EFFECT OF PARASITIZATION BY *APANTELES SYLEPTAE* F., PLANT EXTRACT AND CHEMICAL INSECTICIDE ON THE DIFFERENTIAL HAEMOCYTE COUNTS OF THE OLIVE LEAF MOTH, *PALPITA UNIONALIS* HÜBNER (LEPIDOPTERA: PYRALIDAE)

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INTRODUCTION

Olive, *Oleae europaea* L. (Oleaceaea) is an economically important crop in Mediterranean region and particularly in Egypt. The olive moth, *Palpita unionalis* Hübner (Lepidoptera: Pyralidae) infests flowers, fruit and leaves causing reduction of fruit setting, fruit drop and general weakening of trees. Pest control is a major concern of olive growers and its annual cost is added to associate crop losses contributes substantially to the total cost of olive production. In view of the environmental degradation by toxic pesticides, emphasis nowadays, is being put on natural products for controlling the pest populations. Also, the reduction of pesticides in olive orchards, the result of recent advances in olive pest management, allowed the increase of natural enemies of the pest commonly present in olive orchards and kept moth densities below economic threshold levels (Katsoyannos, 1992 and El-Khawas, 2000).

Insects possess an open circulatory system, which contains various types of haemocytes that perform physiological functions, including protection from pathogens. Due to this reason, the study of haemocytes has become an important area of research. Strand and Noda (1991), El-Maasarawy and El-Sheikh (1993) and Zohdy *et.al.* (2000) studied the haemocytic changes in the host larvae in response to parasitization. The pathological effects of botanicals on haemocytes have been reported in a few insect species by Rizk *et al.* (2001/2002) and Sharma *et. al.* (2003), despite the plants being a source of non-toxic compounds utilized for insect

control. Also, haemocyte changes after chemical treatments examined by Gupta and Sutherland (1968) and Rizk (1991) and Zohdy *et.al.* (2000).

Our investigation has been carried out to study the comparative effects of parasitization by *Apanteles syleptae* F. (Hymenoptera: Braconidae), LC_{50} levels of the botanical (natcom) and the chemical insecticide (diazinox) on quantitative and qualitative differential haemocyte counts (DHCs) of *Palpita unionalis* Hübner (Lepidoptera: Pyralidae).

MATERIAL AND METHODS

P. unionalis culture was started by collection of infested olive flowers from fields. It was reared in the laboratory, at constant temperature of 27±1°C and 65±5% R.H following the technique used by Shehata *et al.* (2003).

The solitary larval endoparasitoid, *A. syleptae* was collected from fields as cocoons during April till June and kept under laboratory conditions in glass vial (10 x 3.5 cm in diameter). The emerged adults were left in vials and provided with honey droplets to serve as food, for 24-hrs until mating. By using an aspirator, eight fertilized parasitoid females were picked from the rearing vials, and introduced into a plastic container containing 100 2nd instar larvae 5 day old of *P. unionalis* (El-Khawas, 2000). To ensure that parasitism took place, larvae were exposed to parasitoid females for six hours then removed. Parasitized larvae of *P. unionalis* were transferred to a clean breeding jar and provided daily with tender fresh olive leaves for feeding. The unparasitized larvae were daily removed whenever found to prevent attacking the parasitized ones. The parasitiod last instar larvae emerged from the host larvae and pupated inside cocoons, which they were collected into new vials until emergence of adults.

Commercial garlic extract (Natcom) was used at concentrations of 0.125, 0.25, 0.5 and1.00 ml. Diazinox (40% and 50% WP, 4 EC, 14% granules) were used at concentrations of 10, 20, 40 and 80 ppm.

Tenders of olive leaves were dipped in the different concentrations of natcom and diazinox and were left to dry before offered to 5-day-old (2nd larval instar) larvae of *P. unionalis*. After 48-hrs of natcom treatments, the survived larvae were transferred to untreated olive leaves. Three replicates of ten larvae each were placed in each cup (7x5cm). Control tests were conducted using leaves dipped in

water only and left to dry. Mortality rate was recorded daily and evaluated after 96-hrs for natcom and 24-hrs for diazinox treatments.

Haemocytes studies:

Five-day-old *P. unionalis* larvae were exposed to females of the endoparasitoid, *A. syleptae*. After 48 and 96-hrs, parasitized and treated larvae with LC_{50} levels of botanical and chemical insecticides were examined for differential haemocyte counts (DHCs). At the same ages, the (DHCs) were investigated for the unparasitized and untreated larvae as control.

