Global Veterinaria 4 (1): 22-33, 2010
ISSN 1992-6197

Biochemical Studies on *Culex pipiens* (L.) (Diptera: Culicidae) Exposed to *Allium satvium, Citrus limon* and *Bacillus thuringiensis israelensis* with Reference to Assessment of the Biosafety on Albino Mice

[1]Rokaya M.A. Saeed, [2]A.A. Zayed, [2]Amira H. El Namaky, [1]Hanan M. Ismail and [1]Heba Y. Mady

[1]Department of Zoology, Women's College for Arts,
Science and Education, Ain Shams University, Cairo, Egypt
[2]Department of Parasitology and Animal Diseases, National Research Center, Dokki, Egypt

Abstract: The present work studied the electrophoretic banding pattern of untreated and treated *Culex pipiens* larvae with plant oil extracts (*Allium satvium, Citrus limon*) and *Bacillus thuringiensis israelensis* and assessment the biosafety of these extracts and bacteria to non-target-organism were also undertaken. A total of 13 protein bands were detected in the untreated larvae. However, after exposure to 10 µl garlic oil extracts, at 12 hrs, the larval SDS dissociated protein was separated into 18 protein bands with 2 shared common protein bands. At 24 hrs. post exposure, there were 17 protein bands were detected showing common 3 protein bands shared with the control. Moreover, at 48 hrs, the numbers of bands recorded were 15 protein bands with 3 shared common protein bands with control. Among treatment with lemon oil, at 12, 24 and 48 hrs. post exposure, the larval SDS dissociated proteins were separated into 9,7,8 protein bands, respectively. These bands included three shared common protein bands with control.While after exposure to 5 µl of *Bacillus thuringiensis israelensis*. The total number of protein bands, at 30, 90 and 150 minutes was 8, 9 and 9 bands, respectively. There were 4, 5 and 7 shared protein bands with control, respectively. Protein pattern of treated fourth instar *Culex pipiens* larvae differ not only between various dosages but also between various time intervals at the same dosage. Intraperitonealy injection of BALB /c strain albino mice with two types of plant oil extracts and *Bacillus thuringiensis israelensis* caused no marked effect on serum alanine transaminase and asparatate transaminase, cholesterol, triglycerides, total protein, bilirubin, albumin and globulin. It was concluded that the use of plant oil extracts (*Allium satvium, Citrus limon*) and *Bacillus thuringiensis israelensis* have great effect on the total protein of *Culex pipiens* larvae with no effect on serum of mice, suggesting that they may be a new promising controlling agents.

Key words: *C. pipiens Citrus limon* · *Allium satvium* · *Bacillus thuringiensis israelensis* · SDS-PAGE

INTRODUCTION

Conventional arthropods control strategy involves application of broad spectrum chemical and pesticides that often produce undesirable effects. The world health organization (WHO) reported that 2, 200 million people in 90 countries were annually exposed to malaria infection and causing an estimated 1.4 - 2.6 million deaths, with more than 90% in African countries alone [1]. This stimulated the search of new eco-friendly vector control tools. Also, most mosquito control programs aimed to deal with larval stages in their breeding sites [2,3]. However, control of malaria and other mosquito borne diseases is becoming increasingly difficult because the effectiveness of vector control has declined due to development of mosquitoes resistance against currently used insecticides. Moreover, potential hazard uses of these chemical insecticides have been associated with toxic residual effects that usually produce environmental pollution [4, 5]. Much effort has been focused on plant extracts or photochemical as potential sources of commercial mosquito-control agents or as lead compounds [6, 7]. Furthermore, plant oil extracts considered as environmentally safe, less hazardous to non-target biota, simple, inexpensive and can be applied effectively by using techniques more suitable for

Corresponding Author: Dr. Ahmed A. Zayed, Department of Parasitology and Animal Diseases, National Research Center, Dokki, Egypt

developing countries [8-15]. Massoud *et al.* [16] determined the biochemical changes in *C. pipiens* (L.) treated with oil and Oleo-resin extracts of *Myrrh commiphora molmol* revealed inhibitory action on the protein contents and loss of certain enzymes which affect the metabolic processes. Lerdthusnee and Chareonviriyaphap [17] compared isoenzyme patterns of 13 fields-collected populations of *A. aegypti* by using starch gel electrophoresis. Three populations were collected before *Bacillus thuringiensis israelenesis (*Bti) application and 10 populations were collected after the Bti treatment. The results revealed that the number of polymorphic loci were lower in Bti treated populations as compared to controls. These results were most likely due to a genetic bottleneck produced by Bti treatment. Heterozygosis was increased in the month following Bti treatment. This probably due to immigration where the control programmed was withdrawn. The present study was carried out to monitor the total protein of *C.pipiens* (L.) treated with *C. limon*, *A. satvium*,Bti. and assessment the biosafety of these extracts and bacteria to non-target-organism were also undertaken.

MATERIAL AND METHODS

Insect: Fourth instars C. *pipiens* (L.) (Culicidae) were selected as the target insect for the present study (Fig. 1a). The fourth instars larvae were collected from nearby untreated sites in and around Cairo. These larvae were maintained for many generations as described by Hafez [18].

Plant Oil Extracts: Two essential oil plants were used in the present study, *A. sativum* (garlic oil extract) and *C. limon* (lemon oil extract). They were obtained from El-Gomhouria Company, Cairo, Egypt.

Tested Bacteria: The entomopathogenic bacteria *Bacillus thuringenesis israelensis* (Bti) in the form of liquid concentration obtained from Chema industries. Chema @ Chema. Com. eg.

Biochemical Study: For determination of biochemical changes, 60 alive 4th instars of *C. pipiens* (L.) were treated with the lethal doses (10 µl) of plant oil extracts and (5 µl) Bti at 12, 24 and 48 hrs and 30, 60 and 150 min post-exposure, respectively. Similar untreated control larval groups were used. The survived 4th instar *C. pipiens* (L.) were collected for characterization of total protein by 10 % sodium dodecyle-sulphate polyacrylamide gel electrophoresis technique (SDS-PAGE) [19].

