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Effect of Teflubenzeron and Lufenuron on Some Biological Aspects of *Chrysopela carnea (Stephens)* Immature Stages

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ABSTRACT

Biological and biochemical effects of LC_{50} of Growth Regulators (IGR's) Teflubenzuron and Lufenuron were studied against green *Chrysopella carmea* (Stephens) under laboratory conditions. Tested compounds were evaluated against fresh first instars by contact and feeding techniques on *Corcyra cephalonica* egg. LC_{50s} were 43.17 and 28.65 ppm, by contact technique for Teflubenzuron, and Lufenuron, respectively, but when the feeding technique was applied, the LC_{50s} were 31.22 and 16.71 ppm for Teflubenzuron, and Lufenuron, respectively. The biological effects of these compounds were studied on larval and pupal periods which resulted from treated first instar. The obtained results showed prolongation in developmental periods of larval and pupal stages compared with control. The latent effect of these compounds on some biochemical parameters in second instar larvae resulted from treated fresh first instar larvae were analyzed for Phenoloxidase activity, total protein, N-acetyl- glucosamine significance, and chitinase activity. The results showed a significant reduction in all biochemical parameters but chitinase was significantly high compared with control. In general, the tested compounds had significant biological and biochemical effects on immature stages of *C. carnea*.

INTRODUCTION

The biological control using predators and parasitoids is one of the latest methods or trends in the field of pest control to preserve the environment and its components and to avoid the use of chemical pesticides and the negative effects that it has on the environment and its components, including human, food and animals. The presence of predators and parasitoids in different field crops has been a subject for many studies of reducing insecticide usage and environmental pollution (WhiteComb and Bell 1964; Dean and Sterling 1992). Sometimes the presence of predators reduces the usage of pesticides.

Chrysoperla carnea (Stephens) (aphid lion) is considered one of the most important and very useful predators especially in the fields affected by the aphids. Aphid lion larvae have a wide range of prey acceptance (Hydron and WhiteComb 1979), which can feed on them such as aphids, whiteflies and eggs of some insects, Due to the polyphagous, the wide geographical distribution, and tolerance to some pesticides (Araujo and Bichao 1990, New

1975), so this predator has received interest and studies as potential biological pest control agent (Hassan *et al*. 1985)

The effectiveness of *C. carnea* as biological control agent has been studied in different field crops, (Hagley and Miles 1987) reported that *C. carnea larvae* gave about 100% Lepidoptran pest control when used along with *Trichogramma* spp. (Rincon- Vitova 1999). In spite of all these benefits, *C. carnea* has almost been eliminated from fields due to the use of some non-selective compounds (Nasreen *et al*. 2005). Now, the importance of biological control has been recognized as a holistic approach for integrated pest management and it can be achieved only by using selective compounds to kill the pest only.

The group of pesticides known as insect growth regulators (IGRs) is potent insecticides, owing to its potent insecticides, which cause inhibiting growth in insects (Ghoneim *et al.,* 2017). Insect Growth Regulators (IGR's) disrupt and impede the life cycle and development of insects. Insect growth and metamorphosis are regulated by hormones produced by endocrine glands; these hormones are transported in the insect hemolymph that helps in the completion of various physiological processes. IGRs cause the abnormal formation of endocuticle that accumulates during the molting process, specifically uridines diphospho-N-acetylglucosamine thereby preventing chitin synthesis and breakdown of the metamorphosis process (Ghoneim *et al.,* 2017, Kandil 2013 and Said *et al.,* 2017 and 2019), this prevents cuticle formation and causes mortality. (Sabry and Abdou, 2016), recorded that IGRs, may be selective in their mode of action and potentially act only on the target species, also showed that activity of IGRs depends on the development and metamorphosis of insects.

The present study aimed to investigate the effect of Teflobenzeron and Lufenuron as IGR compounds on some biological and biochemical parameters of *Chrysoperla carnea* (Stephens) (aphid lion).

