Egypt. J. Agric. Res., 78 (4), 2000.

EFFECT OF DELFIN AND BAYTHROID ON THE TOTAL HAEMOCYTE COUNTS (THCS) AND HAEMOCYTE PERCENTAGE IN HEALTHY AND PARASITIZED LARVAE OF THE COTTON LEAFWORM

NAWAL ZOHDY[1], ALY A. EL-MOURSY[1], ESMAT A. KARES[2], AMINA M. ABDEL-RAHMAN[1] AND MONA B. R. EL-MANDARAWY[2]

1 Department of Entomology, Faculty of Science, Cairo University.

2 Plant Protection Research Institute, Agricultural Research Centre, Dokki, Giza.

(Manuscript received 7 July, 1999)

Abstract

Second instar larvae of *Spodoptera littoralis* were treated with LC_{10} level of delfin for 48 hours and with baythroid for 24 hours. The haemolymph of 5, 7, 9 and 11 days host larvae *i.e.* at 1, 3, 5 and 7 days after parasitism was then investigated.

After 1 days of parasitism with *Microplitis rufiventris* Kok. where the egg stage was inside the host, no significant difference in the total haemocyte count (THC) was found between unparasitized and parasitized larvae. After three days of parasitism, where the first larval instar of the parasite was inside the host larvae, the THCs of the parasitized larvae decreased insignificantly as compared to the healthy ones. While after five or seven days of parasitism where the second or third parasitic larval instars were inside the host, a highly significant decrease in THCs occurred as compared to those of unparasitized larvae. When healthy and parasitized larvae were treated with LC_{10} level of delfin or baythroid, THCs decreased significantly as compared to those of the control at different host larvae.

Larvae of *S.littoralis* have eight types of haemocytes prohaemocytes, phagocytic cells (palsmatocytes, spindle and granular cells), oenocytoids, adipohaemocytes, spherulocytes and cystocytes.

The phagocytic cells (palsmatocytes, spindle cells and granular cells) in parasitized larvae increased as compared to those of unparasitized ones of the same ages. While prohaemocytes of parasitized larvae became reduced in number as compared to those of unparasitized larvae. The reduction in prohaemocytes may be due to prohaemocytes transformation into phagocytic cells, which are responsible for defense against invading organisms emphasizes their against the parasitoid. The spherulocytes and oenocytoids decreased in parasitized larvae than in the unparasitized ones.

After treatment of parasitized larvae with delfin or baythroid, the phagoyctic cells increased more than those of unparasitized and parasitized larvae increased than those of control.

During various developmental instars of *S.littoralis* larvae, the increase in haemocyte counts occurred in plasmatocytes, granulocytes and oenocytoids. While the prohaemocytes did not increase during development.

INTRODUCTION

Blood is an important tissue medium through which invading microorganisms are ultimately carried to sites proper for them, qualitative and quantitative changes in blood occur due to pathogenic microorganisms (Ramakrishnan and Tiwari, 1972).

Rizk (1991) was one of the authors who dealt with the classification of blood cells of *S. littoralis*. Several authors studied the haemocytic defense reactions and changes in the host larvae in response to parasitization (Vinson, 1971; Kitano, 1974; Osman *et al.*, 1982-1983; Strand and Noda, 1991; El-Maasarawy and El-Sheikh, 1993).

Haemocyte changes after treatments with *B.thuringiensis* were examined by Gagen and Ratcliffe (1976) and Kares *et al.* (1992).

Chemical effects of haemocytes of different insects were investigated by Gupte and Sutherland (1968) and Rizk (1991).

The present investigation was carried out to study the effect of *B.thuringiensis* (delfin) and a chemical insecticide (baythroid) on the total haemocyte counts (THCs) and haemocyte percentage of *S. littoralis* healthy and parasitized larvae by Microplitis rufiventris Kok. (at 5, 7, 9 and 11 days of host larvae i.e. 1, 3, 5 and 7 days after parasitism).

MATERIALS AND METHODS

1. Rearing of the cotton leafworm, *Spodoptera littoralis* (Boisd.): The cotton leafworm (the host) was reared under constant conditions of 28±1 ^{o}C and 65±4% R.H. using the technique described by Ibrahim (1974).

2. Rearing of *Microplitis rufiventris* Kok.: The larval endoparasite *Microplitis rufiventris* was reared under the same technique described by Kares *et al.* (1998).

3. Materials used

3.1. Bioinsecticide (Delfin): Delfin, a selective bacterial insecticide containing 53X10^{6} S.U. (Spodoptera Unit) of *B. thuringiensis* var. *kurstaki* / g of product.

3.2. Chemical insecticide (Baythroid): Baythroid 5% E.C. Formulation: Emulsifiable concentrate containing 50g a.i./liter. (Cyfluthrin) Cyano-(4-fluoro-3-phenoxybenzyl)-methyl-3-(2, 2-dichloroethyl)-2, 2-dimethyl-cyclo-propane carboxylate used at a rate of 3.7g a.i./feddan.

