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METABOLIC EFFECTS OF THE IGR'S CHLORFLUAZURON (IKI) AND TWO FORMULATIONS OF TRIFLUMURON (BAY SIR AND SIR 514) IN SCHISTOCERCA GERGARIA FORSK.

By

ABOUEL-ELA; R.G.* HILMY. N.M. ; S.M. ALLAM; A.A.IBRAHIM AND A.D. ABD EL-MAGID**

* Entomol. Dept., Fac. Sci., Cairo Univ.
** Entomol. Dept, Fac. Sci., Benha Univ.

ABSTRACT

Quantitative analysis of the main metabolites (expressed as the total haemolymph and fat body contents of protein, carbohydrate and lipid) were carried out during the 3rd. 5th and 6th day of 4th nymphal instar of *S.gregaria* topically treated with different concentrations of Chlorfluazuron (IKI; EC) and two different formulations of Triflumuron (Bay Sir 8514; EC and Sir 8514; FC) using colouremetric method. The mean values of the total haemolymph and fat body protein contents were reduced in nymphs treated with Triflumuron, whereas the haemolymph and fat body protein contents were increased in Chlorfluazuron treated nymphs. A general increase in carbohydrate and lipid contents of the haemolymph and fat body was also observed in all treated insects.

INTRODUCTION

Many studies have been devoted to the role of insect growth regulators in insect development and a great diversity of functions has become known. Yet, the detailed mode of action, intracellular receptors, the mode of transport in the insect body, and many other aspects are even now not fully known. In the same time there is still some contradiction in the results obtained concerning the effect of these promising control agents on the main metabolities in insects. Accordingly,

the present study deals with the metabolic effects of the chitin synthesis inhibitiors; Chlorfluazuron (IKI) and triflumuron (Bay Sir and Sir 8514) in *Schistocerca gregaria* to clearify the mode of action of these compounds and to clearify the contradicted results about this subject.

MATERIAL AND METHODS

Experimental Insect :

The test insect, *Schistocerca gregaria* Forsk. (Orthoptera, Acrididae), was reared and handled according to the method described by Salem et al., (1983) and Pener et al., (1989) with some modifications. Fresh clover in winter and the leaves of *Sesbania aegyptiace* in summer were used in feeding insects.

Experimental nymphs were segregated from the stock colony at the beginning of the first instar and reared in groups of 100 hoppers per cage (30x30x30)cm) in a walk-in insectary at faculty of Science Benha University. An electric bulb (150 Watt) synchronized to photoperiod of 12 hours was placed in each cage to provide additional heat to maintain an ambient temperature of 32±2°C .

Insect Grwoth Regulators :

Triflumuron (Bay Sir 8514; 8.5%EC) was kindly provided by Prof hammed, M.S.; department of Entomology, Faculty of Science, Ain shams University.

Chlorfluazuron (IKI;5%EC) and Triflumuron (Sir 8514;6.5%FC) were kindly provided by Prof. Zidan, H., Faculty of Agriculture Ein shams University.

IGRs Application :

Three doses of each compound were prepared by dissolving the appropriate concentration in 4 ul of solvent, (IKI and Bay Sir 8514 were dissolved in acetone whereas Sir 8514 was dissolved in water). and were applied topically on the dorsal surface of a newly moulted 4th instar nymph. Each treatment

was replicated three times of ten individuals each. The untreated check groups receive 4ul of solvent only. The treated and untreated insects were kept at constant conditions of 32±2°C and 65±5% R.H.

Haemolymph collection and Purification:

the haemolymph was collected using capillary tubes after incising the hind leg membrane and gently squeezing the body . The haemolymph was transferred to clean dry centrifuge tubes. Few crystals of Phenylthiourea were added to prevent melanization before analysis, then stored at 4 ± 1°C until used. A known volume of the collected haemolymph (0.01 ml) was diluted up to 2 ml with saline solution and purified by centrifugation. Then the supernatant was collected for blood analysis, to determine protein and carbohydrate. The sample used for lipid determination was purified as follows: A known volume of the collected haemolymph (0.012ml) was diluted to 2 ml (1 ml Methanol ± 1 ml Chloroform), with shaking. Then methanol and chloroform was evaporated. After evaporation the contents of the tube was diluted up to 2 ml of ethanol.

Fat body collection and purification :

After the collection of haemolymph, the abdomen of nymphs were dissected and fat bodies around the alimentary canal were collected. 0.02 gm of the collected fat body were homogenized in 2 ml saline solution and the homogenate was purified by centrifugation. The supernatant was collected for estimation of Proteins and carbohydrates. one ml methanol and 1 ml chloroform was used instead of saline solution for the sample used in lipid determination as previously described in haemolymph purification.

