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Changes of cotton leaf worm haemocytes and esterases after exposure to compounds derived from urea and Rice Straw

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ABSTRACT

In the current study, the role of cotton leaf worm haemocytes and esterases in detoxification were recorded. The haemolymph was collected from the 6th instar larvae which treated with LC_{25}, LC_{50}, LC_{70} of three newly compounds extracted from wastes from natural origin, Cyano acetyl urea (CAU), Benzimidazolyl acetyl urea (BAU) from urea and Cyano acetyl urea (CAH) from rice straw as 4th instar larvae. The mean of total haemocytes counts in haemolymph of untreated 6th larval instar was 9430±35.78 cells/mm^3. Data indicated an increase in the total haemocytes of treated ones with CAU, at LC_{25} and LC_{50}, BAU at LC_{25} and CAH at LC_{25}, LC_{50} of CAH the percentages of change were: 19.01, 14.31, 1.90, 21.00 and 14.55, respectively. In addition, the percentages of oenocytoid counts were increased in all concentrations for all compounds except for CAH (at LC_{70}) as it did not change comparing control count. On contrast, data cleared reductions in granulocytes in all concentrations for all compounds except for BAU at LC50 as they increased slightly (15.12%). On the other hand, the results were showed great differences in number of zones of esterase activity and in substrate specificity between treated and untreated samples. For instance, in the case of α - naphthyl acetate, 36 esterase bands were detected in control and treated larval samples with Rf ranging between 0.01 to 0.28. While, in the case of β - naphthyl acetate, 39 esterase bands were detected in control and treated larval samples with Rf ranging between 0.01 to 0.92.

Keywords: *Spodoptera littoralis*- urea derivatives - rice straw- detoxification- haemocytes - esterase patterns.

INTRODUCTION

Haemocytes play an essential role in defending insects and other invertebrates against invaders (Pech and Strand, 1996). Insect cellular defence reactions against invaders include nodule formation and encapsulation (Salt, 1970). Granular haemocytes are characterized by the possession of acidophilic granules which are membrane bounded. These are involved in the detoxification of chemicals and killing of microorganism through encapsulation and phagocytosis (Saxena and Srivastava, 2001; Chapman, 1998). Also, plasmatocytes, prohemocytes and granulocytes play an important role in phagocytosis, cell clumping and wound healing (Gagen and Ratcliffe, 1976; Barakat, 1997and Irving, *et al*., 2005).

Esterases play a role in detoxification of xenobiotics (Shen and Dowd, 1991). They exhibit a greater degree of polymorphism than other enzymes because they act on a class of molecules many of which come directly from external environment (Kojima *et al.,* 1970). The esterases can break an

ester bond with the help of a water molecule. Most enzymes of this class hydrolyze endogenous substances and are important in intermediary metabolism (Sivakumarm and Maya, 1991). Because most insecticides are ester of substituted phosphoric, carbamic, or cyclopropanecarboxylic acids, they are subjected to degradation by esterases (Devonshire, 1991).

The present study aims to investigate the role of the cotton leaf worm haematocytes to detoxify the three newly tested compounds. This was attained by determination the total haemocyte count and the percentage of differential haemocyte count in the larvae after treatment with previous compounds. In addition, this study was concerned with differentiation of *S. littoralis* esterase activity in untreated and treated larvae with tested compounds.

MATERIALS AND METHODS

Experimental compounds:-

Tested compounds [Cyano acetyl urea (CAU), Benzimidazolyl acetyl urea (BAU) and Cyano acetyl hydrosylate (CAH)] were extracted from waste products, from natural origin. The first two compounds were extracted from urea while the latter one was extracted from rice straw. These newly extracted compounds were provided from Industrial Chemistry Division - National Research Center (El-Bohouth Street, Dokki, Egypt).

Haematological studies:-

Collection of hamolymph

This study was carried out on normal and treated 6th instar larvae (resulted from the treated 4th instars larvae with LC_{25} (0.12, 0.19 and 0.015%), LC_{50} (0.52, 0.66 and 0.1%) and LC_{70} (1.57, 1.73 and 0.44%) of CAU, BAU and CAH, respectively). The haemolymph was obtained by amputation of one or two prothoracic legs of the larvae using fine scissors. Gentle pressure was done against the thorax until a drop of haemolymph appeared at the point of amputation. This experiment was done right carefully to avoid contamination of haemopymph from two individuals.

Total haemocytes counts (THCs)

The haemolymph was calculated according to the formula of Jones (1962) as follows:

$$\frac{\text{Number of haemocyte counted per chamber X dilution X depth factor}}{\text{Number of 1mm squares counted}}$$

(Where the depth factor is usually 10).

Differential haemocyte counts (DHCs)

Examined samples of haemolymph from 10 individuals (6th instar larvae) in a given stage made differential haemocyte counts. Whenever possible, a minimum of 100 cells/ 6th instar larvae were investigated. Stained preparations were made according to Arnold and Hinks (1979). The cell shape, cytoplasmic ratio, cytoplasmic inclusions and shape of nucleus were used for classification of haemocytes using the classification scheme of Brehelin and Zachary (1986). The percentages of haemocyte types were calculated by the formula:

$$\frac{\text{Number of each haemocyte type}}{\text{Total number of haemocytes examined}} \text{ X } 100$$

Measurements were replicated 10 times for the examined larvae of both control group and the treated one.

