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Effect of β–ecdysone ingestion on total and differential haemocyte counts (THC & DHC) in the tobacco caterpillar, *Spodoptera litura* FABR. (Lepidoptera: Noctuidae).

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The 5th and 6th instar larvae of *Spodoptera litura* were individually given 0.5, 1.0, 2.0, 4.0 and 6.0 µg of β–ecdysone (moulting hormone) to observe its effect on the total and differential haemocyte counts (THC & DHC). The increase in the concentration of hormone resulted in a progressive fall in THC. The intake of the strongest dose by 5th instar larvae (24 h old) caused about 50% loss of total cells in 6th instar larvae (2-day old). But when 6th instar larvae were treated with 6.0 µg/larva, in the prepupae and pupae (2-day old) there was a loss of 83.3% and 75% respectively as compared to control. On the basis of the data on DHC, it is evident that in moulted 6th instar larvae (2-day old), prohaemocytes were less susceptible to the intake of the strongest dose and showed double values as compared to controls due to the cytotoxic effects in other haemocyte types.

The percentage of plasmatocytes was reduced to less than half and adipohaemocytes were completely destroyed even by the intake of a 1.0 µg dose, whereas podocytes and granular haemocytes showed apparent increase because of their resistance and survival. The population of oenocytoids was reduced to about 5% by the strongest dose. The percentages of cystocytes and spherule cells were unaccountable because of their severe damage and indistinguishable condition even by the intake of the weakest dose.

In the prepupae of treated 6th instar larvae, the effect was also dose-based. With the strongest dose, the percentages of prohaemocytes and oenocytoids were found highly increased due to the massive loss of other cells. The percentage of other types of cells became almost nil. In the affected pupae there were no intact cells except oenocytoids and a DHC was only made on the damaged but recognizable cells of each type.

Keywords: *Spodoptera litura*, β–ecdysone, haemocytes, moulting, pathogenicity, total haemocyte counts (THC), differential haemocyte counts (DHC).

INTRODUCTION

In normal development a moulting hormone (ecdysone) is released in low concentration at precisely controlled rates to promote moulting during growth. Besides natural ecdysones in the form of α and β isomers, several ecdysoids, including those of plant origin (phytoecdysones) have been synthesized. It has been noted that exogenous application of these ecdysones or their analogues results in suppression of metamorphosis and reproduction, but their effect on haemocytes is little known. NISHI (1982) in *Spodoptera litura*, KHAN *et al.* (1984) in the larvae of *S. litura* and nymphs and adults of the grasshopper *Hieroglyphus nigrorepletus* and AHMAD & KHAN (1989) in the cotton stainer bug *Dysdercus cingulatus*, observed a decreasing trend of the THC, whereas RAO *et al.* (1984) found a significant increase in the THC of *S. litura* by injecting β-ecdysone.

In the present investigation the effect of different doses of β-ecdysone fed to larvae of *S. litura*, which are polyphagous pests of several crops, was observed as regards the toxicity on THC and DHC with a view to control the larvae of *S. litura.*

MATERIAL AND METHODS

A stock culture of *S. litura* was maintained at 30 ± 1 °C and 70-40 % R.H. Larvae were provided with tender fresh castor leaves as food.

From this stock newly moulted larvae of 5th and 6th instars were isolated and starved overnight. Doses of 0.5, 1.0, 2.0, 4.0 and 6.0 µg β-ecdysone (in acetone) were sprayed on pieces of castor leaves, measuring 5 x 5 cm and offered individually. After complete ingestion of the treated leaf pieces by each larva, all larvae on one dose were transferred to a separate rearing jar provided with untreated castor leaves as food.

For control, untreated larvae and larvae treated with acetone only, of similar stage and age, were kept in separate rearing jars at the same temperature and humidity.

Total haemocyte counts (THC): Fresh blood of individual larvae was drawn up into a pipette used for counting white blood cells of human beings. This blood was diluted with modified diluting fluid suited for the blood. THC/mm^3 were counted with the help of an improved Neubauer counting chamber described by DARMADY & DAVENPORT (1963) and KOLMER *et al.* (1969). THC was calculated using the formula $^{X}/_{4} \cdot 10 \cdot 20$, where X is the total number of haemocytes in the 4 corner squares of the chamber, 10 is the reverse of the counting chamber depth and 20 is the dilution of the haemolymph.