DHCs were estimated by placing a drop of haemolymph on a clean glass slide and preparing the smear. The air-dried smears were fixed in absolute methyl alcohol for two minutes and left to dry. The smears were then stained in dilute Giemsa's stain, flushed off with water then the film was rinsed in distilled water and gently blotted dry with clean blotted paper. Quantitative DHCs were performed by determining the percentage of each type of haemocytes counted per about 150-200 cells per slide and replicated for 3 times (Arnold and Hinks, 1976). Qualitative DHCs were measured as biometric studies of nuclear and cells diameters by an ocular micrometer. The haemocytes were photographed under the microscope by using magnification of 1000x.

Mortality rates were corrected according to Abbott's formula (1925), the toxicity lines and LC_{50} values were calculated according to Finney (1971). The biometric measurements and the ratios of different haemocytes were statistically analyzed by ANOVA and mean values were separated by the least significant difference (L.S.D.) procedure (Snedecor and Cochran, 1980) at P = 5%.

RESULTS AND DISCUSSION

Effect of Natcom and Diazinox treatments:

Table (1) shows that the corrected mortality rates of *P. unionalis* larvae after 72 hours of fedding on tenders of olive leaves treated for 48 or for 24 hours with natcom and diazinox concentrations, respectively. The LC_{50} values were 0.23 ml and 16.75 ppm for natcom and diazinox treatments, respectively. Fodale and Mule (1990) used 0.03% methidathion, 0.14% fenthion, 0.06% dimethoate, 0.09% carbaryl and *Bacillus thuringiensis* but Albanese *et al.* (2000) sprayed azinphos-methyl and hexaflumuron for control of *P. unionalis* in the field.

Parasitization of *P. unionalis* larvae:

P. unionalis larvae were individually parasitized by the parasitoid *A. syleptae* to ensure parasitization. After 48 and 96-hrs post parasitism (haemolymph samples were taken), the parasitoid in egg and larval stages, respectively. Pinto and Salerno (1994) who found that the duration of egg incubation was about 46-48-hrs and the egg-larval development was an average of 12±0.2 -13.6±0.4 days.

TABLE (I)

Percentage mortality and comparative toxicity of Natcom (after 72 hours of treatments) and Diazinox (after 24 hours of treatments) against 5-day-old larvae of *P. unionalis*.

Concentrations (ml)	% Mortality	LC_{50}	Slope	Concentrations (ppm)	% Mortality	LC_{50}	Slope
Natcom				Diazinox			
0.00	3.33	0.23 ml (0.19:0.27)	2.03	0.00	0.00	16.75 ppm (14.39:19.19)	2.54
0.125	33.33			10	30.00		
0.25	53.33			20	53.33		
0.50	76.67			30	76.67		
1.00	90.00			40	100.00		

Haemocytes:

Seven types of haemocytes were detected in the haemolymph of *P. unionalis* larvae, according to the cell features and cell nucleus size relationships, they were prohaemocytes (PRs), phagocytic cells (plasmatocytes (PLs), spindle cells (SPIs) and granular haemocytes (GRs)), oenocytoids (OEs), adipohaemocytes (ADs) and spherulocytes (SPHs) (Table, 2 and 3 and Fig.1and 2). Arnold and Hinks (1976) recorded the functioning haemolymph cell types in lepidoptera. Zohdy *et.al.* (2000) and Rizk *et al.* (2001/2002) described eight types of haemocytes prohaemocytes, palsmatocytes, granular cells, spindle cells, adipohaemocytes, oenocytoids, spherule cells and cystocytes, in *Spodoptera littoralis*. The same haemocytes were found in *Sesamia cretica* (El-Mandarawy, 1997).

Tables (3 and 4) show the percentages of different haemocyte types after 48 and 96-hrs post parasitism by *A. syleptae* and treatment with LC_{50} levels of botanical extract and chemical insecticide.

Qualitative DHCs determination:

Qualitative DHCs of *P. unionalis* parazitized larvae and that treated with natcom and diazinox treatment caused some morphological changes in blood cells.