Biosafety of Plant Oil Extracts (*A. sativum*, *C. limon*) and Entomopathogenic Bacteria (Bti) to Non-Target Organism: The aim of this experiment was to study the side effect of the plant oil extracts and Bti in albino mice. The animals used throughout this study were male BALB / c strain albino mice. The mice were divided into four groups: the 1st group was injected intraperitonealy with l0µl of *A. sativum* to *C. pipens* lethal dose., the 2nd group was injected intraperitonealy with l0 µl *C. limon* (lethal dose of *C. limon* to *C. pipens*), the 3rd group injected intraperitonealy with 5 µl of Bti (lethal dose for *C. pipiens*)

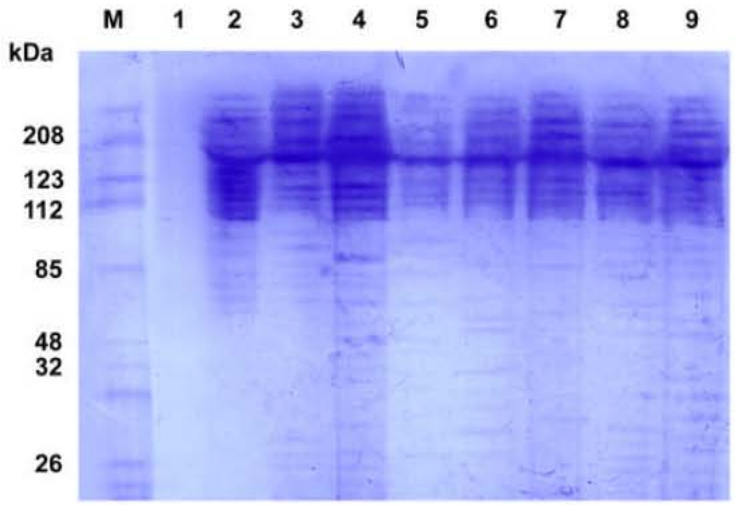


Fig. 1: 10% SDS-PAGE electrophoretic profile for total protein of 4th instar *C. pipiens* (L.) before and post-exposure to plant oil extract and Bti

and non-inoculated control mice (4th group). five mice per each group were used and each group were used at four times. Blood samples were collected after 24, 48 and 72 hrs and the sera were separate at once. Samples were centrifuged at 3000 rpm for 15 min. The serum was stored at 20°C until used for biochemical analysis. Serum alanine transminase (ALT) and asparatate transaminase (AST) activities were determined as method of Reitmam and Frankel [20] using Bio-Merieux kits. Serum total cholesterol level was estimated according to the method of Allain *et al.* [21] using Sentinel Ch. kits. Serum triglyceride level was estimated as technique mentioned by Mc-Gowan *et al.* [22] using Sentinel Ch. Kits. Total Protein in serum was determined using the method of Josephson and Gyllensward [23] and Biocon kits. Total bilirubin in serum was estimated as described by Malloy [24] using quantitative determination of bilirubin IVD. A serum albumin and globulins level was estimated according to method of Douman *et al.* [25].

Statatistical Analysis: The data were computed using analysis of variance procedure (ANOVA) and the significant mean differences between treatment were separated by Duncan`s multiple range test procedure. Values were considered significant when different at $P<0.05$.

RESULTS

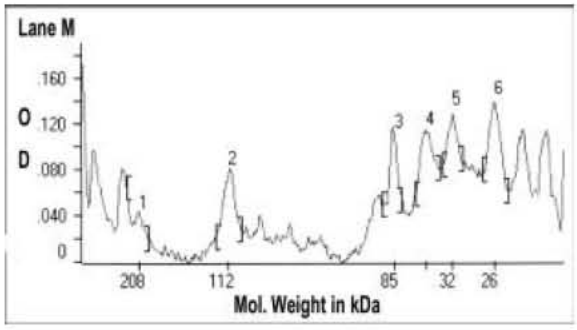
Characterization of Treated Larval Proteins of 4th Instar *C. pipiens* (L.) Using 10% SDS-PAGE

Characterization of Larval Proteins Treated with *A. Sativum*: SDS-PAGE detected 18 protein bands in larvae treated with 10 µl /25L garlic oil extract at 12 hrs post-exposure. The molecular weights of these bands varied from 25.029 - 144.28 kDa. However, only 13 bands were detected in control non-treated larvae having variable higher molecular weights of 22.043 - 284.68 kDa (Figs. 1, 2). It was found that there were two shared common protein bands with molecular weights of 103.34 and 56.602 kDa at three different hours. The band densities were found to decrease as the time of oil extract exposure increased. There were 17 protein bands with molecular weights of 22.639 -280.81 kDa at 24 hrs post-exposure. These bands recorded three shared bands of 103.7, 56.816 and 22.639 kDa with control non-treated larvae. At 48 hrs post-exposure, 15 bands were detected with molecular weights of 22.405 - 280.81 kDa. Other three shared bands were also recorded at 48 hrs post-exposure with molecular weights of 103.77, 56.816 and 22.405 kDa (Table 1, Fig. 3). The denestomorphic electrophotogram (Fig. 3) recorded that the band number 7, 8 and 10 detected at 48 hrs post-exposure had the higher densities reached 2.8, 3.6 and 6.3%, respectively (Table 1). Mean while, at 12 hrs. post-exposure, band number 15, 16 and 18 showed to have the higher densities of 6.9, 9 and 5.4 %, respectively. However, only 2 bands were showed to have higher densities of 12.4 and 7.18 % at 24 hrs. post-exposure.