MATERIALS AND METHODS

Rearing of Host Insect, *Corcyra cephalonica:*

Eggs of *C. cephalonica* were used for mass culturing of the predator. In the laboratory, the larvae of *C. cephalonica* were reared by following a standardized protocol. *C. cephalonica* eggs were obtained from the Biological Control, Laboratory, Cairo University.

Mass Rearing of the Predator *Chrysopela carnea*:

The larvae were obtained from the biological control laboratory of the Faculty of Agriculture, Cairo University, and then mass-rearing for several generations in the laboratory conditions (25 ± 2 °C& 65± 5% R. H.) as described by Hassan, (2014) in the biological laboratory of the Faculty of Agriculture, Menoufia University. Green lacewings *Chrysoperla carnea* larvae were obtained from adults reared on the eggs of *C. cephalonica*. The laid eggs were collected daily and kept under the same conditions. The newly hatched larvae were fed on eggs of *C. cephalonica.* The hatched larvae will be raised to the required stage (1^{st} instar) for treatment.

Tested Insect Growth Regulators (IGR) and Determination of LC50:

1- (Teflubenzuron) Trade name: Nomolt® 15% Suspension Concentrate (SC).
Rate of application: 50 cm^3 / 100 L.
Basic product: BASF Co.
2- (Lufenuron) Trade name: (Match 5% EC). Rate of application: The field rate is 160 ml/feddan.

Effect of Tefubenzuron and Lufenuron was tested against first instar larvae of *C. carnea* under laboratory condition, these compounds were obtained from the Plant Protection Research Institute. Six concentrations of tested compounds were prepared (200,

100, 50, 25, 12.5 and 6.25 ppm). The spraying technique was used for treating Corcyra eggs with previous concentrations. Tested instar larvae of *C. carnea* were released into petri plates containing treated Corcyra eggs. The larvae were allowed to feed on the treated eggs and once they are fed, they were transferred to individual larval rearing trays (Honey comb structure) by providing untreated Corcyra eggs, larval mortality was observed and LC50 was calculated.

Effect of Tested Compounds on Biological Parameters of *Chrysopela carnea* Immature Stages:

After LC50 determination, fifty larvae of *C. carnea were* fed on Corcyra eggs treated with LC50 of tested compounds and were observed until pupation, control larvae were fed on untreated Corcyra eggs which spray with distilled water. The treatments were replicated three times. Observations on larval period, pupa formed and percentage of adult emergence was recorded.

Effect of Tested Compounds on Biochemical Analysis:

Preparation of Samples for Biochemical Assay:

Samples of *C. carnea* larvae treated by LC_{50} of Teflubenzeron and Lufenuron were collected after 8 days from treatment to study the latent effects of the tested compound on the third instar larvae, these samples were homogenized in distilled water. The homogenates were centrifuged at 5000 r. p. min. at $5C^0$ in a refrigerated centrifuge. The supernatants were kept in a deep freezer at $-20C^0$ till used for biochemical assays. Some biochemical analyses were estimated in the Physiological Dept. of Plant Protection Researches Institute.

Analyses Technique:

The colorimetric determination of total soluble protein, in the total homogenate of *C. **carnea*** third larvae was carried out, as described by Bradford (1976). Determination of chitinase activity was prepared according to Bade and Stinson (1981). Determination of N-acetyl- glucosamine by the sensitive method of Waterhouse *et al.* (1961). Phenoloxidase activity was determined according to modification of Ishaaya (1971)

Statistical Analysis:

The recorded data were statistically analyzed by one- way ANOVA test at 0.05 of probability and range test of means used Duncan's multiple using SPSS program (version 11)

RESULTS AND DISCUSSION

Effect of Tested Compounds on Larval Stage:

The LC_{50} values of tested (IGRs) on first instar larvae are given in Table (1), LC_{50} values were 43.17, 31.2, 28.65 and 16.71 ppm for Teflubenzuron and Lufenuron by treating with contact and feeding technique, respectively. These values show that Lufenuron was highly toxic than Teflubenzuron

The data recorded in Table (1) showed that the difference in periods of larvae & pupae, the total period of immature stages and malformed larvae resulted from treated first instar larvae with LC_{50} of Teflubenzeron and Lufenuron. The tested compounds significantly prolonged the duration of the larval and pupal stages than that of control, which led to longest total immature stages periods, in addition to malformations in the third instar larvae.