Biometric measurements of different haemocytes and the nuclear-cell ratio for larvae at the control, 48 and 96-hrs of parasitization are shown in Table. 2 and 3 and fig., 1&2. Parasitization of *P. unionalis* larvae by *A. syleptae* caused irregularity and elongation of cell wall with twisting at the ends of spindle cells. Mitotic divisions in PLs nuclei were observed with badly and faintly stained of the cytoplasm of all cells. These observations confirmed by El-Mandarawy (1997) and Zohdy *et.al.* (2000). The botanical product, natcom caused changes in PLs by inducing the appearance of cytoplasmic vacuoles, moving the nucleus to cell wall with losing its central position and revealed destruction of plasma membrane (Rizk *et.al.*, 2001/2002). Chemical insecticides, diazinox led to various cytopathological changes summarized in: GRs shrinkage, cytoplasmic vacuolization in PLs with irregularity in cell shape as compared to control. Also, a reduction in the nuclear and cell diameter was appeared (Saxena and Saxena, 1985; Rizk, 1991).

Quantitative DHCs determination:

Quantitative DHCs in larvae under the influence of parasitization, natcom and diazinox treatments revealed a decrease of PRs, SPHs and OEs and an increase in PLs, GRs and SPIs at different larval ages (Tables, 4 and 5). Parasitization decreased the ratio of PRs from 56.67 and in the control to 39.55 and 36.00% after 48 and 96-hrs post parasitism, respectively. While the percentage of PLs were 9.66 and 12.78% for unparasitized larvae and 20.67 and 22.89% for parasitized ones after 48 and 96-hrs post parasitism, respectively. After 48-hrs of parasitism, the SPHs and OEs decreased to 4.00 and 4.67% but dropped after 96-hrs of parasitism to 3.89 and 4.11%. The increase of PLs were responsible for defence against the invading organisms thus emphasizing their function against the parasiloid, but a decrease in SPHs and OEs for parasitized larvae, probably due to the consumption of SPHs by parasitoid larvae or the use of OEs in healing wounds. These results agree with those of Zohdy *et.al.* (2000) on *S. littoralis* parasitized by *Microplitis rufiventris* Kok., El-Mandarawy (1997) on *S. cretica* parazitized by the ectoparasitoid *Bracon brevicornis* and El-Maasarawy and El-Sheikh (1993) on *Mythimna loreyi* parasitized by *Meteorus gyrator*.

The botanical extract, natcom decreased the percentages of PRs to 45.00 and 40.44% after 48 and 96-hrs post treatment. But PLs, GRs and SPIs increased to 15.33, 20.33 and 5.22% after 48-hrs post treatment and to 19.78, 21.00 and 6.45 after 96-hrs post treatment, respectively. The ratio of SPHs and OEs represent 5.11 and 5.78% in control and increased to 4.67 and 5.33% after 48-hrs of treatment, while the ratios were 4.00 - 4.67% after 96-hrs of treatment, respectively. The

TABLE (II)

Biometric measurements of different haemocytes of *P. unionalis* larvae after 48 h post parasitization and natcom or diazinox treatments.