Differential haemocyte counts (DHC): The percentage of different types of haemocytes was calculated on the basis of the number of each type of haemocyte in permanent and stained preparations. In each preparation, three areas, each of 1 cm^2, were selected for counting. The criterion for this selection was the uniform distribution of haemocytes and each type of cell at a particular stage was calculated by counting ten separate preparations from the respective number of insects, treated or untreated.

RESULTS

Total haemocyte counts of 6th instar larvae of S. litura following the ingestion of different doses of β-ecdysone by 5th instar larvae.

The treated 6th instar larvae (48 h old) showed dose-dependent damage of cells and reduction of the THC. Following the ingestion of the highest dose, i.e. 6.0 µg, THC was reduced to almost half of the control (Fig. 1) and the cells were mostly unidentifiable due to extreme pathological changes.

Total haemocyte counts of the prepupae and the pupae of S. litura following the ingestion of different doses of β-ecdysone by the 6th instar larvae.

In the affected prepupae, the THC showed almost linear negative correlation with hormone concentration (Fig. 1). When 6th instar larvae were treated with 6.0 µg/larva, the prepupae showed a loss of 83.3 % haemocytes as compared to the controls. A similar trend was seen in pupal blood though only doses above 1 µg were effective and the total loss in THC by the strongest dose was only 75 % (Fig. 1).

Differential haemocyte counts of 6th instar larvae of S. litura following the ingestion of different doses of β-ecdysone by 5th instar larvae.

