hrs exposure time, respectively, followed in decreasing order of toxicity to  $\alpha$ -cypermethrin with the LC<sub>50</sub> values of (46.6, 57.1ppm); (0.86, 1.93ppm); (0.47, 0.557ppm); (0.061; 0.186ppm); (0.042, 0.128ppm); and (0.030, 0.101ppm) after 6, 12, 24, 48, 72, and 96hrs exposure time, respectively, and finally to imidacloprid with the mean LC<sub>50</sub> values of ( $\geq$ 35×10<sup>3</sup>,  $\geq$ 2098×10<sup>3</sup>); ( $\geq$ 35×10<sup>3</sup>, 2098×10<sup>3</sup>, 2098×10<sup>3</sup>, 2098×10<sup>3</sup>ppm); (35×10<sup>3</sup>, 501×10<sup>3</sup>ppm); (104×10<sup>2</sup>,

 $89 \times 10^3$  ppm); ( $100 \times 10^2$ ,  $32 \times 10^3$  ppm) and ( $83 \times 10^2$ ,  $15 \times 10^3$  ppm) for  $2^{nd}$  and  $4^{th}$  larval instars after 6, 12, 24, 48, 72, and 96 hrs time exposure, respectively.

The toxicities of the tested insecticides against *S. lttoralis* were increased as the larval stage decreased.

#### b. In case of A. mellifera:

It is evident from LC<sub>50</sub> values of the tested insecticides that emamectin benzoate was the most toxic to honeybee workers with the mean LC<sub>50</sub> values of 0.399, 0.320, 0.275, 0. 203, 0.193, and 0.184ppm after 6, 12, 24, 48, 72, and 96hrs time exposure, respectively, followed in decreasing order of toxicity to  $\alpha$ -cypermethrin with the LC<sub>50</sub> values of 13.129, 10.878, 5.411, 4.369, 3.861, and 3.267ppm after 6, 12, 24, 48, 72, and 96hrs time exposure, respectively, and finally imidacloprid was the least toxic insecticide with the LC<sub>50</sub> values of 65.33, 37.8, 24.97, 17.29, 14.55, and 10.98ppm after 6, 12, 24, 48, 72, and 96hrs time exposure, respectively.

It is evident from  $LC_{50}$  values that, the toxicities of the tested insecticides against *S*. *lttoralis* and *A. mellifera* were increased as the exposure time increased.

According to toxicity index, the tested insecticides were arranged in decreasing order based on LC<sub>50</sub> values to emamectin benzoate >  $\alpha$ -cypermethrin > imidacloprid.

#### (2) Biochemical studies:

# (1) Effects of tested insecticides on acetylcholinesterase (AChE) activity of *S. lttoralis* and *A. mellifera*:

#### a. AChE of S. littoralis:

The *in vivo* effects of the tested insecticides on AChE activity isolated from *S. lttoralis* 4<sup>th</sup> instars larvae head capsules revealed that imidacloprid at the concentrations of  $125 \times 10^3$ ,  $251 \times 10^3$  and  $501 \times 10^3$ ppm caused 11.2, 16.4, and 26.2% inhibition, subsequently. Also,  $\alpha$ -cypermethrin caused inhibition percentages of 4.2, 6.1, and 8.9% when it was tested at the concentrations of 0.138, 0.276, and 0.552ppm, respectively. On the other hand, emamectin benzoate at the concentrations of 0.095, 0.191, and 0.381ppm caused 56.1, 65.9, and 77.6% activation of *S. lttoralis* AChE activity, respectively.

#### b. AChE of A. mellifera:

The *in vivo* effects of the tested insecticides on AChE activity isolated from the head of surviving honeybee workers (*A. mellifera*) revealed that imidacloprid at the concentrations of 6.2, 12.5, and 24.97ppm caused 6.9, 16.5, and 23.4% inhibition, respectively. Also,  $\alpha$ -cypermethrin caused inhibition percentages of 2.1, 5.1, and 7.5% when it was tested at the concentrations of 1.6, 2.7, and 5.4ppm, subsequently. On the other hand, emamectin benzoate at the concentrations of 0.07, 0.14, and 0.275ppm caused 60, 73.2, 82.8% activation of *A. mellifera* AChE activity, respectively.

The inhibition and activation of *S. lttoralis* and *A. mellifera* AChE activity using imidacloprid,  $\alpha$ -cypermethrin, and emamectin benzoate concentration dependent.

AChE enzyme was effective as a biomarker of exposure of emamectin benzoate insecticides.

# (2) Effects of tested insecticides on antioxidant enzymes activities of *S. lttoralis* and *A. mellifera*:

The *in vivo* effects of the tested insecticides on antioxidant enzymes activities of *S*. *lttoralis* and *A. mellifera* revealed that there was an increase in the catalase (CAT), glutatione-*s*-transferase (GST) and glutathione peroxidase (GP<sub>x</sub>) activities depend on the concentration of insecticides used compared to the control.

#### (3) Effects of tested insecticides on Malondialdehyde level (MDA):

There was a significant decrease in the level of Malondialdehyde (MDA) (LPO indicator) in the case of  $\alpha$ -cypermethrin and imidacloprid on both insects. On the other hand, emamectin benzoate caused slight elevation in the level of MDA in *S. lttoralis* and *A. mellifera* compared to the control.

Obiviously, the treatment of *S. lttoralis* and *A. mellifera* with  $\alpha$ -cypermethrin and imidacloprid in the present study represent a model for reduction of lipid peroxidation and an enhancement of the insect antioxidant system for scavenging ROS resulted due to oxidative stress.

Therefore, this profile of biomarker variation could represent a useful fingerprint to characterize exposure to different groups of insecticides.

Also, the battery of honeybee biomarkers might be a promising option of biomonitor the health of aerial and terrestrial ecosystems and to generate valuable information on the mode of action of pesticides.

The use of biomarker is the promising approach in the assessment of ecosystem health. It might allow the detection of early biological change, which may result in long-term physiological disturbances.