Treatment	Length			Width		
	Cell	Nucleus	Nuclear cell ratio	Cell	Nucleus	Nuclear cell ratio
Prohaemocytes						
Control	8.1a	7.3a	89.60	7.1a	6.1a	86.32
Parasitization	7.0bc	6.6a	93.63	6.4a	5.5b	84.97
Natcom	7.4ab	6.7a	90.14	6.6a	5.3b	80.81
Diazinox	6.2a	4.9b	79.03	5.3b	4.0c	74.38
L.S.D.	0.85	1.01		.69	.60	
Plasmatocytes						
Control	15.0a	10.0a	66.67	14a	9.0a	64.29
Parasitization	15.2a	7.9b	52.19	9.3b	5.1b	55.20
Natcom	13.1b	7.3b	55.87	11c	6.4c	58.18
Diazinox	11c	5.2c	46.97	9.7bc	4.2d	43.64
L.S.D.	1.23	0.87		1.34	0.87	
Granular haemocytes						
Control	11.3a	8.0a	70.80	٩.٢a	6.7a	73.09
Parasitization	16.5b	١٢.٣b	74.34	16.0b	10.5b	65.63
Natcom	13.0c	10.0c	76.92	11.0c	9.0c	81.82
Diazinox	11.2a	7.3a	65.37	9.0a	6.3a	70.00
L.S.D.	0.72	1.17		0.81	0.88	
Spindle cells						
Control	11.7a	7.3a	62.4	8.3a	6.0a	72.00
Parasitization	12.7a	7.2ab	56.8	7.0b	5.0ab	71.43
Natcom	15.5b	6.8b	44.1	5.2c	4.3b	82.17
Diazinox	14.0c	6.1c	43.6	5.7c	4.0b	70.18
L.S.D.	0.98	0.46		0.77	1.27	
Adipohaemocytes						
Control	16.4a	4.3ac	٢٦.٢	16.0a	4.0a	25.00
Parasitization	20.5b	6.0b	٢٩.٢٧	16.5a	4.5a	27.27
Natcom	20.0b	5.3ab	26.50	15.7a	4.0a	٢٥.٤٨
Diazinox	14.0c	4.0c	28.57	7.0b	2.5b	35.71
L.S.D.	0.87	0.09		1.09	0.55	
Spherulocytes						
Control	8.0a	3.9	48.75	6.0a	2.3a	38.33
Parasitization	9.4bc	4.8	51.06	8.3b	3.7bc	44.58
Natcom	10.0b	5.2	52.00	9.9c	4.1b	41.41
Diazinox	9.0c	4.8	53.33	8.6b	3.3c	38.37
L.S.D.	0.85	N.S.		1.01	0.52	
Oenocytoids						
Control	10.0a	5.0	50.00	8.0a	4.2	52.50
Parasitization	9.0ab	4.0	44.44	6.0b	3.0	50.00
Natcom	7.5bc	4.3	57.33	6.7c	3.8	56.72
Diazinox	8.0c	5.0	62.5	7.7a	4.5	58.44
L.S.D.	1.17	N.S.		0.69	N.S.	

Different alphabetical letters indicate significant difference between each two treatments.

TABLE (III)

Biometric measurements of different haemocytes of *P. unionalis* larvae after 96 h post parasitization and natcom or diazinox treatments.

Treatment	Length			Width		
	Cell	Nucleus	Nuclear cell ratio	Cell	Nucleus	Nuclear cell ratio
Prohaemocytes						
Control	9.1a	7.5a	82.05	8.4a	6.9a	81.72
Parasitization	8.6a	6.7b	78.21	7.6b	6.2b	80.68
Natcom	8.3a	7.0ab	83.60	6.5c	5.4c	82.14
Diazinox	6.7b	5.3c	73.89	5.9c	4.3d	72.88
L.S.D.	1.03	0.72		0.78	0.58	
Plasmatocytes						
Control	16.7a	10.5a	62.87	14.9a	9.2a	61.97
Parasitization	14.5b	7.8b	54.02	12.5b	6.7b	53.60
Natcom	16.2a	8.2b	50.41	10.0c	5.6b	56.00
Diazinox	13.0c	5.4c	41.54	9.3c	4.4c	47.31
L.S.D.	1.11	0.79		1.21	1.16	
Granular haemocytes						
Control	13.3a	8. 7a	65.00	11.3a	7.7a	68.14
Parasitization	12.5a	9.0a	72.00	10.0b	8.0a	80.00
Natcom	17.3b	11.9b	68.59	16.0c	11.0b	68.75
Diazinox	13.0a	9.5a	73.08	11.0bc	9.0c	81.8٢
L.S.D.	1.58	1.28		1.24	0.62	
Spindle cells						
Control	15.6a	8.9a	57.05	10.0a	6.3a	63.00
Parasitization	13.3b	8.8a	66.00	7.7b	6.0ab	77.92
Natcom	16.0a	9.0a	56.25	9.0a	5.0bc	55.56
Diazinox	15.0a	7.3b	48.89	7.4b	4.2c	56.76
L.S.D.	1.24	0.67		1.11	1.11	
Adipohaemocytes						
Control	18.0a	4.4ac	24.44	17.0a	3.7a	21.76
Parasitization	25.5b	6.2b	24.31	21.0b	3.0ac	14.29
Natcom	21.5c	6.0ab	27.9١	18.0a	4.5b	25.00
Diazinox	19.0d	5.0c	26.3٢	12.0c	2.9c	24.17
L.S.D.	0.84	1.10		1.97	0.74	
Spherulocytes						
Control	8.5a	4.5a	52.94	6.5a	2.7a	41.54
Parasitization	9.7b	4.7a	48.45	8.7b	4.7b	54.02
Natcom	10.6c	6.0b	56.60	10.2c	5.9c	57.84
Diazinox	11.0c	5.0a	45.45	10.0c	3.8d	38.00
L.S.D.	0.85	0.81		1.04	0.65	
Oenocytoids						
Control	11.2a	5.6	50.00	9.3a	4.3	46.2٤
Parasitization	10.5a	5.7	54.2٩	8.0b	4.0	50.00
Natcom	10.7a	6.0	56.07	9.0ac	4.6	51.11
Diazinox	8.3b	5.0	60.24	8.5bc	4.7	55.29
L.S.D.	0.74	N.S.		0.72	N.S.	