4. Treatments: Experiments were carried out to study the effect of the bioinsecticide (delfin) and the pyrethroid insecticide (baythroid) on the total and differential haemocyte counts (quantitative) of unparasitized *S.littoralis* larvae and those parasitized by different developmental stages of *M.rufiventris* (egg stage; and 1st, 2nd and 3rd larval instars of the parasitoid). Therefore, the haemolymph was taken from:

1. Five days old *S.littoralis* host larvae containing eggs of the endoparasitoid *M.rufiventris*.

2. Seven days old *S.littoralis* host larvae containing 1st instar larvae of the parasite.

3. Nine days old *S.littoralis* host larvae containing 2nd instar larvae of the parasite.

4. Eleven days old *S.littoralis* host larvae containing 3rd instar larvae of the parasite.

5. Treated unparasitized S.littoralis larvae used at the same age of each treated parasitized larvae.

All the above mentioned larvae were treated with the sublethal concentration LC_{10} level of either delfin or baythroid. The suitable age for *M.rufiventris* parasitism was the second instar *S.littoralis* larvae, so, it was of importance to determine LC_{10} level of each of delfin and baythroid at this age (to determine their effects on the haemolymph of unparasitized *S.littoralis* larvae and those parasitized by the different developmental stages of *M.rufiventris*.

6. Control experiments for untreated unparasitized and parasitized larvae.

To determine LC_{10} of delfin after 72 hours from the beginning of treatment and LC_{10} of baythroid after 24 hours against the unparasitized second instar of *S.littoralis* larvae [the same technique and the same concentrations were used by Kares *et al.*, (1998)].

A proleg on the sixth abdominal segment was snipped off using a pair of fine scissors and the haemolymph was allowed to ooze on a clean, grease-free microscopic slide.

5. Determination of total haemocyte counts (THCs): Total haemocyte counts (THCs) is the number of cells per mm^3 of haemolymph. To determine the total haemocyte counts, haemolymph was allowed to flow onto a clean glass slide. A portion of the blood was quickly drawn to 0.5 mark of Thoma white blood cell diluting pipette. This tip was then diluted to mark 11 Tuerk's solution (1-2% glacial acetic acid, slightly

coloured with gentian violet). The haemocytes were counted in the improved Meanbauer ruling. Cells were counted in four corners and multiplied by a factor of 50 to give the number of cells per cubic millimeter. If the cells were clumped or were otherwise unevenly distributed in the chamber, the preparation was discarded. This experiment was replicated 4 times.

6. Determination of differential haemocyte counts DHCs: For determning the differential haemocyte counts, blood films were made by applying one end of a slide to a drop of blood and the slide placed on a leveled surface, holding it with the thumb and index fingers of the left hand. The narrow edge of another slide was placed in the drop and held there till the blood has spread across it. It was then drawn slowly over the whole length of the first slide. The inclination of the second to the first should be 45 and there should be no pressure what so-ever between the two surfaces.

The more slowly one slide is drawn over the other, the thinner is resulting film. Smooth spreading of the film was aided by warming the first slide on the flame of spirit-lamp before applying it to the drop of blood. After the blood was spread, it was dried by being waved rapidly in air to prevent undue shrinkage of the cells.

Staining of films: The dry film was fixed in absolute methyl alcohol for 2 minutes and dried in air. The dry film was well covered with Giemsa stain, flushed off with distilled water then the film was rinsed in distilled water and gently blotted dry with clean blotting paper.

Quantitative analysis of blood haemocytes: Determination of the percentage of each type of haemocyte cells per about 150-200 cells per slide for 3 times.

Statistical analysis: Data obtained for differential haemocyte counts (qualitative) were statistically analyzed using T and F tests at 0.05 and 0.01 levels of probabiltiy differential haemocyte counts (Qauntitative) were statistically analyzed using T-tests at 0.05 and 0.01 levels of probability.

RESULTS AND DISCUSSION

1. Determination of LC_{10} of delfin (after 72 hours) and baythroid (after 24 hours) for unparasitized second instar *S.littoralis* larvae: Table 1 shows that the corrected mortality after 72 hours of the unparasitized second instar *S.littoralis* larvae fed on castor-bean leaves treated for 48 hours with delfin concentrations and for 24 hours with baythroid concentrations. The LC_{50} values of larvae

treated with delfin and baythroid were 10.5 x 10^4 S.U. and 37 ppm, respectively. The calculated LC_{10} level of each of delfin and baythroid from their LC_{50} levels were 2.1x10^4 S.U. and 0.74 ppm, respectively, Table 2.

