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EFFECT OF PYRIPROXYFEN ON LIPID AND CHOLESTEROL CONTENTS IN THE LAST INSTAR NYMPHS OF *SCHISTOCERCA GREGARIA* (FORSK.)

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Abstract

Application of 100, 50, and 25 ug/nymph pyriproxyfen to the 5th instar nymphs of Schistocerca gregaria had remarkably reduced lipid and cholesterol contents in their haemolymph and fat bodies. This reduction was accompanied with a 2 days prolongation in the last stadium. It was therefore, concluded that pyriproxyfen plays the same role of juvenile hormone in suppressing lipid and cholesterol contents in the desert locust.

INTRODUCTION

Hill and Izatt (1974) indicated that Allatectomy of 1 day old adult female of *Schistocerca gregaria* had resulted in an accumulation of lipids in its fat bodies. This action was more likely to be related to the lack of juvenile hormone (JH). El-Gammal *et al.* (1989) showed that the JH - analogue fenoxycarb, decreased cholesterol level in treated 5th instar nymphs of S.gregaria. Baeher et al. (1979) reported that ecdysteroids and JH were never present in high levels during the last two larval instars of *Locusta migratoria.* Stall *et al.* (1983) found that cholesterol showed significant conversion into 2-deoxyecdysone ecdysone and related ecdysteroids in *L. migratoria.*

The present study was conducted to evaluate the effect of pyriproxyfen on lipid and cholesterol contents as indicators of JH-like action in *S.gregaria.*

MATERIALS AND METHODS

Experimental insects

The 5th instar female nymphs of the desert locust *Schistocerca gregaria* Forsk. used in the present investigation were derived from a stock colony maintained at the Locust Research Department, Dokki, Cairo. The stock colony was reared under crowded conditions for several generations as described by Hunter-Jones (1961).

Chemical and application technique

Technical grade of the juvenile hormone analogue, pyriproxyfen (2-[1-methyl-2-(4-phenoxy-phenoxy) ethoxy] pyridine) (Sumitomo Chemical Co., LTD, Osaka, Japan) was used. The compound was dissolved in acetone and applied topically on the ventral part of the abdomen in a volume of 4 u1 using a Hamilton microsyringe (Model NCH701.) Three doses 100, 50 and 25 ug/nymph were applied. For each dose, thirty females of fifth instar nymphs in three replicates were used.

The application was done during the stadium (the 1st and 6th days). The reason was to evaluate the juvenilization effect of the compound on lipid and cholesterol levels in the last nymphal instar of *S. gregaria*. The treated and untreated nymphs were kept in wooden-framed cages (30x30x30 cm) for chemical analysis. All cages were incubated at 32±1°C with 12h photoperiod and 65±5% R.H. Leaves of *Sesabania aegyptiaca* plant were provided as a feeding material.

Estimation of lipid and cholesterol contents in haemolymph and fat bodies

The haemolymph of the treated and untreated nymphs was collected through a fine puncture in the hind leg membrane and transferred into dry centrifuge tube with few crystals of phenylthiourea to prevent its melanization. Thereafter, the nymphs were dissected and the fat bodies collected. This collection was conducted during the last three days of the treated nymphs (8,9 and 10 days) and at day 8 for the untreated nymphs.

Total lipid was estimated by the modified method of Knight et al. (1972), while total cholesterol was determined in both haemolymph and fat body by the enzymatic colourimetric method of Richmond (1973).

RESULTS AND DISCUSSION

Effect of pyriproxyfen on lipid content

Data presented in Table 1 show the effect of pyriproxyfen on lipid content when applied to 1-day old 5th instar female nymphs of S.gregaria. It is apparent that during day 8 the doses of 100, 50 and 25 ug/nymph suppressed lipid content in haemolymph compared with the untreated control. The amounts of lipids were 2.51, 1.45, 1.22 and 2.82 mg/ml for the three doses and the control, respectively. Reduction of lipid concentration continued during day nine. In thfe following day (day 10 however, a remarkable reduction in lipids was observed. The lipids were reduced from 2.82 mg/ml (control value) to 0.66, 0.50 and 0.20 mg/ml for the same doses, respectively.

It is of interest to denote that, JHA application during the 1st day of the 5th instar, had strongly reduced the amount of liplids in haemolymph during the last day of this instar (day 8) and the prolonged days (9 and 10) in a gradual manner.

This sharp reduction was also extended to the amount of lipids in the fat body of the treated nymphs (Table 1) . During day 8, the fat contents were, 32.85 , 31.24, 15.18 and 100 mg/g for the 100,50,25 ug/nymph and the control nymphs, respectively. The reduced amounts of lipids during day 9 of the treated nymphs were, 14.09, 43.08 and 18.05 while they were 15.88, 27.74 and 14.03 mg/g during day 10 for each dose, respectively, compared with control (100 mg /g).