Isozymes analysis:

Esterase bands were separated by Polyacrylamide gel electrophoresis (PAGE) according to the method of (Salama *et al.,* 1992). After

electrophoresis, the gel was soaked in 0.5M borate buffer (pH 4.1) for 90 min. at 4 ^{o}C (Sims, 1965) to lower the pH of the gel from 8.8 to ≈ 7 which the reaction precedes readily. The low temperature minimizes diffusion of the protein with in the gel. The gel then was rinsed rapidly in two changes of double distilled water.

The gel was stained for esterolytic activity by incubation at 25 ^{o}C in a solution of 100 mg α-naphthyl acetate in 2ml acetone (as substrate) and 100mg fast blue RR salt in 200 ml of 0.1M phosphate buffer, pH 6.5 (Sell *et al*. 1974).

The α-naphthyl acetate, which was released on hydrolysis of the substrate, coupled with the dye salt to produce on insoluble pigment at the site of enzyme activity.

β- naphthyl acetate also was used as substrate. After incubation, the gel was destained in 7% acetic acid. Also, the gel was scanned to calculate the relative mobility and concentration of identified bands using Gel-Pro Analyzer.

The similarity index and genetic distance were determined according to (Nei and Li, 1979).

RESULTS

Haematological studies

Changes in total haemocytes counts

Table (1) shows the total number of haemocytes in haemolymph of the 6th instar larvae at sublethal concentrations. The mean of total haemocytes counts in haemolymph of untreated 6th larval instar was 9430±35.78 cells/mm^3. Data indicated an increase in the total haemocytes of treated ones with CAU, at LC_{25} and LC_{50}, BAU at LC_{25} and CAH at $LC_{25,}$ LC_{50} of CAH since haemocytes counts were 11223±23.9, 10780±48.15, 9610±21.21, 11411±49.44 and 10803±36.8 cells/mm^3 respectively. The percentages of change were: 19.01, 14.31, 1.90, 21.00 and 14.55, respectively.

While a reduction was noticed in total haemocytes of treated larvae with: CAU (at LC_{70}), BAU (at Lc_{50}, Lc_{70}) and CAH (at LC_{70}) comparing the control group: 6810±21.21, 8210±26.96, 6590±29.96 and 8810±15.56 cells/mm^3 and the percentages of changes were: -27.78, -12.93, -30.11 and -6.57 respectively.

Table 1: Changes in total haemocytes counts of the 6th larval instar of *S. littoralis* after treatment of 4th larval instar with sub-lethal concentrations of CAU, BAU and CAH.

Treatment	Cont.	CAU 2nd % change			BAU 2nd % change			CAH 2nd % change		
		LC_{25}	LC_{50}	LC_{70}	LC_{25}	LC_{50}	LC_{70}	LC_{25}	LC_{50}	LC_{70}
Total haemocytes counts cells/ mm^3 mean ± S.E.	9430 ± 35.78	11223 ± 23.9	10780 ± 48.15	6810 ± 21.21	9610 ± 21.21	8210 ± 26.96	6590 ± 29.96	11411± 49.44	10803 ± 36.8	8810 ± 15.56
% change	-	19.01	14.31	-27.78	1.90	-12.93	-30.11	21.00	14.55	-6.57

Changes in differential haemocytes counts

Table (2) showed that the mean percentages of plasmatocyte, prohemocyte, granulocyte, spherulocyte and oenocytoid in untreated group were 42, 33, 15, 9 and 1 respectively.

The percentage of plasmatocyte increased relative to control in larvae treated with CAU (at LC_{25}), BAU (at LC_{25}) and CAH (at LC_{25}, LC_{50}, LC_{70}) since these percentages were: 45.55, 45.64, 56.1, 62.0 and 65.12. On the other hand, the percentages of plasmatocytes count decreased relative to control in larvae treated with CAU (at LC_{50}, LC_{70}) BAU (at LC_{50}, LC_{70}): 29.8., 38.16, 37.88 and 28.65 respectively. Also, the percentages of prohaemocyte counts increased relative to control in larvae treated with CAU (at LC_{25}, LC_{50}, LC_{70}) BAU (at LC_{50}, LC_{70}): 34, 44.5, 39.12, 38.0 and 50.3 in respectively. In contrast,

the percentages of prohemocyte count decreased relative to control in larvae treated with CAU (at LC_{25}), and CAH (at LC_{25}, LC_{50}, LC_{70}): 30.2 21.36, 24.0 and 20.0 as compared with control group respectively.

Table 2: Mean percentage of differential haemocytes counts of the 6th larval instar of *S. littoralis* after treatment of 4th larval instar with sub-lethal concentrations of CAU, BAU and CAH.