1.1 Effect of delfin and baythroid on the total haemocyte counts (THCs) of healthy *S.littoralis* larvae and those parasitized by *M.rufiventris*

After the first day of parasitism: Table 3 shows that the total haemocyte counts differed insignificantly in cases of unparasitized and parasitized larvae at the same age, being 8125 and 8085 h/mm3, respectively, with a reduction of only 0.49%. Taking into consideration that the egg incubation period of the parasite *M.rufiventris* was 1.23 days in the same host larvae and at the same temperature (Kares *et al.* (1998). So, it can be concluded that the presence of the parasitoid egg stage did not induce any significant effect on the THCs of the host larvae.

These results are in agreement with those of Vinson (1971) who reported that there was no difference in the YHCs of *Heliothis virescens* larvae and those parasitized by *Cardiochiles nigriceps* three days after parasitism. The author did not also find a significant difference between the THCs means at the fourth instar *Pieris rapae* crucivora larvae and those parasitized by *Apanteles glomeratus*.

THCs of *S.littoralis* larvae treated with LC_{10} levels of delfin and baythroid were 6130 and 5195 h/mm3 for parasitized larvae, respectively, Table 3. The reduction in THCs from control of larvae treated with delfin and baythroid were 24.65 and 36.06% for unparasitized larvae and 25.17 and 34.94% for parasitized larvae, respectively.

After three days of parasitism (where the first larval instar of *M.rufiventris* being inside *S.littoralis* host larvae 7 days old: The THCs decreased insignificantly between healthy and parasitized larvae.

After five and seven days of parasitism (9 and 11 days old of host larvae containing the 2nd or 3rd larvae instars of parasite): The THCs showed a high significant reduction as compared to the unparasitized larvae with those parasitized ones, Table, 3.

The results of THCs reduction in the haemolymph of larvae of *S.littoralis* parasitized by the larval instars of parasitoid are in agreement with those of Osman *et al.* (1982-1983) which show that the THCs considerably decreased by 47.5% in the haemolymph of *S.littoralis* larvae after the 6th day of parasitism by *M.rufiventris*. Vinson

Table 1. Corrected mortality percentage of unparasitized *S.littoralis* larvae fed on castor-bean leaves treated with different concentrations of delfin and baythroid.

Delfin (after 72 hours)		Baythroid (after 24 hours)	
Concentration S.U.	% Cumulative mortality	Concentration ppm	% Cumulative mortality
0.00	3.33	0.00	3.33
$4x10^4$	20.0	20	30.00
$8x10^4$	36.66	30	53.33
$12x10^4$	53.33	40	70.00
$16x10^4$	66.66	50	83.33
$20x10^4$	80.00	60	93.33
$24x10^4$	90.00		

Table 2. Comparative toxicities of the unparasitized *S.littoralis* larvae fed on castor-bean leaves treated with delfin and baythroid.

Treatment	LC_{50}	Slope	Confidence limits (P 0.05)	
			LC_{50}	Slope
Delfin	10.50 S.U.	2.16	12.29 : 8.97	2.72 : 1.71
Baythroid	37.00 ppm	2.21	44.3 : 31.09	3.29 : 1.48

Table 3. Total number of haemocytes/mm3 in haemolymph of healthy *S.littoralis* larvae and those parasitized by *M.rufiventris* treated LC_{10} levels of delfin and baythroid.

Age of host larvae (days)	Days after Parasitization (stage of parasite inside the host)	Treatments	Average±S.E. of number of haemocytes/mm^3		T-test	% Reduction		
			Unparasitized larvae	Parasitized larvae		For treatments compared to control		Between Parasitized and unparasitized larvae
						Unparasitized larvae	Parasitized larvae	
5	1(egg)	Control	8125±721.5a	8085±612.9a	N.S.			
		Delfin	6130±401.3ab	6050±559.4b	N.S.	24.56	25.17	0.49
		Baythroid	5195±408.3b	5260±315.2b	N.S.	36.06	34.94	1.31
		L.S.D.	2129.61	1639.51				+1.25
7	3 (1st larval instar)	Control	10195±652.6a	9075±686.0a	N.S.			
		Delfin	7225±646.9b	6260±621.5b	N.S.	29.13	31.02	10.99
		Baythroid	6275±876.7b	5905±960.6b	N.S.	38.45	34.93	13.36
		L.S.D.	2345.84	2464.2				5.90
9	5 (2nd larval instar)	Control	14255±403.4a	8425±538.2a	H.S.			
		Delfin	10315±907.3b	5955±390.1b	H.S	27.64	29.32	40.90
		Baythroid	8865±191.4b	5005±424.3b	H.S	37.81	40.59	42.27
		L.S.D.	1867.73	1456.56				43.54
11	7 (3rd larval instar)	Control	17375±483.3a	74.05±453.9a	H.S			
		Delfin	12175±537.5b	2780±625.0b	H.S	29.93	62.46	57.38
		Baythroid	9795±832.1c	2075±496.9b	H.S	43.63	71.98	77.17
		L.S.D.	2035.82	1696.43				78.82

(1971) also found that the THCs of *H.virescens* larvae parasitized by *C.nigriceps* decreased significantly after 6 days as compared to those of unparasitized larvae.