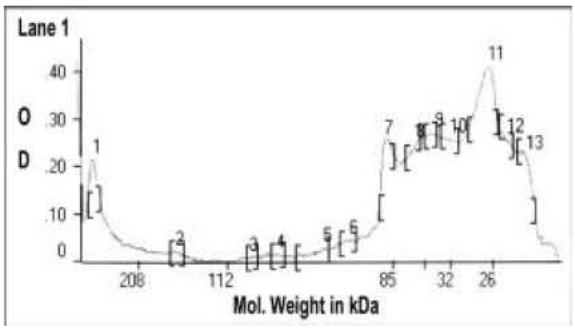
Characterization of Larval Proteins Treated with *C. limon*: SDS PAGE detected only 9 protein bands in protein of larvae treated with10 µl/25L. *C. limon* oil extract at 12 hrs post-exposure. The molecular weights ranged from 25.87 - 103.77 kDa. However, 13 protein bands with molecular weights of 22.0 - 284.68 were separated in proteins of control non-treated larvae. There were three shared common bands having molecular weights of 103.77, 91.74 and 56.81 kDa. It was found that the number of bands was decreased as the exposure times increased. There were 7 and 8 bands at 24 and 48 hrs post-exposure, respectively (Table 5). Two shared common bands with molecular weights of 56.81 and 22.48 were found at 24 hrs. post-exposure compared with the corresponding bands of control non-treated larval proteins (Fig. 1). The denestomorphic electrophotogram recorded that the band number 22 at 12 hrs post- exposure had the higher densities reached 52.18 % (Table 2, Fig. 4). However, at 24 and 48 hrs post-exposures, the band number 20 showed to have the higher densities of 10.9 and 8 %, respectively.

Characterization of Larval Proteins Treated with Bti: SDS PAGE detected variable protein bands in larvae treated with 5 µL/25L Bti bacteria. These bands reached 8, 9 and 9 bands with molecular weights ranged from 22.6 - 103.19, 22.63- 284.68 and 22.04 -284.68 kDa at 30, 90 and 150 min post-exposure, respectively (Table 3, Fig. 5). It was observed that there were four common protein bands shared with the control at 30 min post-exposure. In addition there were 5 and 7 common bands shared with control (non-treated larval proteins) at 90 and 150 min., respectively. The highest band density with 15.23 % was found at 150 min. post-exposure.

Biosafety of *A. sativum*, *C. limon* and Bti Intraperitonally Inoculated to BLAB/c Strain Mice: The levels of Serum alanine transminase (ALT) and asparatate transaminase (AST) showed slight variation in mice serum at different times post inoculation (Table 4). It reached 186, 183 and

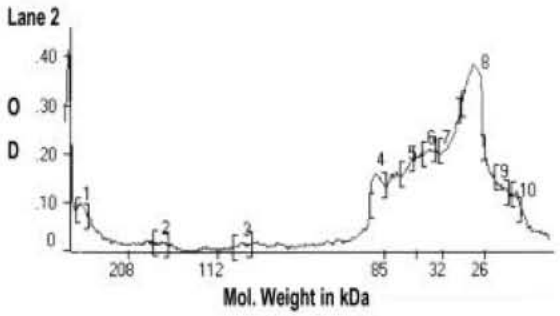


Lane M: Represent molecular weight marker

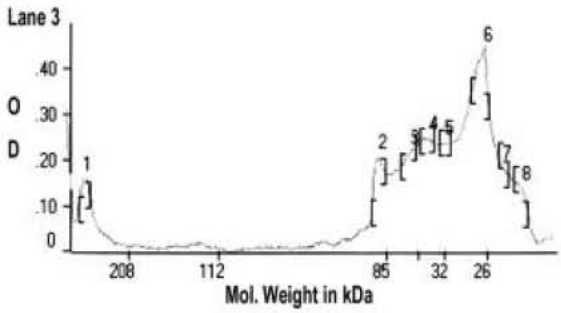


Lane (1): Represent un-treated non-infected 4th instar of *C. pipiens*(L.).

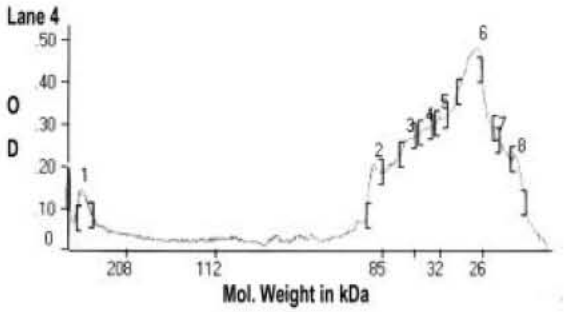
Fig. 2: Gel - Pro Analyzer



Lane (2): At 12 hrs post-exposure.

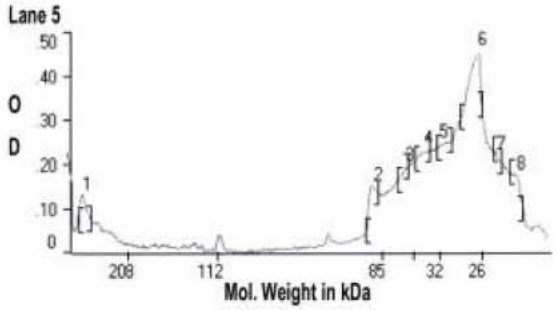


Lane (3): At 24 hrs post-exposure.

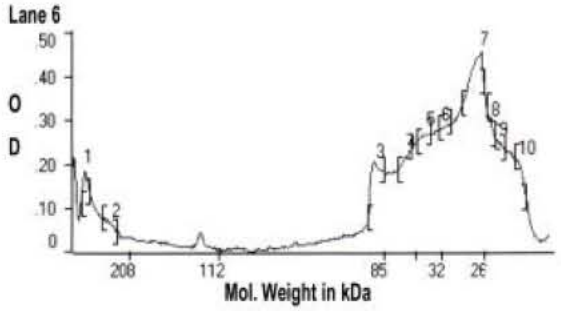


Lane (4): At 48 hrs. post-exposure.

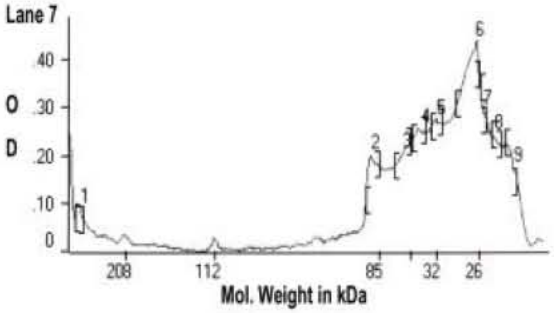
Fig. 3: Lanes (2, 3 and 4): Represent gel- pro-analyzer of 4th instars *C. pipiens* (L.) after exposure to *A. sativum*



Lane (5): At 12 hrs. post-exposure.