Treatments \ Cell type		Plasmatocytes	Prohaemocytes	Granulocytes	Spherulocytes	Oenocytoids
Control		42	33	15	9	1
CAU	LC_{25}	45.55	34	8.78	9.67	2
	LC_{50}	29.88	44.5	12.5	11.28	1.84
	LC_{70}	38.16	39.12	11.38	9.14	2.2
BAU	LC_{25}	45.64	30.2	14.43	8.63	1.1
	LC_{50}	37.88	38	14.12	8	2
	LC_{70}	28.65	50.3	10.5	9.2	1.35
CAH	LC_{25}	56.1	21.36	9.24	12.08	1.22
	LC_{50}	62	24	7	5	2
	LC_{70}	65.12	20	8.7	5.18	1

As to granulocytes, data cleared reductions in all concentrations for all compounds. Granulocytes were decreased to 8.78, 12.5, 11.38, 14.43, 14.12, 10.5, 9.24, 7.0 and 8.7 % for larvae treated with CAU (at LC_{25}, LC_{50}, LC_{70}), BAU (at LC_{25}, LC_{50}, LC_{70}) and CAH (at LC_{25}, LC_{50}, LC_{70}) respectively. The spherulocytes percentages were increased for larvae treated with CAU (at LC_{25}, LC_{50}, LC_{70}) BAU (LC_{70}) and CAH (at LC_{25}) as the percentage were: 9.67, 11.28, 9.14, 9.2 and 12.08 as respectively, compared with control group. On the other hand, the percentages of spherulocyte decreased in larvae treated with BAU (at LC_{25}, LC_{50}) CAH (at LC_{50}, LC_{70}) with percentages were: 8.63, 8.0, 5.0 and 5.18 respectively, as compared with control group.

All the percentages of oenocytoid counts were increased (2.0, 1.84, 2.02, 1.1, 2.0, 1.35, 1.22 and 2.0%) for those treated with CAU (at LC_{25}, LC_{50}, LC_{70}), BAU (at LC_{25}, LC_{50}, LC_{70}) and CAH (at LC_{25} and LC_{50}) except for CAH (at LC_{70}) as it did not change comparing control count.

ISOZYME ANALYSIS

Esterase detected by α-naphthyl acetate:

Table (3) and figure (1) represent α-esterase electrophoretic banding pattern of the 4th instar larvae of *S. littoralis* under different concentrations. A maximum number of 36 bands, which were not necessarily present in all of the studied samples , were detected at approximately Rf ranging between 0.01 and 0.97

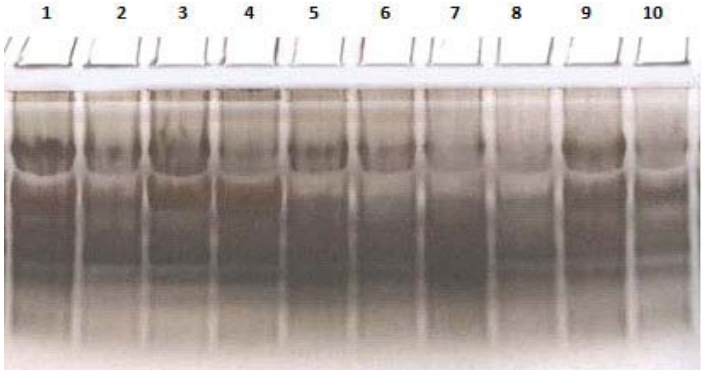


Fig. 1: Electrophoretic α- esterase pattern of larval tissues of *S. littoralis* as control and treated samples with tested compounds.

Table 3: Relative fragmentation (Rf) and % amount of larval tissues α-esterase pattern for CAU, BAU, CAH and control samples of 4th larval instar of *S. littoralis.*