The reduction in THCs of larvae treated with delfin and baythroid after 5 and 7 days of parasitism and the reduction of THCs when comparing the uparasitized larvae with those parasitized are shown in Table 3. The results indicate that there is a highly significant reduction in the THCs when comparing the unparasitized larvae with those parasitized after treatments with delfin and baythroid.

For Bioinsecticide treatments: These results of THCs reduction in the haemolymph of *S.littoralis* larvae treated with delfin are in agreement with those of Gagen and Ratcliffe (1976) who reported that injections of killed *Bacillus cereus* caused highly significant decrease in haemocyte counts in *G.mellonella* and *Pieris brassicae* larvae. Also, Kares *et al.* (1992) found THCs decreased after the treatment of *Pieris rapae* larvae with LC_{10} and LC_{50} levels of bactospeine.

As to chemical insecticide treatments: Results of the present study agree with those of Gupta and Sutherland (1968) using chlordane, which were found to cause a fall in total haemocyte count of *Periplaneta americana.* On the other hand, Rizk (1991) found the THCs of *S.littorlis* larvae treated with fenvalerate or sumithion insignificantly increased.

1.2. Quantitative analysis

1.2.1. For host larvae containing the egg stage of the parasitoid

Table 4 shows the percentages of each type of haemocytes per about 150-200 cells per slide.

After the first day of parasitization: The percentage of prohaemocytes was significatly highly reduced from 59.0% in unparasitized larvae to 36.0% in parasitized larvae. The reduction in prohaemocytes is mostly due to their transformation into phagocyitc cells, which are responsible for defense against invading organisms i.e., emphasize their function against parasitoids. While the percentage of phagocytic cells (plasmatocytes, spindle cells and granular cells) in parasitized larvae was significantly increased than that of unparasitized larvae of the same age.

These results agree with Kitano (1974) who noticed a marked decrease in the proportion of the prohaemocytes from early to middle 4th instar larvae of *Pieris rapae crucivora* parasitized by the egg stage of *Apanteles glomeratus,* whereas a continual

increase was observed in proportion of plasmatocytes till middle of the fourth instar.

After delfin and baythroid treatments: The percentage of phagocytic cells of parasitic larvae increased than that of unparasitized larvae. The phagocyte percentage of both treated unparasitized and parasitized larvae was also higher than that of control of each larva. Delfin treatments significantly increased the phagocytes of parasitized larvae as compared to unparasitized ones, but they insignificantly increased after the larvae were treated with baythroid.

Table 4 shows the increase or reduction in each type of haemocytes from control after treatments with delfin or baythroid.

1.2.2. For host larvae containing 1st, 2nd and 3rd instar larvae of the parasitoid

Prohaemocytes in untreated host larvae: at 7,9 and 11 days old (1^{st},2^{nd} and 3^{rd} larval instar of the parasitoid inside the host), were 50.0% ± 0.25, 43.1%± 0.26 and 36.2% 1.99 for unparasitized larvae and 29.7%±1.91, 22.9%±0.63 and 18.4%±1.86 parasitized larvae. While after delfin treatment, the reduction of prohaemocytes from control was 52.0%, 61.0% and 63.8% for unparasitized larvae and 36.7%, 53.7% and 65.8% for parasitized larvae; but after baythroid treatment being 65.4%, 76.8% and 84.8% for unparasitized larvae and 67.3%, 86.9% and 99.5% for parasitized ones, respectively, Table 5-7.

Phagocytes in untreated host larvae: (plasmatocytes, spindle cells and granular cells) were 24.0%±1.26,27.0%±0.2 and 15.1%±0.51 for unparasitized larvae and 41.5%±2.26, 4.4%±0.07% and 18.5%±0.97 for parasitized ones at 7 days old host host larvae. At 9 days old host larvae, they were 27.0%±0.61, 3.8± 0.18 and 17.0%± 0.09 for unparasitized larvae and 44.3%± 0.37%, 5.0%±0.1 and 20.6%±0.28 for parasitized ones. Moreover, at 11 days old *S.littoralis* larvae, the percentage was 28.9±0.97, 5.0±0.33 and 18.7±0.33 for unparasitized larvae and 46.0±0.57, 6.0±0.49 and 22.0±0.6 for parasitized ones, respectively, Table 5-7.

The increase in plasmatocytes, spindle cells and granular cells of larvae treated with delfin or baythroid from control at 7,9 and 11 days old *S.littoralis* larvae is shown in table 5-7.