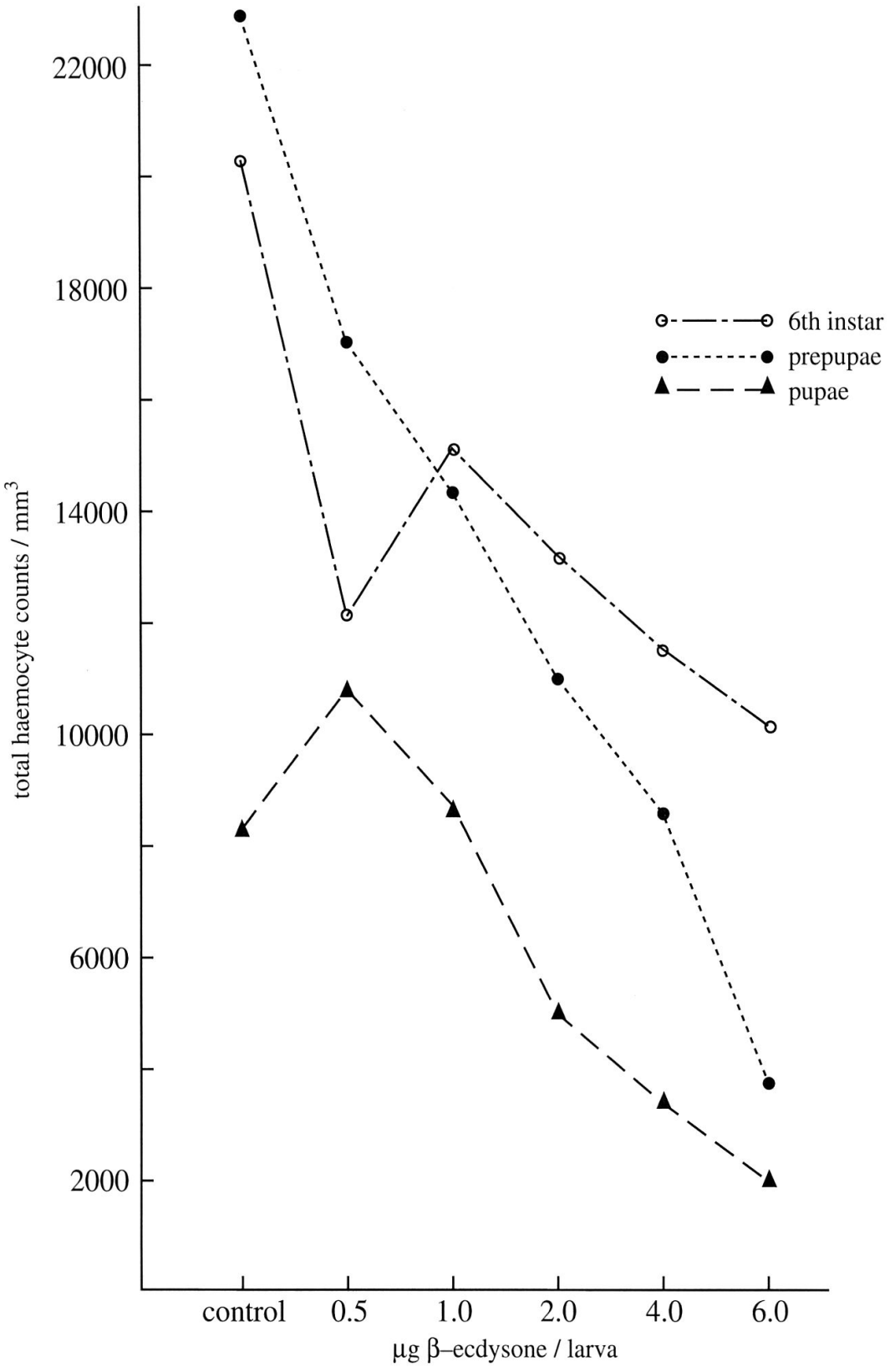

Fig. 1. Total haemocyte counts (THC) of 2 day old 6th instar larvae, prepupae and pupae of *Spodoptera litura* plotted against doses of β-ecdysone fed in the preceding instar.

Low doses of β-ecdysone caused only a small reduction in the percentage of plagmatocytes (PLs). But 2.0 μg of the hormone per larva reduced the population of these cells to about one third of the control (Fig. 2). The prohaemocytes (PRs) were unaffected by any dose and so their percentage appeared higher, showing a double value at the doses of 4.0 and 6.0 μg, when other cells were mostly absent. At the dose of 0.5 μg the percentage of podocytes (POs) decreased from 6.7 % to 2.0 %. The granular haemocytes (GRs) and the adipohaemocytes (ADs) were unaffected. However, whereas the latter were completely destroyed at the dose of 1.0 μg, the number of GRs dropped at that dose. By 2.0 μg and higher doses there was little change in absolute numbers of the GRs; the relative number of these cells increased with respect to other cells, reaching a 21/2 times higher value than the controls. The oenocytoids (OEs) represented a low percentage and were slightly affected by the 0.5 μg dose, but higher doses reduced their population to about 5% (Fig. 2). The percentages of the cystocytes (CYs) and spherule cells (SPs) were unaccountable because of their severe damage and indistinguishable condition even with the smallest dose of 0.5 μg (Tab. 1).

Differential haemocyte counts of prepupae and pupae of S. litura following the ingestion of different doses of β*-ecdysone by 6th instar larvae.*

In the prepupae of treated 6th instar larvae the percentage of GRs was not significantly affected by different doses of β-ecdysone, whereas the percentage of PLs increased and became 8 times that of the controls with 6.0 μg treated larvae, because of the damage to some other cell types. The OEs were most resistant and their percentage increased proportionately 15 times to that of controls even after the ingestion of the highest doses (Fig. 2; Tab. 2). The percentage of PLs dropped sharply after the ingestion of 0.5 μg/larva and became almost negligible, i.e. 2.8 % from 61 % at the 6.0 μg dose. The percentage of POs showed an irregular decrease at the dose of 0.5 μg/larva (Fig. 2). The ADs decreased at the dose of 0.5 μg and became nil at 1.0 μg and higher doses. DHC in affected pupae were made on the basis of the number of cells of each type, which were damaged but still recognizable. However, there were no intact cells except OEs (Tab. 3).

The percentage of PRs was apparently unchanged because these cells existed in damaged condition. The PLs were most susceptible and their percentage dropped even at the 0.5 μg dose. Similarly, the percentage of POs decreased even after 0.5 μg and became half of its control population at the highest dose. On the other hand, GRs were unaffected and so their number increased with respect to the total number of surviving cells. The percentage of OEs dropped at the doses of 0.5 μg/larva, increased at 1.0 and 2.0 μg and dropped again at 4.0 and 6.0 μg doses (Fig. 2).

Since SPs and CYs as well as intact ADs were not seen in the smears of control pupae, the effect of β-ecdysone could not be studied in these cells.