Band No.	Rf	Control		CAU						BAU						CAH					
		C	Am. %	LC_{25}	Am. %	LC_{50}	Am. %	LC_{70}	Am. %	LC_{25}	Am. %	LC_{50}	Am. %	LC_{70}	Am. %	LC_{25}	Am. %	LC_{50}	Am. %	LC_{70}	Am. %
1	0.01	-		-		+	3.14	-		-		-		-		-		-		+	0.88
2	0.02	+	1.96	-		-		-		-		+	1.75	+	2.19	+	0.94	+	1.23	-	
3	0.03	-		+	2.76	-		+	3.54	+	3.06	-		-		-		-		-	
4	0.10	-		-		+	6.06	-		-		-		-		-		-		-	
5	0.11	+	4.68	+	3.52	-		+	4.96	+	6.58	+	6.49	+	6.82	-		-		-	
6	0.14	-		-		-		+	2.23	-		-		-		-		-		-	
7	0.21	-		-		-		-		-		-		-		-		-		+	11.73
8	0.22	-		-		-		-		-		-		+	12.51	-				-	
9	0.23	+	17.08	-		+	13.82	-		-		+	14.00	-		+	18.70	-		-	
10	0.24	-		-		-		-		+	15.17	-		-		-		-		-	
11	0.25	-		+	15.55	-		-		-		-		-		-		+	19.49	-	
12	0.26	-		-		-		+	10.69	-		-		-		-		-		-	
13	0.42	-		-		-		-		-		-		-		-		+	26.25	+	26.70
14	0.43	-		-		-		-		+	12.64	+	11.34	-		-		-		-	
15	0.44	+	20.08	-		+	21.38	-		-		-		-		+	18.25	-		-	
16	0.45	-		+	21.01	-		+	27.90	-		-		+	11.28	-		-		-	
17	0.53	-		-		-		-		-		+	20.38	-		-		+	13.94	+	14.95
18	0.56	+	14.55	+	14.75	-		-		+	19.15	-		+	10.45	+	15.24	-		-	
19	0.57	-		-		+	11.47	-		-		-		-		-		-		-	
20	0.58	-		-		-		+	9.27	-		-		-		-		-		-	
21	0.60	-		-		-		-		-		-		-		-		-		+	18.14
22	0.64	-		-		-		-		+	17.26	+	21.15	-		+	22.56	-		-	
23	0.65	+	18.80	-		-		-		-		-		+	22.05	-		-		-	
24	0.66	-		+	20.60	-		-		-		-		-		-		+	15.13	-	
25	0.67	-		-		+	16.20	+	17.48	-		-		-		-		-		-	
26	0.72	-		-		-		-		-		+	19.13	-		+	19.81	-		+	17.61
27	0.73	-		-		-		-		-		-		+	25.28	-		-		-	
28	0.74	-		-		+	12.78	+	18.04	+	19.77	-		-		-		+	16.38	-	
29	0.75	+	16.31	+	15.84	-		-		-		-		-		-		-		-	
30	0.82	-		-		+	9.80	-		-		-		-		-		-		-	
31	0.90	-		-		-		-		-		-		-		-		-		+	9.99
32	0.91	+	6.53	-		-		-		-		-		-		-		-		-	
33	0.92	-		-		-		-		-		-		-		-		+	7.58	-	
34	0.94	-		+	5.97	-	5.34	-		-		+	5.76	+	4.42	+	4.51	-		-	
35	0.95	-		-		-		-		+	6.37	-		-		-		-		-	
36	0.97	-		-		-		+	5.89	-		-		-		-		-		-	

The maximum number of bands was nine and observed in LC_{50} and LC_{70} of (CAU), whereas the minimum number was seven and recorded in LC_{25}, LC_{50} and LC_{70} of (CAH). The resulted profile comprises 36 polymorphic bands . The α-esterase pattern revealed 16 unique bands and scored as follows: one in control sample at Rf value of 0.91; three in samples of LC_{50} of (CAU) at Rf values of 0.10, 0.57 and 0.82; four in samples of LC_{70} of (CAU) at Rf values of 0.14, 0.26, 0.58 and 0.97; two in samplesof LC_{25} of (BAU) at Rf values of 0.24 and 0.95; two in samples of LC_{70} of (BAU) at Rf value of 0.22 and 0.73; one in samples of LC_{50} of (CAH) at Rf values of 0.92 and three in samples of LC_{70} of (CAH) at Rf values of 0.21, 0.60 and 0.90. The similarity indecies (S.I.) and genetic distances (G.d.) were recorded between control and other treated samples as shown in Table (4). S.I. between LC_{25}, LC_{50} and LC_{70} of (CAU), LC_{25}, LC_{50} and LC_{70} of (BAU) and LC_{25}, LC_{50} and LC_{70} of (CAH) samples and their control were 0.38, 0.24, 0.18, 0.25, 0.38, 0.50, 0.53, 0.13, and 0.00 , respectively

Table 4: Similarity index and genetic distance between treated and untreated samples α-esterase larval tissue of *S. littoralis*

S.I

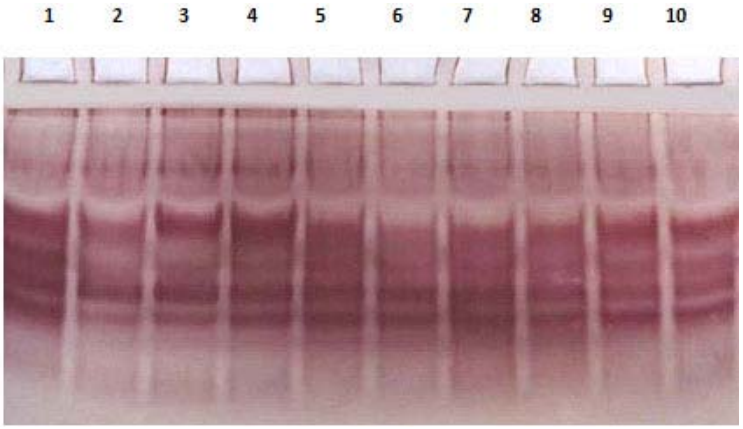
G.d

Samples		Cont.	CAU			BAU			CAH		
			LC_{25}	LC_{50}	LC_{70}	LC_{25}	LC_{50}	LC_{70}	LC_{25}	LC_{50}	LC_{70}
Cont.		-	0.38	0.24	0.18	0.25	0.38	0.50	0.53	0.13	0.00
CAU	LC_{25}	0.62	-	0.12	0.35	0.25	0.25	0.50	0.26	0.26	0.00
	LC_{50}	0.76	0.88	-	0.22	0.12	0.12	0.23	0.37	0.00	0.12
	LC_{70}	0.82	0.65	0.78	-	0.35	0.12	0.23	0.00	0.12	0.00
BAU	LC_{25}	0.75	0.75	0.88	0.65	-	0.38	0.25	0.26	0.13	0.00
	LC_{50}	0.62	0.75	0.88	0.88	0.62	-	0.38	0.66	0.26	0.26
	LC_{70}	0.50	0.50	0.77	0.77	0.75	0.62	-	0.40	0.13	0.00
CAH	LC_{25}	0.47	0.74	0.63	1.00	0.74	0.34	0.60	-	0.14	0.14
	LC_{50}	0.87	0.74	1.00	0.88	0.87	0.74	0.67	0.86	-	0.25
	LC_{70}	1.00	1.00	0.88	1.00	1.00	0.74	1.00	0.86	0.75	-