From these results, we may conclude that the phagocytes (plasmatocytes, spindle cells and granular cells) in parasitized larvae increased as compared to those of

unparasitized ones at the same ages, in contrast to a reduction in prohaemocytes. This change is supposed to be due to prohaemocytes transformation into phagocytic cells to resist the endoparasitoid inside the host larvae. These results agree with those of Osman *et al.* (1982-1983) on the 4th instar *S.littoralis* larvae parasitized by *M.rufiventris* and El-Maasarawy and El- Sheikh (1993) on haemocytes of maize worm 4th instar *Mythimna* (=*leucania loreyi* (Dup.) parasitized by the braconid wasp, *Meteorus gyrator* Thun, (larval instar inside host) indicated that granulocytes and plasmatocytes increase in the presence of foreign bodies or the endoparasitoids. The conspicuous increase of plasmatocytes which remains high until the prelast day of host's life being responsible for defense aganst the invading organisms emphasizes their function against the parasitoids. Vinson (1971) found that there was no change in the relative proportion of plasmatocytes and prohaemocytes in *Heliothis virescens* unparasitized and those parasitized by *Cardiochiles nigriceps* Viereck through the period of 8 days after parasitism, also Strand and Noda (1991) on *Pseudoplusia includens* parasitized by *Microplitis demolitor* noticed that in vitro spreading behaviour of haemocytes was altered significantly by parasitism, where spreading of plasmatocytes remained suppressed through the course of parasitoid development.

The percentage of oenocytoids, adipohaemocytes, spherule cells and cystocytes in untreated larvae at 7,9 and 11 days old is shown in Table 5 to 7

From these results, it is shown that the spherulocytes and oenocytoids decreased in parasitized larvae than in unparasitized larvae. These data agree with those of Osman *et al.* (1982-1983) on *S.littoralis* parasitized by *M.rufiventris* and El-Maasarawy and El-Sheikh (1993) on *Mythimna loreyi* parasitized by wasps of *Meteorus gyrator* who stated that spherulocytes decreased drastically in parasitized larvae, probably due to parasitoid consumption. The oenocytoids also decreased after parasitism due to their use in healing wounds.

The increase or reduction in oenocytoids, adipohaemocytes, spherule cells and cystocytes from control at 7,9 and 11 days old host larvae in each treatments is shown in Tables 5-7.

The statistical analysis for t-test between unparasitized and parasitized larvae for each treatment is give in Table 5,6 and 7.

During various developmental instars of *S.littoralis* larvae, the increase in haemocyte count occurred for plasmatocytes, granulocytes and oenocytoids. While the prohaemocytes 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 and coagulocytes did not increase during development.

Kares *et al.* (1992) noticed that bactospiene treatments at LC_{10} and LC_{50} levels lead to a decrease in the number of prohaemocytes in the 4th instar *Pieris rapae* than that of control, where the plasmatocytes were the dominant type of cell.

Rizk (1991) found that after insecticide treatments, the haemocyte percentages of 4th instar *S.littoralis* larvae treated with sumithion and fenvalerate were affected significantly as compared to those of control. A significant difference was also found between the different types of haemocytes.

Table 4. Percentage of the different haemocyte types of 5 days old *S.littoralis* larvae unparasitized and those parasitized by egg stage of *M.rufiventris* and then treated with delfin and baythroid.

Type of cells	Unparasitized larvae			Parasitized larvae			T-test between unparasitized and parsitized larvae		
	Control	Delfin	Baythroid	Control	Delfin	Baythroid	Control	Delfin	Baythroid
Prohaemocytes % R	59.0.±0.68	30.1±0.42 -49.0	21.9±2.1 -62.9	36.0.±2.74	24.6±1.2 -31.7	12.5±0.62 -65.3	H.S.	S.	S.
Plasmatocytes % I	22.5±0.73	36.0±0.54 60.0	40.7±0.11 80.0	40.4±3.14	41.0±1.40 1.5	46.5±1.0 15.1	H.S	S.	S.
Spindle cells % I	0.00	3.0±0.77 0.00	4.1±0.53 0.0	1.1±0.66	3.2±0.66 190.9	5.0±0.93 354.5	N.S.	N.S.	N.S.
Granular cells % I	12.9±0.47	22.0±0.67 70.5	23.9±1.24 85.3	18.1±0.25	25.6±0.78 41.4	28.1±0.55 55.2	H.S.	S.	S.
Oenocytoids % I	0.0	3.9±0.9 0.0	2.5±0.56 0.0	0.1±0.03	1.2±0.26 19.0	2.3±0.19 37.3	H.S.	N.S.	N.S.
Adipohaemocytes % I	3.2±0.39	3.5±0.65 9.4	3.8±0.42 18.8	3.3±0.59	3.3±1.0 0.0	4.2±0.61 2.7	N.S.	N.S.	N.S.
Spherule cells % R % I	0.8±0.29	1.5±0.27 78.5	1.8±0.38 125.0	1.2±0.28	1.0±0.53 -16.7	1.4±0.37 16.7	N.S.	N.S.	N.S.
Cystocytes % I	1.6±0.05	0.0±0.0 0.0	1.3±0.15 -18.8	0.0±0.0	0.0±0.0 0.0	0.0±0.0 0.0	H.S.	-	H.S.