The haemolymph lipid contents of the resulting 5th instar female nymphs following hormonal treatment with the same doses when the nymphs were 6-day old, were also estimated during days 8,9 and 10 (Table 2). It could be shown that the amounts of this metabolite were reduced by the three doses used. They amounted to 0.37, 0.61 and 0.81 mg/ml during day 8 for the doses 100, 50 and 25 ug/nymph, respectively. The control was 2.82 mg/ml. During day 9, these amounts reached 0.68, 1.0 and 1.77 mg/ml, while in day 10, they showed 0.07, 1.24 and 0.45 mg/

ml for the same doses, respectively. It is therefore evident that the three doses had reduced lipid contents in the haemolymph of treated nymphs during the three periods of analysis.

The fat body lipid contents were also affected following the same hormonal treatmens (Table 2). In the 8th day, the lipid amounts were 22.02, 30.4 and 17.17 mg/g for the doses 100 , 50 and 25 ug/nymph, respectively, compared to 100 mg/g for the unreated control. In the nineth day these amounts were reduced to 40.47, 14.67 and 20.45 mg/g for the three tested doses, respectively. The last day of prolongation (day 10) induced a strong decrease in fat body lipid contents after teatment with the same doses (10.54, 11.9 and 14.7 mg/g, in respect).

It could be concluded that the application of the hormone to one or six day old 5th instar nymphs had reduced the lipid contents of both haemolymph and fat body during the last day of the stadium and the following prolonged two days (9 and 10) of the treated nymphs. This could be considered as evidence for juvenilization and solitarization. El-Gammal (1979) demonstrated that isolation in S.gregaria female nymphs had reduced the lipid content in haemolymph and fat body. Carbohydrates and protein however were increased. Similar results were also obtained when 5th instar nymphs of *S. gregaria* were treated with fenoxycarb, (El-Gammal *et al.* 1989).

This behaviour could be due to the indirect effect of the JH analogue on natural JH level in the treated nymphs. Plantevin *et al*, (1991) found that the level of haemolymphatic JH was much higher in fenoxycarb treated larvae of *Bombyx mori* than in the control.

Effect of JHA pyriproxyfen on cholesterol content

Table 3 shows that the application of JHA within the 1st day of the 5th instar female nymphs increased shightly the haemolymph cholesterol at the higher doses (100 and 50 ug/nymph). The two doses induced, 0.44 and 0.66 mg cholesterol/ml haemolymph in comparison with 0.36 mg/ml of the control nymphs during day 8 of this instar. However, the lowest dose (25 ug) decreased the cholesterol level to 0.19 mg/ml as compared with the control (0.36 mg/ml). During day 9 of the stadium, cholesterol concentrations in haemolymph were considerably reduced to 0.23, 0.29 and 0.12 mg/ml for the doses 100, 50 and 25 ug/nymph, respectively in comparison with 0.36 for the untreated control. This reduction continued on 10th day, where the three doses reduced the cholesterol level to 0.37 , 0.22 and 0.17 mg/ml,

respectively.

Moreover, the three doses (100, 50 and 25 ug) induced a remarkable reduction in the fat body cholesterol. Their amounts were 1.12, 0.70 and 0.31 mg/g fat body on day 8, 1.68, 1.50 & 1.69 mg/g on day 9 and 1.00 , 0.87 & 0.69 mg/g on day 10 for each dose, respectively, compared to 3.26 for control nymphs.

Table 4 shows the effect of treatment with JHA during the 6th day of the 5th instar female nymphs on cholesterol content. The cholesterol concentrations in their haemolymph on day 8 were 0.24, 0.33 and 0.39 mg/ml compared to 0.36 mg for the control for the same doses respectively, thus indicating a slight reduction in the level of haemolymph cholesterol. On day 9, a gradual reduction was noticed. The concentrations were lowered to 0.15, 0.30 and 0.33 mg/ml for each dose, respectively, while in the 10th day, the haemolymph cholesterol levels were sharply reduced reaching 0.28, 0.13 and 0.17 mg/ml compared to 0.36 mg/ml for the control.

Cholesterol levels were strongly reduced in the site of its synthesis (fat body) when the same doses were used. The fat body cholesterol contents were 0.42 , 0.94 and 0.45 mg/g day 8 for the doses of 100, 50 and 25 ug, respectively in comparison to 3.62 mg/g in the control. On the 9th day, cholesterol level was a little higher than on day 9 though it was still lower than the control. The cholestrol concentrations amounted to 1.18, 2.12 and 2.58 mg/g for the three doses, respectively. Contrary to what was expected for day 10, cholestrol tended to decrease again to 1.13, 0.25 and 0.90 and 0.90 mg / g compared to the values obtained in day 9 (Table 4).