ɛrase detected by β-naphthyl acetate:

Electrophoretic pattern of β-Esterase was obvious through 39 bands, with Rf ranged from 0.01to 0.92 as shown in Table (5) and Figure (2). The maximum number of bands was 11 and observed in LC_{50} of (BAU), whereas the minimum number was eight and recorded in LC_{70} of (CAH). The resulted profile comprises 39 polymorphic bands. The (β-est) pattern revealed 17 unique bands and scored as follows: six in control samples at Rf values of 0.20, 0.27, 0.49, 0.59, 0.66 and 0.83; one in samples of LC_{50} of (CAU) at Rf value of 0.40; one in samples of LC_{70} of (CAU) at Rf value of 0.09; one in samples of LC_{25} of (BAU) at Rf values of 0.71; one in samples of LC_{50} of (BAU) at Rf value of 0.48; two in samples of LC_{70} of (BAU) at Rf value of 0.12 and 0.89; two in samples of LC_{25} of (CAH) at Rf value of 0.13 and 0.87 and three in samples of LC_{70} of (CAH) at Rf values of 0.60, 0.67 and 0.88.

Fig. 2: Electrophoretic β-esterase pattern of larval tissues of *S. littoralis* as control and treated samples with tested compounds.



For legends and abbreviations, see the foot note of figure (1)

Table 5: Relative fragmentation (Rf) and % amount of larval tissues β-esterase pattern of CAU, BAU, CAH and control samples of 4th larval instar of *S. littoralis.*

Band No.	Rf	Control		CAU						BAU						CAH					
		C	Am. %	LC_{25}	Am. %	LC_{50}	Am. %	LC_{70}	Am. %	LC_{25}	Am. %	LC_{50}	Am. %	LC_{70}	Am. %	LC_{25}	Am. %	LC_{50}	Am. %	LC_{70}	Am. %
1	0.01	+	2.79	+	3.56	+	4.34	-		-		-		-		-		-		-	
2	0.03	-		-		-		+	4.12	+	3.13	-		-		+	2.01	+	1.05	+	1.00
3	0.04	-		-		-		-		-		+	2.53	+	2.42	-		-		-	
4	0.09	-		-		-		+	5.48	-		-		-		-		-		-	
5	0.10	+	3.19	-		+	3.79	-		+	7.57	-		-		-		-		+	3.14
6	0.11	-		+	6.57	-		-		-		+	6.32	-		-		+	2.65	-	
7	0.12	-		-		-		-		-		-		+	7.64	-		-		-	
8	0.13	-		-		-		-		-		-		-		+	6.24	-		-	
9	0.20	+	7.93	-		-		-		-		-		-		-		-		-	
10	0.21	-		+	11.63	-		-		+	8.53	+	6.51	+	8.30	-		-		+	8.87
11	0.22	-		-		+	11.59	+	8.41	-		-		-		+	8.30	+	9.40	-	
12	0.27	+	4.09	-		-		-		-		-		-		-		-		-	
13	0.28	-		+	8.19	+	6.81	+	6.37	+	6.20	+	7.48	-		+	7.83	-		-	
14	0.29	-		-		-		-		-		-		+	7.04	-		+	6.25	-	
15	0.38	+	18.28	-		-		-		-		-		-		-		-		+	24.04
16	0.40	-		-		+	19.89	-		-		-		-		-		-		-	
17	0.41	-		+	23.63	-		+	24.03	+	15.24	+	7.61	-		+	16.17	-		-	
18	0.42	-		-		-		-		-		-		+	16.47	-		+	23.37	-	
19	0.48	-		-		-		-		-		+	8.01	-		-		-		-	
20	0.49	+	20.10	-		-		-		-		-		-		-		-		-	
21	0.52	-		-		-		-		+	13.86	-		-		-		-		+	14.82
22	0.53	-		-		-		+	8.69	-		+	9.78	-		+	11.61	+	14.04	-	
23	0.54	-		-		+	9.74	-		-		-		+	10.95	-		-		-	
24	0.59	+	20.39	-		-		-		-		-		-		-		-		-	
25	0.60	-		-		-		-		-		-		-		-		-		+	16.18
26	0.61	-		+	17.97	-		+	16.25	+	16.41	-		-		-		+	14.74	-	
27	0.62	-		-		+	19.07	-		-		+	20.05	+	15.41	+	15.99	-		-	
28	0.66	+	17.62	-		-		-		-		-		-		-		-		-	
29	0.67	-		-		-		-		-		-		-		-		-		+	24.14
30	0.70	-		+	13.93	+	13.00	+	11.24	-		+	17.18	+	16.60	+	15.72	+	22.14	-	
31	0.71	-		-		-		-		+	15.30	-		-		-		-		-	
32	0.77	-		+	7.10	+	5.78	-		+	8.95	-		-		-		-		-	
33	0.78	-		-		-		+	8.13	-		+	8.59	+	7.82	+	8.87	-		-	
34	0.83	+	5.80	-		-		-		-		-		-		-		-		-	
35	0.87	-		-		-		-		-		-		-		+	7.25	-		-	
36	0.88	-		-		-		-		-		-		-		-		-		+	7.83
37	0.89	-		-		-		-		-		-		+	7.35	-		-		-	
38	0.91	-		+	7.42	-		+	7.29	-		+	5.93	-		-		+	6.37	-	
39	0.92	-		-		+	6.00	-		+	4.82	-		-		-		-		-	