In the normal larvae, prepupae and pupae of *S. litura*, the THC revealed increasing numbers up to the prepupal stage, showing a maximum of 24664 haemocytes per mm^3, whereas the number of cells markedly declined. In the pupal stage this may be due to histolysis of haemocytes during metamorphosis.

DISCUSSION

The present data reveals that oral intake of the strongest dose of β-ecdysone by 5th and 6th instar larvae, respectively, caused about 50 % loss of total haemocytes in 6th instar larvae and 75 % to 80 % in prepupae and pupae. The toxicity of

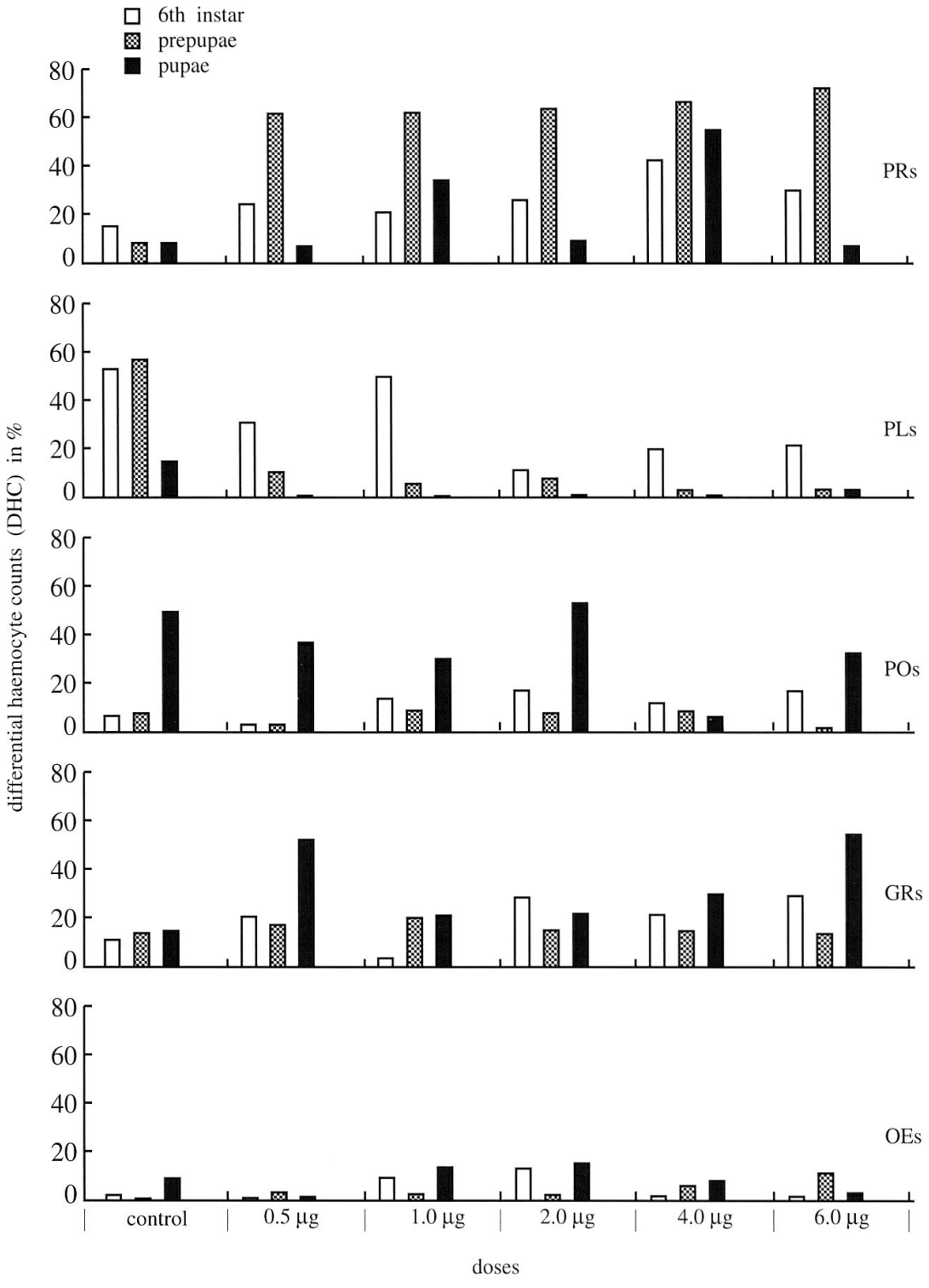

Fig. 2. Comparative changes in the percentage of different types of haemocytes (differential haemocyte counts, DHC) of 6th instar larvae, prepupae and pupae of *Spodoptera litura* fed with various doses of β-ecdysone, as in Fig. 1.