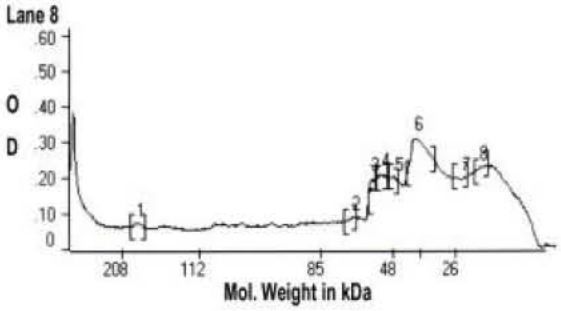


Lane (6): At 24 hrs. post-exposure.

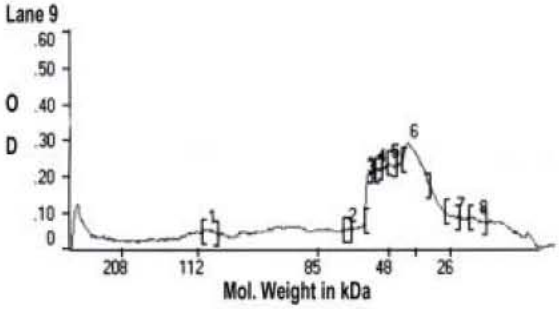


Lane (7): At 48 hrs. post-exposure.

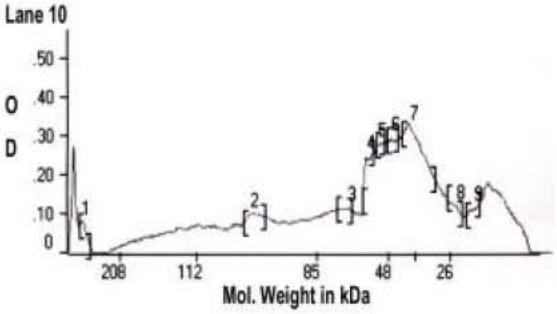
Fig. 4: Lanes (5, 6 and 7): Represent gel- pro-analyzer of 4th instars *C. pipiens* (L.) after exposure to *C. limon*



Lane (8): At 30 min. post-exposure.



Lane (9): At 90 min. post-exposure.



Lane (10): At 150 min. post-exposure.

Fig. 5: Lanes (8, 9 and 10): Represented gel- pro-analyzer of 4th instar *C. pipiens* (L.) after exposure to Bti.

Table 1: Electrophoretic total protein profile of 4th instar *C. pipiens* (L.) after exposure to 10 µl *A. sativum* (garlic oil extract)

	Intervals in hrs.							
	Control		12 hrs.		24 hrs.		48 hrs.	
Band No.	Mol-Wt	Conc. %	Mol-Wt	Conc. %	Mol-Wt	Conc. %	Mol-Wt	Conc.%
1	284.68	4.44			280.81	4.111	280.81	5.1682
2					229.53	1.778		
3								
4	160.71	0.524	144.28	0.991				
5			127.72	0.8134	123.01	1.0718		
6	107.98	0.1957	117.38	0.826				
7	*103.34	0.379	109.19	1.2505	109.19	0.9734	109.19	2.843
8	95.553	1.0722	*103.77	0.904	*103.7	0.6138	*103.77	3.568
9	91.45	1.393	101.16	1.215	101.98	0.848	101.98	1.695
10	86.162	6.418	98.88	1.74	98.886	1.123	98.886	6.297
11	*56.602	6.363	94.73	2.028				
12	41.13	5.659	91.111	2.48				
13	32	7.3001	88.603	2.111	90.4	1.487	90.159	2.21
14	26.56	18.937	76.319	2.883	76.67	1.4914	78.49	1.584
15			66.004	5.975	66.004	1.72511	66.004	2.561
16	24.163	9.805	*56.816	9.015	*56.816	2.976	*56.816	2.2014
17	*22.043	8.559	51.978	7.6914	51.978	3.204	51.978	0.869
18			46.015	5.404	46.015	2.335	46.015	0.704
19			39.86	6.0361	40.539	3.015	40.883	0.496
20			34.52	11.435	34.528	12.418	34.528	5.635
21			25.029	5.0397	25.029	7.185	25.029	0.1409
22					*22.639	7.67	*22.405	4.694

Table 2: Electrophoretic total protein profile of 4th instars *C. pipiens* (L.) after exposure to 10 µl *C. limon* (lemon oil extract)

	Intervals in hrs							
	Control		12 hrs.		24 hrs.		48 hrs.	
Band No.	Mol-wt	Conc. %	Mol-wt	Conc. %	Mol-wt	Conc. %	Mol-wt	Conc.%
1	284.68	4.44						
2								
3							183.26	2.1237
4	160.71	0.524						
5								
6	107.98	0.1957						
7	*103.34	0.379						
8	95.553	1.0722	*103.77	1.413				
9	*91.45	1.393						
10	86.162	6.4189	98.886	1.122				
11	*56.602	6.363						
12	41.13	5.659	*91.742	1.3289				
13	32	7.3001						
14	26.56	18.937						
15					66.004	2.267	66.004	1.914
16	24.163	9.805	*56.816	8.102	*56.816	5.17	*56.816	3.236
17	*22.043	8.559	51.978	11.57	51.978	6.190	51.978	6.277
18			46.015	7.1906	46.015	4.3405	46.015	5.006
19								
20			34.528	11.97	34.528	10.905	34.528	8.007
21			25.029	1.257	25.029	5.1642	25.029	3.054
22			25.872	52.182	*22.483	7.707	*22.483	5.938

Table 3: Electrophoretic total protein profile of 4th instar *C. pipiens* (L.) after exposure to 5 *µl B. thuringensis israelenisis* (Bti)