The similarity index (S.I.) and genetic distance (G.d.) were recorded between control and other treated samples as shown in Table (6). S.I. between LC_{25}, LC_{50} and LC_{70} of (CAU), LC_{25}, LC_{50} and LC_{70} of (BAU) and LC_{25}, LC_{50} and LC_{70} of (CAH) samples and their control were 0.11, 0.21, 0.00, 0.11, 0.00, 0.00, 0.00, 0.00 and 0.24 respectively.

Table 6: Similarity index and genetic distance between treated and untreated samples β-esterase larval tissue of *S. littoralis*

S.I

Samples		Cont.	CAU			BAU			CAH		
			LC_{25}	LC_{50}	LC_{70}	LC_{25}	LC_{50}	LC_{70}	LC_{25}	LC_{50}	LC_{70}
Cont.		-	0.11	0.21	0.00	0.11	0.00	0.00	0.00	0.00	0.24
CAU	LC_{25}	0.89	-	0.50	0.53	0.52	0.63	0.21	0.31	0.44	0.12
	LC_{50}	0.79	0.50	-	0.30	0.40	0.28	0.30	0.40	0.21	0.11
	LC_{70}	1.00	0.47	0.70	-	0.40	0.57	0.20	0.80	0.63	0.11
BAU	LC_{25}	0.89	0.46	0.60	0.60	-	0.29	0.10	0.30	0.21	0.33
	LC_{50}	1.00	0.37	0.72	0.43	0.71	-	0.48	0.57	0.42	0.11
	LC_{70}	1.00	0.79	0.70	0.80	0.90	0.52	-	0.30	0.31	0.11
CAH	LC_{25}	1.00	0.69	0.60	0.20	0.70	0.43	0.70	-	0.50	0.11
	LC_{50}	1.00	0.56	0.79	0.37	0.79	0.58	0.69	0.50	-	0.12
	LC_{70}	0.76	0.88	0.89	0.89	0.89	0.89	0.89	0.89	0.88	-

G.d

DISCUSSION

Effects of cyano acetyl urea (CAU), benzimidazolyl acetyl urea (BAU) and cyano acetyl hydrosylate (CAH) on Total haemocyte counts (THCs).

Data indicate that the response of total haemocyte counts (THCs) were negatively-related to concentrations of tested compounds, since THCs decreased with increase in the concentrations. THCs have been increased after treatments with LC_{25} and LC_{50} of cyano acetyl urea (CAU), LC_{25} of benzimidazolyl acetyl urea (BAU) & LC_{25} and LC_{50} of cyano acetyl hydrosylate (CAH). The increase in THCs could be attributed to the enhanced encapsulation of foreign/toxic molecules through process of melanization; melanin deposition during encapsulation is commonly initiated by haemocytes and/or phenoloxidase enzyme circulation in the plasma (Nappi and Christensen, 2005; Rolff and Siva-jothy, 2002). In addition, THC is also positively correlated with the rate of phagocytosis, nodule formulation, encapsulation, recognition of foreign bodies and wound healing (Lavine and Strand, 2002). Total number of haemocytes in haemolymph is likely to reflect the capability of immune system to deal with pathogens or chemical molecules (Kraaijeveld *et al.,* 2001).

Many insects posses populations of sessile haemocytes (Wigglesworth, 1972 and Ratcliffe and Gagen, 1976) which might be activated in response to infection. *Manduca* posses hemopoietic organs which produce haemoytes to the circulation and a small measurable population of circulating gametocytes that have been observed undergoing mitosis in other several insects (Jones, 1977). So, the remarkable increase in THCs may be due to the release of sessile haemocytes and the activation of mitotic division of the haemocytes. This finding coincided with that reported by Osman *et al*., (1984) in haemolymph of the larvae of *S. littoralis* after treatment with Dimilin; Horohov and Dunn (1982) on *M. sexta* larvae and Guzo & Stoltz (1987) who worked on *Orgyia leucostigma* larvae and found that the nodulation of smaller objects such as yeast cells were accompanied by a rapid and sustained increase in THCs. Also, Bakr *et al*., (2007) who observed increase in THCs of 6th larval instar of *S. littoralis* after treated with flufenoxuron and chlorfluazuron. Moreover, many authors reported that the increase of total circulating haemocytes counts could be considered as an immune response against pathogens (Chu *et al*., 1993; Ford *et al*., 1993 Anderson *et al*., 1995; Ordas *et al*., 2000 and Hassan & Ibrahim 2010).