Chemical Analysis of Metabolites :

Quantitative analysis of the main metabolites (expressed as the total haemolymph and fat body contents of protein, carbohydrate and lipid) were carried out during the 3rd, 5th and 6th day of the treated and check groups, using

colorimetivic methods . three pools of each treatment were utilized and divided into three equal samples, each for assay of one metabolite.

Protein content was determined by Folin Ciocalteau reagent, according to the method described by Lowery et al., (1951),, Lipid content was determined using the method of frings et al., (1972), whereas the total carbohydrate content was determined according to Singh and Sinha (1977).

RESULTS AND DISCUSSION

Total protein :

Our results revealed that, the mean values of the total haemolymph and fat body protein contents were reduced in 3rd. 5th and 6th day of the 4th nymphal instar treated with Bay Sir 5814 and Sir 5814 (Table, 1). these results may indicate that both formulations of Triflumuron inhibited the anabolism of the treated insects, where the metabolic activity is mostly of catabolic pattern. Bakr et al., (1991) obtained similar action of Bay Sir 8514 on *Musca domestica*. Fenoxycarb (El-gammal et al., 1988) and Precocene II (Hassan, 1990), induced similar decrease in protein content of *S.gregaria.*

On the other hand, the present results revealed an increase in haemolymph and fat body protein contents in IKI treated nymphs. the high level of protein may be due to the direct inhibitory action of IKI (as benzophenyl urea) on chitin synthesis and thus non utilized protein will be accumulated in fat bodies . These results are in agreement with what have been found by Hassan (1990) who found that IKI increased the haemolymph and fat body protein levels in 8-days old 5th instar nymphs of *S.gregaria,* and El-gammal, et al., (1988) who treated last nymphal instar of *S.gregaria* with Fenoxycarb, and found that the IGR caused an increase in total protein during the first two periods of 5th nymphal instar.

In the present study it may be also noticed that the haemolymph and fat body protein contents in all treated and

Table (1): Haemolymph and Fat Body Protien Content of 4th Instar Nymphs of *S.gregaria* topically treated with BAY SIR 8514, SIR 8514 and IKI under Lab. Conditions (temp. 32±2°C & RH. 65±5%).

IGRs	DOSE ug/nymph	HAEMOLYMPH PROTEIN CONTENT (mg/ml. Haem.)						FAT BODY PROTEIN CONTENT (mg/g.Fat B.)					
		During 3rd Day		During 5th Day		During 6th Day		During 3rd Day		During 5th Day		During 6th Day	
		Protein Content ±S.E.	Change%	Protein Content ±S.E.	Change%	Protein Content ±S.E.	Change%	Protein Content ±S.E.	Change%	Protein Content ±S.E.	Change%	Protein Content ±S.E.	Change%
BAY SIR 8514	1.15	18.61±0.97	49.9	118.56±1.91	17.3	55.49±0.6	50.5	6.93±0.73	45.5	12.41±0.63	31.2	7.99±0.24	38.2
	0.98	29.25±1.65	21.2	120.56±1.4	15.9	64.17±1.15	42.8	9.38±1.01	26.3	13.99±0.45	22.5	10.5±0.62	18.8
	0.75	31.08±0.61	16.3	130.54±1.52	8.9	69.13±0.98	16.1	11.78±1.17	7.4	17.45±0.6	3.3	10.64±0.4	17.7
SIR 8514	0.13	25.95±2.69	30.1	106.51±2.53	25.7	96.96±1.2	13.6	7.9±1.07	37.9	12.71±0.77	29.5	6.34±1.17	51
	0.098	31.02±1.38	16.5	112.2±3.99	21.7	96.17±1.4	14.3	10.96±0.38	13.8	13.49±0.89	25.2	7.49±1.69	42.1
	0.085	34.01±0.83	8.4	133.33±1.2	7	106.1±0.91	5.4	11.36±0.26	10.7	14.01±0.35	22.3	9.77±0.41	24.4
IKI	1.0	55.53±0.8	49.6	150.99±0.23	5.4	112.16±1.29	8.9	16.45±0.45	29.3	33.47±0.46	85.5	14.32±1.33	10.8
	0.75	61.48±0.67	65.6	187.31±0.19	30.7	114.19±1.38	1.8	54.73±1.63	330.3	72.76±1.53	303.3	21.87±1.38	69.1
	0.55	73.85±1.29	98.9	191.38±1.1	33.5	142.14±0.34	26.7	24.42±1.81	91.9	41.7±2.01	131.2	16.5±1.49	27.6
	Check	37.13±1.38	–	143.31±0.018	–	112.18±1.05	–	12.72±0.41	–	18.04±0.67	–	12.93±0.99	–

Significance (P<0.05) High Significance (P<0.01) Very High Significance (P<0.001).

untreated groups tens to decrease during the 6th day of 4th nymphal instar . This result is in agreement with Hill and Goldsworthy (1968) who found that, the protein content of the haemolymph and fat body of 1st nymphal instar of *L.migratoria* increased during the instar and decreased during the period of ecdysis. This decrease might be due to the utilization of protein in the pre-ecdysial growth of the new cuticle.