			Interval in min.					
	Control		30 min		90 min		150 min	
Band No.	Mol-Wt (kDa)	Conc. %	Mol-Wt (kDa)	Conc. %	Mol-Wt (kDa)	Conc. %	Mol-Wt (kDa)	Conc.%
1	*284.68	4.44			*284.81	1.568	*284.68	2.559
2								
3								
4	160.71	0.524						
5								
6	107.98	0.195						
7	*103.34	0.379	*103.19	2.5543				
8	95.553	1.0722						
9	91.45	1.393						
10	*86.162	6.418			*86.886	4.048	*86.162	4.769
11	*56.602	6.363					*56.602	
12	*41.13	5.659					*41.13	
13	*32	7.3001					*32	5.867
14	*26.56	18.937	66.004	1.3957	66.004	3.1136	*26.562	15.232
15			*56.816	7.212	*56.816	8.274		
16	*24.163	9.805	51.978	8.294	51.978	5.675		
17	*22.043	8.559	*41.015	6.375	*41.015	7.9405		
18								
19			34.528	11.101	34.528	10.475	25.065	9.899
20			25.029	1.879	25.029	1.95	24.163	5.25
21			*22.639	4.271	*22.639	2.929	*22.04	60.06

*: Shared protein bands

Mol. wt.: Molecular weight kDa::Kilo Dalton

Table 4: Effect of injection lethal dose of *A. satirum, C. limon* oil extracts and Bti on the activity of ALT and AST of BALB/c strain mice

	Groups of injected mice (mean±SE)							
	Control		*C. limon* oil		*A. sativum* oil		Bti	
Time in hrs.	ALT	AST	ALT	AST	ALT	AST	ALT	AST
24	182.6±0.8	65.3±0	186.0±1.2	64.3±0.3	185.0±0.5	64.3±0.3	184.0±0.5	62.3±1.2
48	182.6±0.8	65±0	183.0±0.5	65±0.5	185.6±0.3	64±0.57	183.0±0.5	65±0.57
72	182.6±0.8	65±0.1	182.6±0.8	65±0	182.6±0.8	64±0.3	184.0±0	65±

•Values are mean ± SE of 20 individuals for each group

•P> 0.05 non significant.

Table 5: Effect of injection lethal dose of *A. sativum*, *C. limon* oil extracts and Bti on the serum total protein level of BALB/c strain mice

	Groups of injected mice (mean±SE))			
Time in hrs	Control	*C. limon* oil	*A. sativum* oil	Bti
24	5.9 ± 0.3	6.4 ± 0.2	6.1 ± 0.12	6.4 ± 0.2
48	5.9 ± 0.3	6.0 ±.06	5.7 ± 0.2	6.2 ±.05
72	6.0 ± 0.3	6.0 ± 0	6.1 ±.05	6.1 ±.05

•Values are mean ± SE 20 individuals for each group.

•P> 0.05 non significant

Table 6: Effect of injection lethal dose of *A. sativum*, *C. limon* oil extracts and Bti on serum albumin, globulin and bilirubin levels of BALB/c strain mice

	Groups of injected mice ((mean±SE)											
	Control			*C. limon* oil			*A. sativum* oil			Bti		
Time in hrs	Albumin	Globulin	Bilirubin	Albumin	Globulin	Bilirubin	Albumin	Globulin	Bilirubin	Albumin	Globulin	Bilirubin
24	3.3±.05	2.2±0.1	0.2±.01	3.9±.05	2.1±.08	0.2±.03	3.7±0.15	2.3±.05	0.2±0	3.9±0.15	2.4±0.1	0.3± 0
48	3.3±.05	2.2±0.1	0.2±.03	3.8±.12	2.4 ± 0.1	0.2±.03	3.2±.3	2.2±.06	0.2 ±0	3.2 ± 0	2.4 ±0.18	0.2±.01
72	3.3±0.5	2.2±0.1	0.2±.03	3.3±.06	2.4±.06	0.2±.03	3.3±0.5	2.2±.03	0.2±.01	3.7±0	2.6±.05	0.27± 0

Values are mean ± SE of 20 individuals for each group.

P> 0.05 non significant.

Table 7: Effect of injection lethal dose of *A. sativum*, *C. limon* oil extracts and Bti on serum triglycerides and total cholesterol of BALB/c strain mice

	Groups of injected mice (mean±SE)							
	Control		*C. limon* oil		*A. sativum* oil		Bti	
Time in hrs	Cholesterol	Triglycerides	Cholesterol	Triglycerides	Cholesterol	Triglyceride	Cholesterol	Triglycerides
24	111±.1	135.6±0.8	112±1.3	137±0.3	112±.5	136±0.5	111±0	135±0.57
48	111±0.1	135.6±0.8	112±0.5	136±0.5	112±0.6	137±0.5	111±.2	136±0
72	111±0.1	135.6±0.8	111±0	135±0.5	111±.05	135±0	111±0	135±.5

•Values are mean ± SE 20 individuals for each group.

•P> 0.05 non significant.

182.6 for ALT, 64.3, 65 and 65 for AST at 24, 48 and 72 hrs post inoculation with *C.limon*, respectively. However, *A. sativum* oil extract recorded ALT and AST levels of 185 and 64, respectively. Moreover, Bti included variable levels in serum ALT and AST reaching 184, 183, 184 and 62.3, 65and 65, respectively. Serum ALT and AST levels of control non-inoculated mice recorded 182.6 and 65.3 at 24, 48 and 72 hrs, respectively.

No significant effect (P>0.05) was recorded in serum total protein in mice intraperitonealy inoculated with 10 µl oil extracts and 5 µl Bti, compared with control mice. Serum total protein reached 6.4, 6.0 and 6.0, 6.1, 5.7 and 6.1 and 6.4, 6.2 and, 6.1 in mice inoculated with *C. limon*, *A. sativum* oil extract and Bti at 24, 48 and 72 hrs post inoculation (Table 5).