% R.: Percentages reduction from control
% R.: Percentages increase from control

Table 5. Percentage of the different haemocyte types of 7 days old *S.littoralis* larvae unparasitized and those parasitized by 1st larval instar of *M.rufiventris* and then treated with delfin and baythroid.

Type of cells	Unparasitized larvae			Parasitized larvae			T-test between unparasitized and parsitized larvae		
	Control	Delfin	Baythroid	Control	Delfin	Baythroid	Control	Delfin	Baythroid
Prohaemocytes % R	50.0.±0.25	24.0±0.61 -52.0	17.3±0.46 -65.4	29.7.±1.91	18.8±1.46 -36.7	9.7±0.67 -67.3	H.S.	S.	S.
Plasmatocytes % I	24.0±1.26	36.8±0.56 53.3	41.4±1.05 72.5	41.5±2.26	42.5±1.27 2.4	46.4±0.88 11.8	H.S	S.	S.
Spindle cells % I	2.7±0.20	3.9±0.28 44.4	4.9±0.66 81.5	4.4±0.07	4.7±0.58 6.8	4.9±0.92 11.4	S.	N.S.	S.
Granular cells % I	15.1±0.51	23.7±0.86 57.0	26.0±1.0 72.2	18.5±0.97	25.7±0.40 38.9	30.4±0.34 64.3	S.	N.S.	N.S.
Oenocytoids % I	1.6±0.26	4.2±0.16 162.5	4.6±1.68 187.5	1.3±0.45	1.7±0.45 30.8	2.8±0.55 115.4	N.S.	N.S.	N.S.
Adipohaemocytes % I	3.7±1.27	4.8±0.18 29.7	4.0±0.04 8.1	3.5±0.77	4.6±1.51 31.4	4.3±0.14 22.9	N.S.	N.S.	N.S.
Spherule cells % R, % I	2.1±0.57	1.7±10.34 -38.5	1.9±1.05 -9.5	1.1±0.42	1.2±0.2 9.1	1.5±0.8 36.4	N.S.	N.S.	N.S.
Cystocytes % I	0.8±0.20	1.1±0.07 +37.5	0.0±0.0 0.0	0.0±0.0	0.8±0.24 0.0	0.0±0.0 0.0	S.	N.S.	N.S.

% R.: Percentages reduction from control
% R.: Percentages increase from control

Table 6. Percentage of the different haemocyte types of 9 days old *S.littoralis* larvae unparasitized and those parasitized by 2nd larval instar of *M.rufiventris* and then treated with delfin and baythroid.

Type of cells	Unparasitized larvae			Parasitized larvae			T-test between unparasitized and parsitized larvae		
	Control	Delfin	Baythroid	Control	Delfin	Baythroid	Control	Delfin	Baythroid
Prohaemocytes %R	43.1.±0.26	16.8±1.05 -61.0	10.0±1.36 -76.8	22.9±0.63	10.6±1.52 -53.7	30±0.55 -86.9	H.S.	S.	H.S
Plasmatocytes % I	27.0±0.61	41.8±1.24 54.8	45.1±0.60 67.0	44.3±0.37	48.6±1.05 9.7	50.0±0.92 12.9	H.S	S.	S.
Spindle cells % I	3.8±0.18	4.6±0.47 21.1	5.0±0.08 31.6	5.0±0.10	5.8±0.46 16.0	7.1±0.30 42.0	H.S	N.S.	H.S
Granular cells % I	17.0±0.09	23.8±0.48 40.0	27.3±0.68 60.6	20.6±0.28	27.3±0.16 32.5	31.1±0.72 51.0	H.S	H.S	S.
Oenocytoids % I	2.6±0.04	4.0±0.81 16.5	5.0±2.04 92.3	1.3±0.53	1.7±0.48 30.8	3.0±0.44 130.8	N.S.	N.S.	N.S.
Adipohaemocytes % I	3.7±0.44	4.8±0.47 29.7	4.4±0.71 18.9	3.6±0.31	4.7±0.32 30.6	4.3±0.2 23.3	N.S.	N.S.	N.S.
Spherule cells % R, % I	2.3±0.63	2.1±0.47 -8.7	1.9±0.34 -17.4	1.0±0.17	1.2±0.25 20.0	1.6±0.71 60.0	N.S.	N.S.	N.S.
Cystocytes % I	0.5±0.05	1.3±0.16 160.0	1.3±0.24 160.0	1.3±0.28	0.0±0.00 0.0	0.0±0.0 0.0	S.	H.S.	H.S.