Total carbohydrate :

The data in table, (2) indicate that, the three used formulations of compounds induced a significant increase in total haemolymph and fat body carbohydrate contents during the three days of investigation. This increase may be due to a decrease in the uptake of carbohydrates in different physiological procially chitin synthesis. It could be concluded that the used IGRs acted mainly on the fat bodies of treated 4th instar nymphs leading to strong accumulation of carbohydrates in this tissue.

Many investigators recorded an increase in carbohydrate content in insects treated with Adipokinetic hormone (Van Marrewjk et al., 1984) and JHA (Abou El-Ela, et al., 1990).

Total Lipid.

Insects preferentially store lipids in the fat body rather than glycogen as an energy source. The storage of lipids has adaptive advantage especially for relatively small insects. This is because, an isoelectric quantity of lipids occupies less storage place than the equivalent amount of gylycogen and in addition the metabolism of lipids generates more water and energy than carbohydrates (Chippendale, 1973).

In the present study (Table,3) , a genearl increase was observed in haemolymph and fat body lipid contents of 4th instar nymphs treated with the used IGRs. This observation

Table (2): Haemolymph and Fat Body Carbohydrate Content of 4th Instar Nymphs of *S. gregaria* topically treated with BAY SIR 5814, SIR 8514 and IKI under Lab. Conditions (temp. 32±20°C & RH. 65±5%)

IGRs	DOSE ug/nymph	HAEMOLYMPH CARB. CONTENT (mg/ml. Haem.)						FAT BODY CARB. CONTENT (mg/g.Fat B.)					
		During 3rd Day		During 5th Day		During 6th Day		During 3rd Day		During 5th Day		During 6th Day	
		Carb. Content ±S.E.	Increase %	Carb. Content ±S.E.	Increase %	Carb. Content ±S.E.	Increase %	Carb. Content ±S.E.	Increase %	Carb Content ±S.E.	Increase %	Carb Content ±S.E.	Increase %
BAY SIR 8514	1.15	36.51±0.81*	26.4	54.76±0.66	5.2	57.3±1.1	6.8	27.53±1.31□	318.4	36.32±0.89*	21.2	51.45±1.41*	45
	0.98	39.52±0.69*	36.8	53.81±0.72	3.4	60.97±1.37*	13.6	44.15±0.68□	570.9	74.35±0.89□	148.2	50.5±1.04*	42.3
	0.75	48.73±0.94□	68.7	58.41±1.11*	12.2	55.87±0.44	4.1	40.57±1.24□	516.6	53.84±0.65□	79.7	84.91±0.92□	39.3
SIR 8514	0.13	31.75±1.06	9.9	55.27±0.52	6.2	54.22±0.91	1.1	19.71±0.86*	199.5	37.48±1.21*	25.1	49.2±1.05*	38.7
	0.098	35.71±0.6*	23.6	62.06±0.67*	19.2	62.9±0.58*	17.2	8.33±1.64	26.6	30.8±1.35	2.8	36.49±1.12	2.8
	0.085	29.4±0.32	1.8	59.01±1.08*	13.3	60.32.1±0.67*	12.4	16.25±0.99*	146.9	39.99±0.74*	33.5	59.1±0.95□	66.6
IKI	1.0	42.22±1.14*	46.2	54.44±0.72	4.6	57.62±0.49*	7.4	26.81±1.37□	307.4	35.06±1.06*	17	50.47±0.84*	42.2
	0.75	32.22±1.64	11.6	57.37±0.36*	10.2	68.09±1.59*	26.9	53.57±1.95□	714.1	73.69±1.87□	145.9	52.19±0.79□	47.1
	0.55	36.19±1.28*	25.3	61.43±1.02*	18	56.24±0.83	4.8	84.88±1.88□	1189.9	91.11±2.3□	204.1	95.06±2.27□	167.9
	Check	28.88±1.94	--	52.06±1.95	--	53.65±1.65	–	6.58±1.49	–	29.96±1.6	--	35.48±1.79	–

* Significance (P<0.05) *High Significance (P<0.01) □Very High Significance (P<0.001).

Table (3): Haemolymph and Fat Body Lipid Content of 4th Instar Nymphs of *S.gregaria* topically treated with BAY SIR 8514, SIR 8514 and IKI under lab. Conditions (temps. 32±2°C & RH. 65±5%).