Different alphabetical letters indicate significant difference between each two treatments.

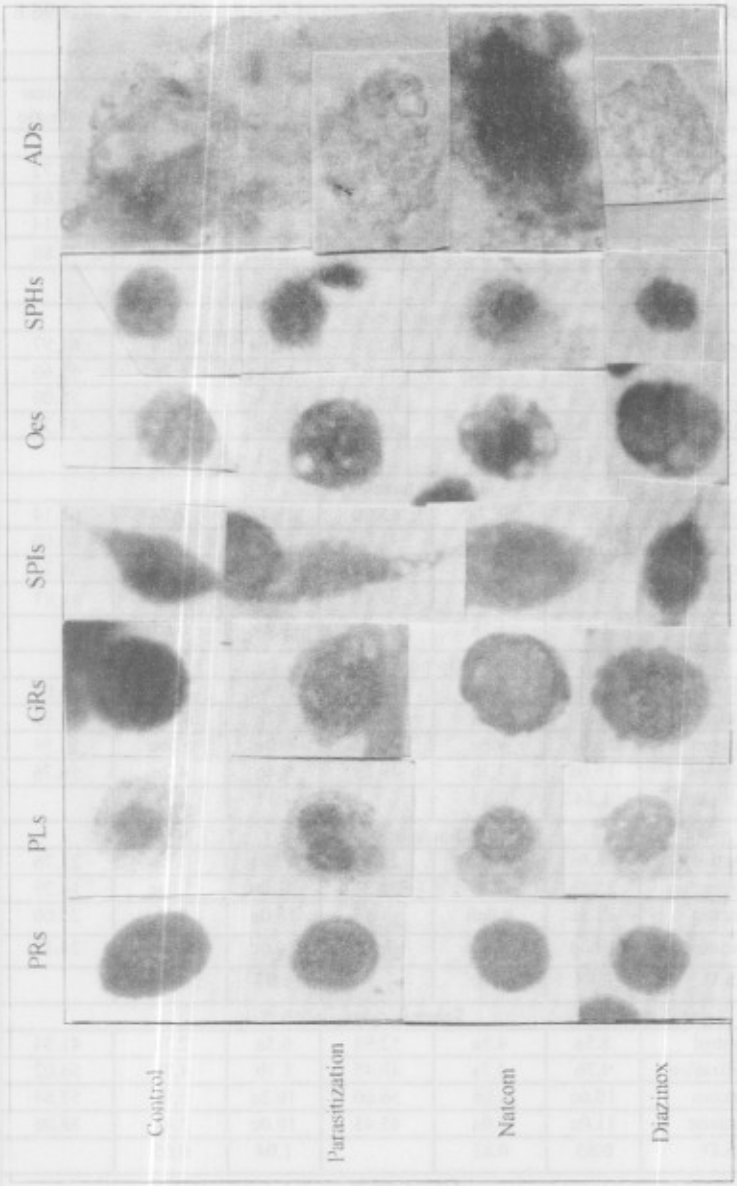

Fig. (1): Different haemocyte types of *P. unionalis* larvae after 48 h post parasitization by *A. sylleptae* or treatments with natcom and diazinox (1000X).

respect ratios of ADs were 4.11 and 3.67% after 48 and 96-hrs of treatment (Tables, 4 and 5). Takeda and Kitano (1971) confirmed our foundation and stated that PLs, GRs and SPIs of *Pieris rapae* increases might be due to their roles in phagocytosis and prohaemocytes that decreased and transformed to phagocytic cells. Rizk *et al.*

(2001/2002) found reduction in the PRs of *S. littoralis* treated with azadrachtin and margosan-O than in control.