Tab 1. Relative differential haemocyte counts (DHC) in the 6th instar larvae of *Spodoptera litura* fed with different doses of β-ecdysone in the 5th instar. Tests: t values between two consecutive DHC means; * = significant at 5 % level (tabulated value of t value at four degrees of freedom: 2.776).

Dose µg / larva	Prohaemocytes mean ± S.E.	t value	Plasmatocytes mean ± S.E.	t value	Podocytes mean ± S.E.	t value	Granular haemocytes mean ± S.E.	t value	Adipohaemocytes mean ± S.E.	t value	Oenocytoids mean ± S.E.	t value	Cystocytes mean ± S.E.	t value	Spherule cells mean ± S.E.	t value
Control	15.25 ± 0.03		57.4 ± 1.10		6.7 ± 0.43		11.8 ± 0.95		4.4 ± 0.87		2.8 ± 0.06		0.9 ± 0.23		0.75 ± 0.03	
0.5	24.1 ± 3.13	0.95	32.9 ± 4.27	1.94	2.0 ± 0.90	2.14	20.8 ± 2.10	3.62*	18.3 ± 3.59	3.38*	1.7 ± 0.21	2.58	0 0	0	0 0	0
1.0	21.5 ± 3.27	1.24	52.9 ± 7.56	3.04*	15.2 ± 2.29	1.34	2.0 ± 1.14	3.72*	0 0	0	8.4 ± 2.04	1.61	0 0	0	0 0	0
2.0	27.8 ± 5.79	1.83	12.3 ± 2.26	1.67	18.1 ± 1.30	1.91	28.9 ± 3.20	1.72	0 0	0	12.9 ± 1.83	3.35*	0 0	0	0 0	0
4.0	43.7 ± 4.85	1.97	21.7 ± 5.50	1.61	12.2 ± 2.31	1.61	21.0 ± 2.78	1.66	0 0	0	1.2 ± 0.50	0.82	0 0	0	0 0	0
6.0	30.8 ± 2.55		21.8 ± 3.11		17.6 ± 2.33		28.5 ± 3.34		0 0		1.4 ± 0.16		0 0		0 0	

Tab 2. Relative differential haemocyte counts (DHC) in the prepupae of *Spodoptera litura* fed with different doses of β-ecdysone in the 6th instar. Tests as mentioned in Tab. 1.

Dose µg / larva	Prohaemocytes mean ± S.E.	t value	Plasmatocytes mean ± S.E.	t value	Podocytes mean ± S.E.	t value	Granular haemocytes mean ± S.E.	t value	Adipohaemocytes mean ± S.E.	t value	Oenocytoids mean ± S.E.	t value	Cystocytes mean ± S.E.	t value	Spherule cells mean ± S.E.	t value
Control	9.0 ± 1.14		61.0 ± 1.05		8.4 ± 0.64		15.0 ± 0.71		4.2 ± 0.67		0.7 ± 0.08		0.7 ± 0.04		1.0 ± 0.06	
0.5	63.0 ± 2.88	0.52	12.0 ± 0.79	1.83	2.0 ± 0.72	2.17	17.0 ± 0.71	1.49	3.0 ± 0.72	3.05*	3.0 ± 0.47	1.54	0	0	0	0
1.0	63.6 ± 2.10	1.26	6.4 ± 0.32	2.08	9.0 ± 2.61	1.94	19.0 ± 1.30	1.81	0	0	2.0 ± 0.47	0	0	0	0	0
2.0	66.0 ± 1.30	1.21	9.0 ± 1.02	1.08	8.0 ± 0.39	1.08	15.0 ± 1.41	0.78	0	0	2.0 ± 0.47	1.94	0	0	0	0
4.0	68.4 ± 2.38	1.63	3.3 ± 1.67	0.43	9.3 ± 2.09	3.15*	14.0 ± 2.25	0.95	0	0	5.0 ± 1.30	2.72	0	0	0	0
6.0	74.0 ± 2.35		2.8 ± 0.92		0 0		12.7 ± 1.00		0 0		10.5 ± 0.35		0 0		0 0	

Tab 3. Relative differential haemocyte counts (DHC) in the pupae of *Spodoptera litura* fed with different doses of β-ecdysone in the 6th instar. Tests as mentioned in Tab. 1.