% R.: Percentages reduction from control
% R.: Percentages increase from control

Table 7. Percentage of the different haemocyte types of 11 days old *S.littoralis* larvae unparasitized and those parasitized by 3rd larval instar of *M.rufiventris* and then treated with delfin and baythroid.

Type of cells	Unparasitized larvae			Parasitized larvae			T-test between unparasitized and parsitized larvae		
	Control	Delfin	Baythroid	Control	Delfin	Baythroid	Control	Delfin	Baythroid
Prohaemocytes % R	36.2±1.99	13.1±0.43 -63.8	5.5±0.28 -84.8	18.4±1.86	6.3±0.79 -65.8	0.1±0.05 -99.5	H.S.	H.S.	H.S.
Plasmatocytes % I	28.9±0.97	44.3±0.35 53.3	46.0±0.57 60.6	46.0±0.57	49.3±0.61 7.2	51.2±0.61 11.3	H.S	H.S.	H.S.
Spindle cells % I	5.0±0.33	5.6±0.55 12.0	6.3±0.16 26.0	6.0±0.49	7.0±0.5 16.7	8.4±0.2 40.0	N.S	N.S.	S.
Granular cells % I	18.7±0.33	24.1±0.66 28.9	28.7±0.44 53.5	22.0±0.6	29.4±0.91 33.6	31.2±0.43 41.8	H.S	H.S	H.S.
Oenocytoids % I	3.5±0.6	4.2±0.17 20.0	5.2±0.1 48.6	1.1±0.10	1.9±0.45 72.7	3.1±0.29 181.8	S.	H.S.	H.S.
Adipohaemocytes % I	4.1±0.43	5.0±0.17 22.0	4.5±0.31 -9.8	3.9±1.55	4.7±0.36 20.5	4.4±0.78 12.8	N.S.	N.S.	N.S.
Spherule cells % R, % I	3.5±2.45	2.4±0.78 -31.4	2.1±0.11 -40.0	1.0±0.48	1.4±0.31 40.0	1.7±0.37 70.0	N.S.	N.S.	N.S.
Cystocytes % I	0.0±0.0	1.4±0.19 0.0	1.3±0.06 0.0	1.6±0.31	0.0±0.00 0.0	0.0±0.0 0.0	H.S.	H.S.	H.S.

% R.: Percentages reduction from control
% R.: Percentages increase from control

REFERENCES

1. El-Maasarawy. S.A.S. and M.A.K. El-Sheikh. 1993. Haemocytes changes in maize worm, *Mythimna* (=*leucania loreyi* parasitized by the braconid wasps *Mateorusgyrator* Thun. Bull. Ent. Soc. Egypt. 71:19-31.

2. Gagen, S.J. and N.A. Ratcliffe. 1976. Studies on the in vivo cellular reactions and fate of injected bacteria in *Galleria mellonelle* and *Pieris brassica* larvae. J. Invertebr. Pathol. 28:17-24.

3. Gupta, A. P. and D.J. Sutherland. 1968. Effects of sublethal doses of chlordane on the haemocytes and midgut epithelium of *Periplaneta americana.* Ann. Entomol., Soc. Amer., 61 (4): 910-918.

4. Hazarika, L.K. and A.P. Gupta. 1987. Variations in haemocyte populations during various developmental stages of *Blatella germanica* (L.) (Dictyoptera, Blatellidae). Zool. Sci., 4 (2):307-313.

5. Ibrahim, A.A. 1974. Studies on the biological control of *Spodoptera littoralis* (Boisd.)in A.R.E. Ph. D. Thesis, Faculty of Agriculture, Cairo University 282 pp.

6. Kares, E.A., A.M. Abdel-Rahman, A.A.El-Moursy and M.B.R. El-Mandarawy.1992. Qualitative and quantitative studies on the haemolymph of *Artogea rapae* (L.) larvae after a bioinsecticidal and gamma radiation treatment. Egypt, J. Biol. Pest Contol. 2 (2):131-141.

7. Kares, E.A., A.A. El-Moursy, N. Zohdy, A.M. Abdel-Rahman and M.B. R.El-Mandarawy. 1998. Biological and toxicological studies on the parasitoid *Microplitis rufiventris* Kok. Egypt. J. Agric. Res. 76 (4): 1944-1513.

8. Kitano, H. 1974. Effects of the parasitization of a braconid, *Apanteles*, on the blood of its host, *Pieris.* J. Insect. Physiol. 20:315-327.

9. Osman, S.E., I. A. Rawash , F. El-Gayar and M.M. El-Samadisi. 1982-1983. The effect of the larval endoparasite *Microplitis rufiventris* Kok. (Hym., Braconidae) on the blood picture and cuticle formation of *spodoptera littoralis* (Boisd.) larvae. Bull.Ent. Sco. Egypt, Econ. Ser., 13:183-189.