The Effect on Albumin, Globulin and Bilirubin: No significant (P>0.05) effect was also showed in serum albumin, globulin and bilirubin level of mice inoculated with 10 µl oil extracts and 5 µl Bti (Table 6). It was found that the level of albumin, globulin and bilirubin levels in mice inoculated with *C. limon* oil reached 3.9, 2.1 and 0.2, 3.8, 2.4 and, 0.2 and 3.3, 2.4 and, 0.2 at 24, 48 and 72 hrs post inoculation, respectively. In the same time, these parameter levels recorded 3.7, 2.3 and 0.2, 3.2, 2.2 and 0.2 and 3.3, 2.2 and 0.2 at 24, 48, 72 hrs post inoculation, respectively in mice injected with *A. sativum*. Moreover, nearly same levels were found in mice injected with Bti.. However, in control mice, the level of the same corresponding parameters recorded 3.3, 2.2 and 0.2, 3.3 and 2.2, 0.2 and 3.3, 2.2 and 0.2 at 24, 48 and 72 hrs, respectively.

The Effect on Serum Cholesterol and Triglycerides: No significant effect was also recorded in serum cholesterol and triglycerides in mice injected with 10 µl oil extracts and 5 µl Bti (Table 7). It was found that *A. sativum*, *C. limon*, oils and Bti induced nearly the same level of cholesterol and triglycerides ranged from 111-112 and 135-137 at all time of inoculations (24, 48 and 72 hrs.), respectively. The level of these parameters in control mice also measured each 111 and 135 at all time of inoculations, respectively.

DISCUSSION

Mosquitoes can transmit a number of diseases-causing organisms to human and animals. These diseases includes: encephalitis, dengue fever, filariasis, yellow fever and malaria [26]. Resistance of mosquitoes to insecticides was considered to be a recent evolutionary adaptation to environmental changes. Response of

mosquitoes to sequential application of chemical insecticides for their control extended to less than a century.. Therefore, use of biological control is recommended to avoid this resistance [27 - 29]. In the present study the 4th instar *C. pipiens* (L.) exposed to plant oil extracts (*C. limon* and *A. sativum*) and entomopathogenic bacteria (Bti) showed remarkable changes in the total protein after treatment. Comparing the protein bands for treated larvae with the oil extracts and untreated larvae showed the appearance and absence of some bands and reduction in the intensity of others. This may be due to the toxic action of the *A. sativum* and *C. limon* plant oil extracts which inhibit the synthesis and expression process of these protein bands. This run in full agreement with Massoud *et al.*[16] who stated that oleo-resin extracts was found to induce a significant higher toxic action to *C. pipiens* (L.).The electrophorteic analysis of total proteins, lipoproteins and glycoproteins revealed inhibitory action of the used plant extracts on the protein content. Larvicidal activity of the oleo- resin was explained as to be related to the loss of certain enzymes inhibited by these extracts which affect the metabolic processes. In the present study, electrophoretic banding patterns of non-treated *C. pipiens* larvae were separated by 10% SDS- PAGE into 13 protein bands with Mol. weight ranging from 22.04 - 284.68 kDa. However, after exposure to 10µl garlic oil extracts, at 12 hrs, the larval SDS dissociated protein was separated into 18 protein bands. There were 2 shared common protein bands. At 24 hrs post-exposure, there were 17 protein bands appeared and a common 3 protein bands shared with control. This study demonstrated the presence of three common protein bands shared between control and 24 hrs post-treated larvae with *A. sativum* and *C. limon*. These finding agreed with the previous detained by other author [16]. These authors showed that common protein bands of 39.640 and 41.2 kDa were produced shared with that of treated larvae with oleo-resin extracts. The larvae exposed to solvent (cremophore) which differs only in the amount was being lower in case of oleo-resin treated larvae. Moreover, they found that the untreated larvae (exposed to water and solvent one) developed 10 and 13 protein bands, respectively. Dawer *et al.*[30] found that a band having a molecular weight of 200 kDa was absent in haemolymph of mosquitoes, (*An. stephenis* and *An. culicifacies*) exposed from 1 - 7 days to volatiles of neem seed. This band was absent in the haemolymph and ovaries of 4 and 7 days old and blood-fed females not exposed to neem odor. Moreover, they found that long and continuous exposure to volatiles of neem proved to be most effective in suppressing or impairing vitellogenessis and oviposition. The degree of impairment was related to the duration of exposure and probably to cuticle absorption of organ sulfur constituents of the neem volatile [30]. The present study demonstrated the presence of new bands at different time of intervals after exposure to Bti. Moreover, there were shared proteins bands with control larvae were detected. Therefore, these bands can be considered as the specific band of *C. pipiens* (L.). This was in agreement with finding of Masoud *et al.*[16] who showed an identical protein band (44.0198 kDa) in all lanes representing protein extracted from *C. pipiens* larvae either treated with both oil and oleo-resin extracts or non treated ones. However, this specific band differs in the amount of proteins in spite having the same molecular weight, being lower in case of normal non-treated larvae than in case of larvae treated with extract [16].

In the present study, assessment of biosafety of Bti and plant oil extracts (*A. sativum, C. limon*) were evaluated. The results showed insignificant increase in serum ALT, AST, cholesterol, total protein, bilirubin, albumin and globulin levels after interperitoneal injection of plant oil extracts and Bti. These results agree with other authors [31- 34] who showed that no change was recorded in activities of serum ALT and AST enzyme levels. Similarly, the treatment of adult white rats with garlic powder or extract resulted insignificant decrease in activities of GOT and GPT [35-39]. Many authors showed that garlic did not affect on cholesterol level [40-45]. The present work declared that injection of BALB/c albino mice with plant oil extracts induced no changes in total protein, albumin and globulin level. This is run with Superko and Kraouss [45] who reported that oral administration of wistar albino rats with raw garlic (4% of diet) for 14 days had no significant change in serum total proteins, albumin and globulins levels. However treatment of rats with garlic powder or extract induced significant decrease in total protein, albumin and globulin levels [44,47]. In the present work the tested doses of entomopathogenic bacteria (Bti) had no detectable effect on BALB/c albino mice serum and nearly control level that observed at the end of the experimental period.. In addition, Nassar [48] found that the aqueous extracts of two natural marine toxins had no acute or chronic marked effect on mice as serum acetyl cholinesterase (AchE) which gave more or less nearly the same level of AchE activity at the end of decapitation periods. No doubt, the toxic action of the natural product as in all insecticides can be attributed to many factors as

active ingredient, type of natural toxin, solvent of extract, doses and time of exposure as well as the stage and behavioral characters of the insect [49].