Dose µg / larva	Prohaemocytes mean ± S.E.	t value	Plasmatocytes mean ± S.E.	t value	Podocytes mean ± S.E.	t value	Granular haemocytes mean ± S.E.	t value	Adipohaemocytes mean ± S.E.	t value	Oenocytoids mean ± S.E.	t value	Cystocytes mean ± S.E.	t value	Spherule cells mean ± S.E.	t value
Control	6.8		16.5		51.5		15.5		0		9.7		0		0	
	± 0.66		± 0.74		± 1.07		± 0.51		0		± 0.41		0		0	
0.5	6.4		1.7		37.2		52.5		1.0		1.2		0		0	
	± 1.55		± 0.49		± 3.10		± 1.93		± 0.60		± 0.13		0		0	
		2.75		1.36		1.26		4.19*		1.93		2.54		0		0
1.0	34.4		0.9		31.4		21.2		0		12.1		0		0	
	± 6.73		± 0.48		± 5.01		± 2.05		0		± 3.64		0		0	
		2.44		2.04		2.29		0.22		0		0.89		0		0
2.0	10.0		0		54.0		21.0		0		15.0		0		0	
	± 3.78		0		± 4.60		± 6.95		0		± 4.59		0		0	
		2.29		0		3.74*		1.10		0		1.54		0		0
4.0	57.0		0		7.0		29.0		0		7.0		0		0	
	± 16.23		0		± 2.90		± 7.94		0		± 2.93		0		0	
		2.45		2.81*		3.32*		2.04		0		1.94		0		0
6.0	6.5		3.5		33.0		54.0		0		3.0		0		0	
	± 2.65		± 0.99		± 2.39		± 1.73		0		± 0.81		0		0	

β-ecdysone in haemocytes is evident by the cytopathological observations reported here and by AHMAD (1992). The reduction of the THC values is clearly dose-dependent. NISHI (1982) also observed the decreasing trend of THC in *S. litura* after injecting β-ecdysone. Similar observations were made by KHAN *et al.* (1984) in larvae of *S. litura* and nymphs and adults of *Hieroglyphus nigrorepletus*, and by AHMAD & KHAN (1989) in *D. cingulatus*. However, WERNER & JONES (1969) found in *Galleria mellonella* larvae low populations of haemocytes even after the injection of saline, and RAO *et al.* (1984) noticed a significant increase of haemocyte numbers in *S. litura* after injecting β-ecdysone. A similar observation was made by GUPTA & SUTHERLAND (1968) in *Periplaneta americana* by using the insecticide chlordane.

On the basis of the data on differential haemocyte counts, it is evident that the prohaemocytes in *S. litura* larvae were less susceptible to the intake of the strongest dose of β-ecdysone as compared to the pathogenicity developed in other cells. The percentage of plasmatocytes, on the other hand, decreased appreciably after the intake of β-ecdysone. The podocytes were generally affected by a 2.0 µg dose. The granulocytes were less influenced when compared to the former. Among all the types of cells the adipohaemocytes were most adversely affected, even by the lowest dose, whereas the oenocytoids showed much resistance. However, the total number of these cells was small throughout development.

The effect on DHC after exogenous application of β-ecdysone has only been recorded before by NISHI (1982) in *S. litura* (injection method) and by AHMAD & KHAN (1989) in *Dysdercus cingulatus* by topical application of β-ecdysone and makisterone A (a phytoecdysone). The results were similar to the present data.

Since the haemocytes are sites of intermediary metabolism and storage of metabolites in the insect body, any physiopathological condition in these cells certainly causes failure in the normal functioning. Therefore, it is suggested that *S. litura* might be controlled successfully by the application of this hormone, if economically feasible.

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