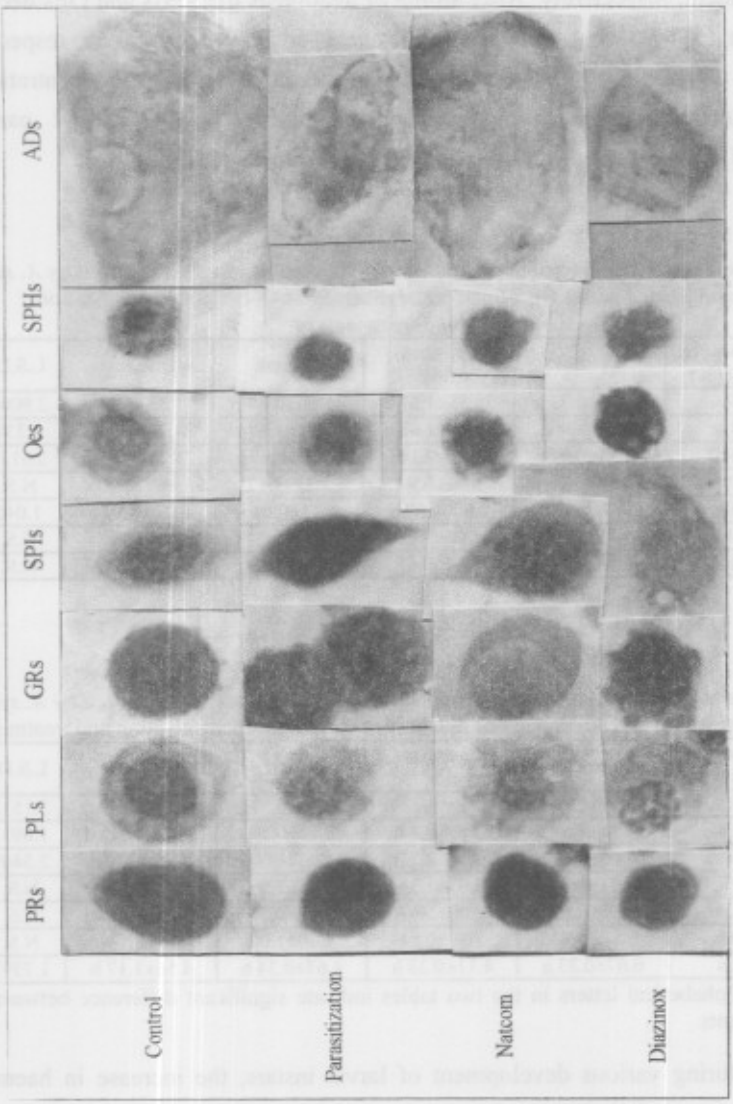

Fig. (2): Different haemocyte types of *P. unionalis* larvae after 96 h post parasitization by *A. sylepiae* or treatments with natcom and diazinox (1000X).

As compared to the chemical insecticide, diazinox caused more decrease or increase in the percentage of different cell types followed by parasitization of natcom treatment, in the two developmental stages. The percentage PRs decreased

to 31.33 and 28.00% than control, at the same time the PLs ratios increased to 25.22 and 25.33% after 48 and 96-hrs post treatment, respectively. GRs and SPIs increased to 25.22 and 6.67 after 48-hrs post treatment and to 26.22and 8.67% after 96-hrs post treatment, respectively. After 48-hrs of treatments the SPHs and OEs decreased to 3.67 and 4.67 % but after 96-hrs they reached 3.89 and 4.56 %, respectively. Ambrose and George (1996) stated that the effect of sub-lethal concentrations of three insecticides namely monocrotophos, dimethoate and methyl parathion decreased the number of PRs of *Acanthaspis pedestris* Stall.

TABLE (IV)

Percentage of differential haemocyte counts for *P. unionalis* larvae parasitized by *A. syleptae* and treated with LC_{50} of natcom or diazinox at 48-hrs post-parasitization and treatments.