In conclusion, the use of plant oil extracts (*Allium Satvium, Citrus Limon*) and *Bacillus thuringiensis israelensis* had no chronic or acute unfavorable effects on serum of mice. This suggested that they may be considered as new promising controlling agents which reduce the using of chemical insecticides which behave as alkyl-ting, carcinogenic, cytotoxic and mutagenic agent.

REFERENCES

1. Mittal, P.K., 2003. Bio larvicides in vector control: challengers and prospects. Journal of Vetrinary Borne Diseases, 40: 20-32.
2. El-Hag, E.A., H.E. Nadi and A.A. Zaitioon, 1999. Toxic and grow with retarding effects of three plant extracts on *C. pipiens* larvae (Diptera: Culicidae).Phytother Research, 13: 388-392.
3. World Health Organization (WHO), 1992. Vector resistance to pesticides. Report to the WHO expert committee on Vector biology and control. Geneva, Switzer land World Health Organization.
4. Chandre, F., F. Darriet, M. Darder, A. Cuany, J.M.C. Doannio, N. Pasteur and P. Guillet, 1998. Pyrethroid resistance in *C.quinquefasciaus* from West Africa. Medical Veterinary Entomology, 12: 359-366.
5. Penilla, R.P., A.D. Rodriguez, J.L. Hemingway, J.I. Torres and J. Arredondo, 1998. Resistance management strategies in malaria vector mosquito control Baseline data for a large scale filed trial against *Ano.* albimanus in Mexico. Medical Veterinary Entomology, 12: 217-233.
6. Arnason, J.T., B.J.R. Photogene, P. Morand, K. Imire, S. Iyengar, F. Duval, C. Soucy-breau, J.C. Westiuk, N.H. Scaiano, B. Hasspiele and A.E.R. Downe, 1989. Naturally occurring and synthetic thiophenes as photo activated insecticides pp. 164-172, in Insecticides of plant origin, Eds. J.T. Arnason, B.J.R. philogene and Morand P. ACS symp. Ser. No.387, American Chemistry Society, Washington D.C.
7. Park, I.K., S.G. Lee, A.C. Shin, J.D. Park and Y.J. Ahn, 2002. Larvicidal activity of isobutyl amides identified in *Piper nigram* fruits against three mosquito species. J. Agriculture Food Chemistry, 50: 1866-1870.
8. Naqvi, N.H., K. Akhtar and M.A. Azmi, 1991. Toxicity of NFD (natural Fraction of Winter neem leaves against *Sitophilus oryzae* L.) exposed impregnated filter paper and its effect on phosphatases and protein metabolites. Acta- Biologica emcoviensia series Zoologia, 33: 49-58.
9. Soliman, B.A. and L.S. El-Sherif, 1995. Larvicidal effects of some plant oils on mosquito, *C. pipiens* L. (Diptera: Culcidae). J. Egyptian Germany Society Zoology, 16: 161-169.
10. Morsy, T.A., A. Shaukry, S.A.M. Mazyad and K.A. Makled, 1998. The effect of volatile oils of *Chenopodium ambrosioides* and *Thymws vulgaris* against the larvae of *Lucilia sericata* (Meigen). J. Egyptian Society Parasitology, 28: 503-10.
11. Shoukry, I.F.I., 1996. Morphological and hisopathological effects induced by two natural volatile oils on the house fly, *M. domestica* (L.) (Diptera; Muscidae) Proc. 1st Conf. Role of science in development of Egyptian Society and Environment, 21-23 October 1996. Zagazig University, Benha Branch, Egypt.
12. Khalaf, A.A. and K.T. Hussein, 1996. Potency and other toxicological activities of some plant extracts against the cowpea weevil, *Callosobruchus maculates*. Ain Shams Scientific Bulletin, 34: 389-404.
13. El-Gougary, O. A., 1998. Insecticidal and synergistic activity of *Atriplex halims* L. extracts. J. Egyptian Society Parasitology, 28: 191-196.
14. Shoukry, I.F.I. and K.T. Hussein, 1998.Toxicity and biochemical effects of two plant volatile oils on the larvae of the greater wax moth *Galleria mellonella* L. (Pyralidae: Lepidoptera). J. Egyptian Germany Society Zoology, 27: 99-116.
15. Abdel-Aal, A.A., N.I. Hamad, S.A. Okasha and E.A.A. Shaalan, 1998. Studies on the biological control and ecology of mosquitoes in Aswan Governorate: Evaluation of *B. sphaericus* strain ABG 6232 as biological larvicide's. Assiut - Veterinary-Medical. Journal, 39: 1-16.
16. Massoud, A.M., I.M. Labib and M. Rady, 2001. Biochemical changes of *C. pipiens* larvae treated with oil and oleo-resin extracts of myrrh *Commiphora molmol*. J. Egyptian Society Parasitology, 31: 517-529.
17. Lerdthusnee, K. and T. Chareonviriyaphap, 1999. Comparison of isozyme patterns of *A. aegypti* collected from pre - and post - *B. thuringiensis israelens's* treatment sites in Thailand. J. American Mosqitoe Control Associate, 15: 48-52.