10. Ramakrishnan, N. and L.D. Tiwari. 1972. Observations on the blood cells of *Prodenia litura* F. in relation to nuclear polyhedrosis. Indian J. Ent. 34 (4): 290-293.

11. Rizk, S.A. 1991. Effect of gamma radiation and some insecticides on the cotton leafworm, *Spodoptera littoralis* (Boisd.). M.Sc. Thesis, Fac. Sci., Cairo Univ.

12. Strand, M.R. and T. Noda. 1991. Alternations in the haemocytes of *Pseudoplusia includens* after parasitism by *Microplitis demolitor*. J. Insect. Physiol. 37 (11): 839-850.

13. Vinson, S.B. 1971. Defense reaction and haemocytic changes in *Heliothis virescens* in response to its habitual parasitoid *Cardiochiles migriceps*. J. Invertebr. Pathol. 18: 84-100.

تأثير الدلفين والبايثرويد علي العد الكلي ونسبة كل خلية في دم يرقات دودة ورق القطن غير المتطفل عليها وتلك المتطفل

نوال زهدي[1]، علي علي المرسي[1]، عصمت عبد الملك كارس[2]، أمينة محمد عبد الرحمن[1]، مني برسوم المندراوي[2]

١ قسم علم الحشرات -كلية العلوم - جامعة القاهرة - الجيزة.
٢ معهد بحوث وقاية النباتات - مركز البحوث الزراعية - الدقي - جيزة.

عند معالجة العمر اليرقي الثاني لدودة ورق القطن بالتركيز القاتل لـ ١٠٪ من اليرقات لمدة ٤٨ ساعة بالدلفين أو المعالجة لمدة ٢٤ ساعة بالبايثرويد ثم بفحص الدم للأعمار ٥، ٧، ٩، ١١ يوما ليرقات العائل ١ ، ٣، ٥ ، ٧ يوما بعد التطفل عليها بطفيل الميكروبليتس روفيفنترس أظهرت النتائج أنه:

بعد اليوم الأول من التطفل (حيث يكون الطفيل داخل العائل في طور البيضة) لوحظ عدم وجود فروق معنوية لعدد الدم الكلي بين اليرقات غير المتطفل عليها وتلك المتطفل عليها، بينما بعد ثلاثة أيام من التطفل يكون الطفيل داخل العائل في طوره الأول. يقل العدد الكلي لخلايا الدم بصورة غير معنوية، ولكن بعد اليوم الخامس والسابع من التطفل حيث يوجد الطفيل داخل العائل في طوره الثاني والثالث. قل العدد الكلي للدم بدرجة معنوية عالية و ذلك بين اليرقات غير المتطفل عليها وتلك المتطفل عليها، كما أنة عندما عولجت اليرقات غير المتطفل عليها وتلك المتطفل عليها بالتركيز القاتل لـ ١٠٪ من اليرقات، قل العدد الكلي لخلايا الدم بدرجة معنوية مقارنة بتلك غير المعاملة عند مراحل العمر المختلفة.

إحتوي دم يرقات دودة ورق القطن علي الخلايا الأولية الملتهمة (الخلايا البلازمية والخلايا المغزلية و الخلايا الحبيبية) والخلايا الأنوسيتية والخلايا الدهنية و كذلك الخلايا الكروية والخلايا الحوصلية.

ولوحظ أن نسبة الخلايا الملتهمة (الخلايا البلازمية والخلايا المغزلية والخلايا الحبيبية) في دم اليرقات المتطفل عليها أكثر منها في غير المتطفل عليها عند نفس العمر. ولقد تبع هذا تناقص في نسبة الخلايا الأولية نتيجة تحولها إلي خلايا ملتهمة مسئولة عن الدفاع عن الأجسام الغريبة فتزيد فاعليتها ضد الطفيل داخل اليرقة. كما أنه لوحظ حدوث تناقص في الخلايا الكروية والأنوسيتية في دم اليرقات المتطفل عليها عن مثيلتها في اليرقات غير المتطفل عليها، بينما حدث بعد معالجة اليرقات بالدلفين أو البايثرويد تزايد للخلايا الملتهمة في دم اليرقات المتطفل عليها أكثر من تلك غير المتطفل عليها. كما كانت خلايا دم اليرقات المعالجة غير المتطفل عليها وتلك المتطفل عليها أكثر منها في تلك غير المعالجة.

خلال فترة النمو المختلفة ليرقات دودة ورق القطن، لوحظ حدوث زيادة في الخلايا البلازمية و الخلايا الحبيبية والخلايا الأنوسيتية، بينما حدث تناقص في نسبة خلايا الدم الأولية خلال نمو اليرقات.