IGRs	DOSE ug/nymph	HAEMOLYMPH LIPID CONTENT (mg/ml. Haem.)						FAT BODY LIPID CONTENT (mg/g.Fat B.)					
		During 3rd Day		During 5th Day		During 6th Day		During 3rd Day		During 5th Day		During 6th Day	
		Lipid Content ±S.E.	Increase %	Lipid. Content ±S.E.	Increase %	Lipid Content ±S.E.	Increase %	Lipid Content ±S.E.	Increase %	Lipid Content ±S.E.	Increase %	Lipid. Content ±S.E.	Increase %
BAY SIR 8514	1.15	26.15±0.99	11.1	29.89±0.83	4.3	28.23±0.78	3.4	176.65±1.68□	180.7	192.36±1□	29.6	154.48±1.49•	11
	0.98	25.84±1.86	9.8	28.99±0.25	1.2	27.89±0.68	2.2	168.06±1.67□	167	187.15±0.71□	26.1	145.58±0.85•	4.6
	0.75	28.23±0.93•	19.9	32.4±0.69•	13.1	29.69±0.62•	9.2	91.63±0.68□	45.6	159.65±1.83•	7.6	142.76±0.78•	2.6
SIR 8514	0.13	32.5±0.51•	38.1	36.98±0.54•	29.1	29.17±0.68•	6.9	141.79±2.61□	125.3	251.9±1.27□	69.8	172.05±0.94•	23.9
	0.098	25.21±0.99•	7.1	32.19±0.65•	12.4	28.44±0.87	4.2	127.39±2.02□	102.4	298.78±3.31□	101.3	149.59±1.25•	7.5
	0.065	27.29±1.27•	15.9	33.23±1.7•	16	31.87±0.31•	16.8	92.98±1.72□	47.7	200.43±1.65□	35.1	178.48±1.1□	28.2
IKI	1.0	27.31±0.68•	16	31.85±0.71•	11.2	27.39±0.31	0.4	170.64±1.58□	171.1	302.89±1.08□	103.4	162.76±1.63□	16.9
	0.75	26.04±0.46	10.6	31.23±1.06	9	30.73±0.38•	12.6	149.06±0.83□	136.8	230.56±1.54□	54.8	159.72±1.19□	14.8
	0.55	29.5±1.04•	25.3	32.92±0.79•	14.9	28.2±0.91	3.3	332.23±1.71□	427.9	374.25±2.6□	151.3	240.1±2.53□	72.5
	Check	23.54±1.27	–	28.65±0.68	–	27.29±0.42	–	62.94±1.5	–	148.39±1.12	–	139.18±1.08	–

•Significance (P<0.05) •High Significance (P<0.01) □Very High Significance (P<0.001).

may be explained by the fact that, the IGRs increased the conversion rate of carbohydrate to lipid. Leading to a high level of lipid in the haemolymph and fat body of the treated nymphs. It was also noticed that, the used IGRs affected mainly the fat body. Similar effect of IKI of 5th nymphal instar of S. gregaria was observed by Hassan, (1990) . An increase in total lipid content following allatectomy of 1- day old females of *S.gregaria* was reported by (Hill and WEZzat, 1974) . Also application of Adipokinetic hormone against *L.migratoria* induced a similar increase in lipid content (Stone and Mordue, 1980, Loughton, 1987 and Pener et al., 1989).

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تأثير منظمات النمو الحشري ، الكلورفلوازورون (آي كيه آي) وصورتين من الترايفلومورون (باي سير وسير ٨٥١٤) على نواتج الأيض في حشرة الجراد الصحراوي شيستوسيركا جريجاريا فورسكال.

*رفعت غريب أبوالعلا ، **ناهد محمد حلمي ، سومية محمد علام ،

عبدالوهاب عبدالمقصود إبراهيم ، عبلة دسوقي عبدالمجيد .

قسم علم الحشرات – كلية العلوم جامعة القاهرة .

** قسم علم الحشرات – كلية العلوم – جامعة بنها .

تم إجراء تحليل كمي باستخدام طريقة القياس الضوئي للنواتج الأيضية الرئيسية . المحتوي الكلي للبروتين والمواد الكربوهيدراتية والدهون في كل من الليمف الدموي والجسم الدهني) خلال اليوم الثالث والخامس والسادس من حياة العمر الحوري الرابع لحشرة الجراد الصحراوي شيستوسيركا جريجاريا التي عوملت بطريقة التطبيق السطحي بتركيزات مختلفة من الكلورفلوازورون (آي كيه آي ، سائل) وتحضيرين من الترايفلومرون (باي سير ٨٥١٤ سائل ك وسير ٨٥١٤ ك مستحلب) . وقد أظهرت النتائج أن المحتوي الكلي للبروتين قد انخفض في كل من الدم والجسم الدهني للحوريات التي عوملت بالترايفلومورون وازداد في الحوريات التي عوملت بالكورفلوازورون.

وأظهرت النتائج زيادة عامة في المحتوي الكلي للمواد الكربوهيدراتية والدهون في الدم والجسم الدهني لجميع الحوريات التي عوملت بمنظمات النمو الثلاثة .