18. Hafez, G.A., 2000. Extended effect of *B. thuringiensis* H-14 on *C. pipiens* adults surviving larval treatment. J. Egyptian Society Parasitology, 30: 377-386.
19. Davis, B.J., 1964. Disc electrophoresis in methods and application to human serum protein. Academic Science, 121: 404-427.
20. Reitman, S. and S. Frankel, 1957. Colorimetric method for the determination of serum transaminase activity. American Journal Clinical Pathology, 28: 56-59.
21. Allain, C.C., L.S. Poom, C.S. Chan, W. Richmond and P.C. Fu, 1974. Enzymatic colorimetric method for determination of total cholesterol. Clinical Chemistry, 20: 470-471.
22. Mc-Gowan, M.W., J.D. Artiss, D.R. Strandbergh and B. Zak, 1983. Enzymatic colorimetric method for determination of triglycerides. Clinical Chemistry, 29: 538-539.
23. Josephson, B. and A. Gyllensward, 1975. Colorimetric determination of total protein (Biuret method). Scandinavian Journal Clinical Laboratory Investment, 9: 29-34.
24. Malloy, H.T., 1937. The determination of the bilirubin with the photoelectric colorimeter. J. Biological Chemistry, 112: 481-791.
25. Douma, B.T., W.A. Waston and H.G. Biggs, 1971. Albumin standards and measurements of serum albumin with bromocresol green. Clinical Chemistry Acta, 31: 87-96.
26. Gouge, H.D., A.K. Smith, C. Olson and P. Baker, 2001. Mosquitoes. Cooperative Extension, Collage of Agriculture and Life Sciences, the University of Arizona.
27. Hakim, G., 1996. Status of malaria office in Lebanon - Announcement from the Lebanese Epidemiological Association, EPi News, pp: 3-8.
28. Zayed, A.A., 1999. Role of external parasites on the productivity of farm animals and their control measures.. J. Applied Veterinary Science National Research Center, 1: 197-225.
29. Abdalla, M.S. and O. Galal, 1996. Effect of volatile oils and a aqueous extracts from some Egyptian plants on alloxan diabetics. J. Egyptian Germany Society Zoology, 21: 237-249.
30. Dawar, R.H., S. Garg, S.F. Basir and G.P. Tawar, 1996. Effect of volatiles from Neem and other natural products on gonotrophic cycle and oviposition of *Anopheles stephensi* and *An. culicifacies* (Diptera: Culicidae). J. Medical Entomology, 33: 195-201.
31. Poopathi, S., J.F. Charles and C.N. Roux, 2002. Alternative method for preservation of mosquito larvae to study binding mechanisms of *B. sphaericus* binary toxin. J. Invertberate Pathology, 79: 132-134.
32. El-Habbak, M.M.E., K. Saleh, M.S. Arbib, A.G. Hegazi and H. Sofy, 1989. Influence of garlic (A. *sativum*, L.) On some biological and biochemical changes in Japanese quail with special reference to its hypercholesterolemia activity. Archive-fur-Geflugelkunde., 53: 73-79.
33. Hikino, H., M. Tohkin, Y. Kiso, T. Namiki, S. Nishimura and K. Takeyama, 1986. Anti-hepatototxic action of *A. sativum* bulls. Plant Medicine, 58: 63-68.
34. Lee, B.J., E.J. Sung, M.S. Lee, I.H. Jang and H.B. Lee, 1994. Effects of garlic on cadmium accumulation in tissue of dog's. Kor. J. Veterinary Research, 34: 635-645.
35. Bogin, E. and M. Abrams, 1979. The effect of garlic extract on the activity of some enzymes. Fd. Cosmet. Toxicoogy, 14: 417-419.
36. Devaki, T., S. Venmadhi and P. Govindaraju, 1992. Alteration in protein metabolism in ethanol injected rats treated with garlic oil. Medical Science Research, 20: 725-727.
37. Sheo, H.J., K.S. Kim and D.L. Jung, 1994. Effect of garlic on toxicity of mercury in rat. J. Korean Society Food Nutrition, 23: 908-915.
38. Ahmed, R.S. and S.B. Sharma, 1997. Biochemical studies on combined effects of garlic (*A. sativum*, Linn.) and ginger (*Zingber officinale*, Rosc) in albino rats. Indian Journal Expermental Biology, 35: 841-843.
39. Liue, Y.l., J.R. Chen and M.J. Shieh, 1998. The effects of garlic powder on the hypolipidermic function and ant oxidative status in hamsters. Nutrition Science Journal, 23: 171-187.
40. Heinle, H. and E. Betz, 1994. Effect of die tarry garlic supplementation in a rat model of a therosclerosis. Arzneim Forsch., 44: 614-617.
41. Alder, A.J. and B.J. Holub, 1997. Effect of garlic and fish oil supplementation on serum lipid and lipoprotein concentration in hypercholesterolemic. American Journal Clinical Nutrition, 65: 445-450.
42. Isaacsohn, J.L., M. Moser, E.A. Stein, K. Dudley, J.A. Davey, E. Liskov and H.R. Black, 1998. Garlic powder and plasma lipid and lipoproteins: a multicenter, randomized, placebo controlled trait. Arch. International Medical, 158: 1189-1194.

43. Abramovitz, D., S. Gavr, D. Harats, H. Ievkovitz, D. Mirelmen, T. Miron, S. Eilat Adar, A. Rabinkov, M. Wilchek, M. Elder and Z. Vereb, 1999. Allicin-induced decrease in formation of fatty streak (a therosclerosis) in mice fed a cholesterol rich diet. Coron. Artery disease, 10: 515-519.
44. Ali, M., K.K. Al-Qattan, F. Al-Enezi, R.M. Khanafer and T. Mustafa, 2000. Effect of allicin from garlic powder on serum lipids and blood pressure in rats fed with a high cholesterol diet. Prostagl Leukotr. EssenFatty acids, 62: 253-259.
45. Superko, H.R. and R.M. Kraouss, 2000. Garlic powder: effect on plasma lipids, post prandial lipemia and low - density lipoprotein particle size high-density lipoprotein Sub class distribution lipoprotein J. American Cardiology, 35: 321-326.
46. Oboh, G., 2005. Hepatoprotective property of ethanolic and aqueous extracts of fluted pumpkin (Telfairia *occidentalis*) leaves against garlic - induced oxidative stress. J. Medical Food, 8: 560-563.
47. Sodium, O., P.K. Joseph and K.T. Augusti, 1984.Certain biochemical effects of garlic oil on rats maintained on high fat-high cholesterol diet. Experientia, 40: 78-80.
48. Nassar, M.M.I., 2000. Assessment of two natural marine toxins (*Microcystis aeruginosa* and *Parasicyonis actinostoloides*) for the control of some medical and agriculture insects with reference to the action on mice. Journal of the. Egyptian Society Parasitology, 30: 631-641.
49. Litchfield, J.T. and F. Wilcoxon, 1979. A simplified method of evaluation dose effect experiments. Journal of Pharmacology and Expermental Therapy, 95: 99-113.