On the other hand, THCs reduced after treatments with LC_{70} of cyano acetyl urea (CAU), LC_{50} and LC_{70} of benzimidazolyl acetyl urea (BAU) & LC_{70} of cyano acetyl hydrosylate (CAH). This drop in haemocytes number may be due to haemocytes engagement in nodule formation as that recorded by Abu El-Magd (1992). Such a decrease in the total number of haemocytes were reported by Ratcliffe and Gagen (1976) and Chain and Anderson (1982), they found that the THCs in *Galleria mellonella* dropped rapidly and immediately after injection of bacteria, and they commented that the decrease was almost entirely due to the depletion of plasmatocytes.

Effects of cyano acetyl urea (CAU), benzimidazolyl acetyl urea (BAU) and cyano acetyl hydrosylate (CAH) on differential haemocyte counts.

In the present study, five main haemocytes have been found in 6th larval instar haemolymph. They are plasmatocytes, prohemocytes, granulocytes, spherulocytes and oenocytoids. Description was based on the basis of cytological parameters such as cell shape, size, nuclear cytoplasmic

ratio and cytoplasmic inclusions, as well as staining affinity. Similar observations were also obtained by Miller and David (2000) who described five types of haemocytes in the 6th instar larvae of tobacco homworm haemolymph; plasmatocytes, prohemocytes, granulocytes, spherulocytes and oenocytoids. Similar observations were reported by Jian *et al.* (2003) in the larvae of *Ostrinia furnacalis.* However, Osman *et al*., (1984) described four types of haemocytes in the haemolymph of the 4th instar larvae of *S. littoralis* (prohemocytes, plasmatocytes, spherulocytes and oenocytoids).

The percentage of plasmatocyte increased relative to control in larvae treated with CAU (at LC_{25}), BAU (at LC_{25}) and CAH (at LC_{25}, LC_{50}, LC_{70}). Increasing in plasmatocytes might be the result of normal mitosis (Mall and Gupta, 1982) and less transformation of these cells into other haemocytes as these cells are involved in phagocytosis directly (Chapman, 1998). This increase could be due to the activation and proper functioning of haemopoietic organs. These organs are responsible for the production of plasmatocytes as reported by Tiwari *et al*., (2002).

Also, the percentages of prohaemocyte counts increased relative to control in larvae treated with CAU (at LC_{25}, LC_{50}, LC_{70}) BAU (at LC_{50}, LC_{70}), this stimulation of prohaemocytes may be attributed to the activation of haemopoieses and consequently the haemopoietic organs start to produce the prohaemocyte (Ayaad *et al.,* 2001). In contrast, the percentages of prohemocyte count decreased relative to control in larvae treated with CAU (at LC_{25}), and CAH (at LC_{25}, LC_{50}, LC_{70}). The prohaemocytes serves as stem cells in the haemolymph (Siliva *et al.,* 2002) and reduction in prohaemocytes could be correlated to the greater transformation of prohaemocytes into other type of cells which play their role in phagocytosis (Saxena and Srivastava, 2001; Bhatti,2002; Lavine and Strand, 2002).

Granular haemocytes are characterized by the possession of acidophilic granules which are membrane bounded. These are involved in the detoxification of chemicals and killing of microorganism through encapsulation and phagocytosis (Saxena and Srivastava, 2001; Chapman, 1998 and Steinhaus, 1949). The decrease observed in granulocytes after exposure to all concentrations for all compounds might be correlated with greater role played by granulocytes in detoxification through phagocytosis (Jose and Martin, 1989; Kurihara *et al*., 1992).

The spherulocytes percentages were increased for larvae treated with CAU (at LC_{25}, LC_{50}, LC_{70}) BAU (LC_{70}) and CAH (at LC_{25}). On the other hand, the percentages of spherulocyte decreased in larvae treated with BAU (at LC_{25}, LC_{50}) CAH (at LC_{50}, LC_{70}). Granular cells and spherule cells were reported to be the main cell types responsible for the rapid removal of foreign particles from the circulation via nodule formation in *Galleria* sp (Gagen and Ratcliffe, 1976). These cell types have been demonstrated to contact with test particles to spherule cells (Ratcliffe, 1975) and de-granulation of granular cells to produce sticky matrix involved in nodule formation (Gagen and Ratcliffe, 1976).

Results reveal that, all the percentages of oenocytoid counts were increased. Oenocytoids produce phenoloxidase and phenylalanine hydroxylase, (Hillyer and Christensen 2002; Hillyer *et al.,* 2003 and Johnson *et al.,* 2003) which are rate-limiting enzymes in the humoral melanization pathway (Infanger *et al.,* 2004 and Shiao *et al.,* 2001). Melanin deposition in arthropods is associated with wound repair, nodule formation and encapsulation (Ayaad *et al.,* 2001). The proportional percentage increase of

oenocytoids in treated larvae makes the latter explanation clear.