It seems that the application of pyriproxyfen had induced an inhibitory effect on cholesterol level in the fat body of the treated nymphs leading to a low efflux rate of this metabolite to the haemolymph. These results indicate that the prolongation which occurred in the teated nymphs up to days 9 and 10 compared to the normal duration of this instar (8 days) may be due to a lack in ecdysone synthesis. Rees (1985) stated that insects used cholesterol as a precursor for ecdysone. Sall *et al.* (1983) found that cholesterol had always yielded significant conversions into 2-deoxy ecdysone, and related ecdysteroids in L.migratoria. El-Gammal *et al.* (1989) reported a significant decrease in cholesterol and lipid contents of the 5th instar nymphs following treatment with the JHA, fenoxycarb. The results are also in agreement with Baehr *et al.* (1979) who demonstrated that ecdysteroids and juve-

Table 1. Effect of JHA (S-31183) on lipid contet when applied to 1-day old 5th instar nymphs of *S. gregaria*.

Dose of JHA ug/nymph	mg lipid/ml haemolymph ± S.D.			mg lipid/g fat body ± S.D.		
	During day-8	During day-9	During day-10	During day-8	During day-9	During day-10
100	2.51±0.2	0.90±0.04	0.66±0.02	32.85±0.85	14.09±0.25	15.88±0.54
50	1.45±0.14	0.88±0.02	0.50±0.10	31.24±0.18	43.8±0.14	27.74±0.65
25	1.22±0.07	0.40±0.07	0.22±0.01	15.18±0.16	18.05±0.38	14.03±0.95
control	2.82±0.23	-------	-------	100.0±4.39	-------	-------

Table 2 . Effect of JHA (S-31183) on lipid contet when applied to 6-day old 5th instar nymphs of *S. gregaria*.

Dose of JHA ug/nymph	mg lipid/ml haemolymph ± S.D.			mg lipid/g fat body ± S.D.		
	During day-8	During day-9	During day-10	During day-8	During day-9	During day-10
100	0.37±0.05	0.68±0.07	0.07±0.01	22.02±0.72	40.47±1.77	10.54±0.13
50	0.61±0.09	1.00±0.20	1.24±0.19	30.40±0.85	14.67±0.61	11.90±26
25	0.81±0.03	1.77±0.15	0.45±0.04	17.17±0.66	20.45±0.39	14.70±0.61
control	2.82±0.23	-------	-------	100.0±4.38	-------	-------

Table 3. Effect of treatment with JHA (S-31183) on lipid contet when applied to 1-day old 5th instar nymphs on their cholesterol content .

Dose of JHA ug/nymph	mg cholesterol/ml haemolymph ± S.D.			mg cholesterol/g fat body ± S.D.		
	During day-8	During day-9	During day-10	During day-8	During day-9	During day-10
100	0.44±0.3	0.23±0.04	0.37±0.06	1.12±0.04	1.68±0.02	1.00±0.03
50	0.66±0.02	0.29±0.02	0.22±0.03	0.70±0.03	1.50±0.02	0.87±0.02
25	0.19±0.04	0.12±0.02	0.17±0.02	0.31±0.03	1.69±0.05	0.69±0.02
control	0.36±0.02	--------	--------	3.26±0.07	--------	--------

Table 4. Effect of treatment with JHA (S-31183) on lipid contet when applied to 6-day old 5th instar nymphs on their cholesterol content .

Dose of JHA ug/nymph	mg cholesterol/ml haemolymph ± S.D.			mg cholesterol/g fat body ± S.D.		
	During day-8	During day-9	During day-10	During day-8	During day-9	During day-10
100	0.24±0.03	0.15±0.04	0.28±0.02	0.42±0.02	1.18±0.02	1.13±0.03
50	0.33±0.02	0.30±0.04	0.13±0.02	0.94±0.04	2.12±0.03	0.25±0.03
25	0.39±0.04	0.33±0.03	0.17±0.03	0.45±0.03	2.58±0.02	0.90±0.04
control	0.36±0.02	--------	--------	3.26±0.07	--------	--------

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تأثير مشابه هرمون الحداثة غير التربينى (اس – ٣١١٨٣) على محتوى حوريات العمر الخامس للجراد الصحراوى شستوسير كا جريجاريا

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إنخفض مستوى الدهون والكولسترول فى الهيمولف والاجسام الدهنية لحوريات العمر الخامس بصورة واضحة نتيجة للمعاملة بثلاثة جرعات (٢٥ ، ٥٠ ، ١٠٠ ، ميكروجرام / حورية) من المشابة الهرمونى (أس ٣١١٨٣) . إرتبط هذا الانخفاض بزيادة فى طول العمر الخامس للحوريات المعاملة الى يومين.

ويوضح هذا أن المركب يقوم بنفس تأثير هرمون الحداثة الطبيعى فى خفض مستوى الدهون والكولسترول فى حوريات الجراد .

respectively.

Moreover, the three doses (100, 50 and 25 ug) induced a remarkable reduction in the fat body cholesterol. Their amounts were 1.12, 0.70 and 0.31 mg/g fat body on day 8, 1.68, 1.50 & 1.69 mg/g on day 9 and 1.00 , 0.87 & 0.69 mg/g on day 10 for each dose, respectively, compared to 3.26 for control nymphs.

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