Haemocyte types (%)	Control	Parasitization	Natcom	Diazinox	L.S.D.
PRs	56.67±0.33 a	39.55±1.67 b	45.00±0.88 c	31.33±1.34 d	2.9006
PLs	9.66±0.88 a	20.67±1.67 b	15.33±1.67 c	24.22±1.34 d	2.331
GRs	15.33±0.67 a	23.33±2.34 b	20.33±0.67 c	25.22±1.17 b	2.6139
SPIs	4.67±0.33	5.11±0.84	5.22±1.17	6.67±1.33	N.S.
ADs	2.78±0.19 a	2.67±0.67 a	4.11±0.84b	4.22±0.19 b	1.040
SPHs	5.11±2.00	4.00±1.00	4.67±1.33	3.67±0.58	N.S.
OEs	5.78±1.34	4.67±0.67	5.33±0.34	4.67±0.33	N.S.

TABLE (V)

Percentage of differential haemocyte counts for *P. unionalis* larvae parasitized by *A. syleptae* and treated with LC_{50} of natcom or diazinox at 96-hrs post parasitization and treatments.

Haemocyte types (%)	Control	Parasitization	Natcom	Diazinox	L.S.D.
PRs	49.89±2.84 a	36.00±0.33 b	40.44±0.51 c	28.00±1.67 d	3.1512
PLs	12.78±1.51 a	22.89±0.84b	19.78±0.39 c	25.33±0.33 d	1.6931
GRs	16.00±0.33 a	24.45±2.27b	21.00±1.00 c	26.22±1.02 b	2.5409
SPIs	6.11±1.17	6.67±1.34	6.45±1.95	8.67±0.67	N.S.
ADs	3.33±0.67	2.00±1.67	3.67±0.58	3.33±0.67	N.S.
SPHs	5.22±0.84	3.89±0.84	4.00±1.00	3.89±1.84	N.S.
OEs	6.67±0.33 a	4.11±0.38 b	4.67±0.34 b	4.56±1.17 b	1.7397

Different alphabetical letters in the two tables indicate significant difference between each two treatments.

During various development of larval instars, the increase in haemocyte counts occurred for plasmatocytes, granulocytes and oenocytoids. PRs did not increase linearly during development. This agrees with Hazarika and Gupta (1987) who indicated that quantitative and qualitative changes in haemocyte population of *Blatella germanica* (L.) occurred with the ontogenic developed from one instar to the next, till reaching the adult stage, but the prohaemocytes did not increase during development.

Finally, we can conclude that parasitization, plant extract and chemical insecticide controlled pests by the suppression of their defence reactions, where DHCs revealed a decrease in PRs, SPHs and OEs with an increase of the phagocytic cells (PLs, GRs and SPIs). Chemical insecticide caused more changes on the shape and percentage of the haemocytes followed by the parasitization then the plant extract treatments. The increase or decrease of the different cell types became more with the passage of time after treatments.

SUMMARY

The quantitative and qualitative differential haemocyte counts (DHCs) were studied on the olive moth, *Palpita unionalis* Hübner (Lepidoptera: Pyralidae), for 48 and 96-hrs post parasitization by *Apanteles syleptae* F.(Hymenoptera: Braconidae) (for egg and larval stages of parasitoid inside host) or that treated with LC_{50} levels of the botanical product (natcom) and the chemical insecticides (diazinox). Seven types of haemocytes were recognized in the haemolymph of *P. unionalis* larvae, according to the cell features and cell nucleus size relationships, they were prohaemocytes (PRs), phagocytic cells (plasmatocytes (PLs), spindle cells (SPIs) and granular haemocytes (GRs)), oenocytoids (OEs), adipohaemocytes (ADs) and spherulocytes (SPHs). Qualitative DHCs were measured as a biometric studies of nuclear and cells diameters of different haemocytes and the ratios between nuclei and cells were determined in the parasitized and treated *P. unionalis* larvae as compared to control after 48 and 96-hrs. According to the different larval treatments, some morphological changes as the abnormalities in the blood cells were occurred. Parasitization caused irregularity and elongation of cell wall with twisting at the ends of spindle cells. Natcom caused changes in PLs by inducing the appearance of cytoplasmic vacuoles, moving the nucleus to cell wall with losing its central position. Diazinox led to cytopathological changes as shrinkage in and vacuolization in PLs. Also, reduction in the nuclear and cell diameter occurred resulting to the different treatments. Quantitative DHCs in larvae under different treatments revealed a decrease of PRs, SPHs and OEs and an increase in phagocytic cells (PLs, GRs and SPIs) at different larval ages. Quantitative and qualitative changes in haemocyte counts occurred with the ontogenic developed from one instar to the next, till reaching the adult stage, but the prohaemocytes did not increase linearly during development.

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