Isozymes:-

The esterases are very large class of enzymes, all of which can break an ester band with the help of a water molecule. Most enzymes of this class hydrolyze endogenous substances and are important in intermediary metabolism (Sivakumarm and Maya, 1991), but can also play a role in detoxification of xenobiotics (Shen and Dowd, 1991). Because most insecticides are ester of substituted phosphoric, carbamic, or cyclopropanecarboxylic acids, they are subjected to degradation by esterases (Devonshire, 1991). These esterases can often be separated into isozymes with different substrate specificities. Insect esterase is very diverse and can include monomer, dimmers and multimers, which means that their relative molecular mass can cover a wide range (Dauterman, 1985).

Also, esterase activity examined because of the importance of carboxylesterases in the catabolism of juvenile hormone (Slade and Zibitt, (1972); Whitmore *et al*., (1972); Ajami and Riddiford, (1973). JH esterase appears to be an important mode of regulation of JH titers in Lepidoptera (Venkatesh *et al*., 1987). The change in haemolymph JH metabolic activity appears to result from changes in JH specific esterases as 1 - naphthyl acetate esterase activity follows a different pattern during the reproductive /parental cycle (Scott *et al*., 2001).

In the present work polyacrylamide gel electrophoresis showed great differences in number of zones of esterase activity and in substrate specificity between treated and untreated samples. These findings are in full agreement with El-Bermawy, (2004) who analyzed esterases from body extracts of 6^{th} larval instar and newly formed pupa of *S. littoralis* produced from treated 2^{nd} larval instar by four botanicals using polyacrylamide gel electrophoresis and two substrates. Also, Hassan and Mohamed (2008) used polyacrylamide gel electrophoresis to detect the forms of esterase in the last larval instar of *P. gossypiella* treated as newly hatched larvae with LC_{50} of three volatile oils (*Petroselinum sativum, Coriandrum sativum* and *C. citratus*). The results showed great differences in number of zones of esterase activity and in substrate specificity between treated and untreated samples. Hassan and Abdel-hafez (2009) revealed that esterases were differed than normal pattern in 6^{th} larval instar of the cotton leaf worm, *Spodoptera littoralis* treated with two acetylcholine receptor modulator (Spinosad and Radiant). Helmy *et al.*, (2010) showed significantly high difference in AchE activity in all stages of *Culex pipiens* emerged from larval treatment with black liquor more than white liquor except pupal stage only showed significantly high difference in white liquor treatment more than black liquor.

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ARABIC SUMMERY

التغيرات الحادثة لخلايا دم وانزيم استيريز دودة ورق القطن بعد التعرض لمركبات مستخلصة من اليوريا وقش الارز

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اجريت هذه الداسة لتسجيل دور كل من خلايا الدم وانزيم الاستيريز لازالة السموم الناتجة من معاملة العمر اليرقى الرابع لدودة ورق القطن بثلاث مركبات حديثة مستخرجة من النفايات وهى :(سيانو اسيتيل يوريا وبنزيميزادوليل اسيتيل يوريا وهما مستخلصين من اليوريا) و(سيانو اسيتيل هيدروسيليت وهو مستخلص من قش الأرز) بتركيزات مختلفه. حيث سجلت التغيرات للعدد الكلى لخلايا الدم و كان متوسط العدد الكلي لكريات الدم في اليرقات غير المعامله هو 35.78±9430 خلية/ مم^3 . بينما ادت المعاملة الى زيادة في العدد الكلي لخلايا الدم في تلك اليرقات المعاملة ب مركبات السيانو اسيتيل يوريا عند LC_{25}, LC_{50} وبنزيميزادوليل اسيتيل يوريا عند LC_{25}و السيانو اسيتيل هيدروسييت عند LC_{25} LC_{50} كانت بنسب 21.00 ,1.90 ,14.31 ,19.01و 14.55 بالتتابع. كما لوحظ ان الخلايا الحبيبية سجلت انخفاضا في جميع التركيزات فيما عدا عند تركيز LC_{50}لمركب بنزيميزادوليل اسيتيل يوريا حيث زادت نسبتها قليلا حيث زادت بنسبة 15% فقط. وعلى النقيض زادت نسبة الخلايا الانوسيتيية في جميع اليرقات المعاملة فيما عدا عندLC_{70} للسيانواسيتيل هيدروسييت حيث لم تتغير بالمقارنة باليرقات غير المعاملة. وقد أظهر هذه الدراسة أنشطة ونظم انزيم الاستيريز لانسجة الطور اليرقى الاخير لدودة وق القطن و أوضحت النتائج أن 36 حزمة انزيمية فى العمر اليرقى الاخير اظهرت نشاط تجاه مادة اساس الفا اسيتات النفثالين بالنسبة لليرقات المعاملة و الغير معاملة وتراوح معامل الهجرة بين (0.01 و 0.28). بينما فى حالة الاستيريز باستخدام البيتا اسيتات النفثالين كمادة تفاعل وجد ان الفصل الكهربائى لانسجة اليرقة لكل من العينات المعاملة والغير معاملة تحتوى على 39حزمة من الخمائر مع معامل هجرة يتراوح بين (0.01و0.92).