Larval periods were 11.1, 10.2 and 8.8 days/ larva by contact technique for Teflubenzuron, Lufenuron and control, respectively. Also, larval periods were 12.8 and 11.1 compared with control 8.8 days/larva when the first instar larvae were treated with Teflubenzuron and Lufenuron by feeding technique. It was observed that there was no significant difference between the tested compounds, but there was a significant difference

between treatments and control. This may be the same chemical composition and therefore the same mode of action.

The results also indicated that the pupal periods were 9.6and 8.3 days/larva for Teflubenzeroun and Lufenuron compared with control 8.6 days/ larva, respectively when contact technique was used for treatment. These periods were 10.3, 9.6 and 8.6 days/larva for previous compounds compared with control 8.6 days /larva when the feeding technique was used for treatment.

Total immature stage periods also were recorded in the table (1), these values were 20.1 and 19.5 days for Teflubenzeroun and Lufenuron respectively when contact technique was used compared with control 17.4 days. when the feeding technique was used these values were 21.1 and 20.7 days compared with control 17.4 days.

Previous results indicate that the tested compounds generally caused significant prolongation in both larval and pupal periods. (Kandil *et al.*, 2013; Ghoneim 2017 and Said *et al.*, 2017 and 2019). In addition to increased incidence of morphological malformations, (Tanani and Bakr 2018).

It was clear from the previous results that the treatment of the first instar larvae with LC50 affected the number of the next second and third instars, as well as the number of pupae, this occurred due to the increase in the death rates and the failure of the moulting process which prevented the formation of the good cuticle, (Khatter, 2014, Gado *et al.*, 2015, Tanani and Ghonim, 2018).

Table 1: Effect of LC_{50} value of Teflubenzuron and Lufenuron on some biological parameters of *Chyrisopela carnea* immature stages.

Treatments	Contact technique						
	LC_{50}	1st instar	2ed instar	3rd instar	Larval Period (day)	Pupal period (day)	Total period of immature stages(day)
Teflubenzuron	43.17	2.6 ± 0.1	3.6 ± 0.2	4.9 ±0.5	11.1 ±1.1 a	9.6 ±1.8 a	20.1 ±2.3 a
Lufenuron	28.65	2.3 ± 0.4	3.3 ± 0.4	4.6 ±0.3	10.2 ±1.3 b	9.3 ± 1.3 a	19.5 ±2.1 b
control	-	2.3 ± 0.2	2.6 ± 0.3	3.9 ±0.4	8.8 ± 1.5c	8.6 ± 1.2 c	17.4 ±1.6 c
N		-	-	-	3	3	3
F value	-	-	-	-	6.331	4.332	12.351
P-value	-	-	-	-	0.002**	0.014*	0.033*
LSD 5%	-	-	-	-	0.22	0.142	1.27
	Feeding technique						
Treatments	LC_{50}	1st instar	2ed instar	3rd instar	Larval Period (day)	Pupal period (day)	Total period of immature stages(day)
Teflubenzuron	31.22	3.6 ± 0.1	4.6 ± 0.4	5.9 ±0.4	12.8 ±1.7 a	10.3 ±1.2 a	21.1 ±1.5 a
Lufenuron	16.71	3.2 ± 0.2	4.6 ± 0.5	5.6 ±0.7	11.1 ±1.2 b	9.6 ±1.4 b	20.7 ± 1.4 b
control	-	2.3 ± 0.2	2.6 ± 0.3	3.9 ±0.4	8.8 ±1.5 c	8.6 ±1.2 c	17.4 ± 1.6 c
N		-	-	-	3	3	3
F value	-	-	-	-	5.411	3.613	14.624
P-value	-	-	-	-	0.011*	0.021*	0.041*
LSD 5%	-	-	-	-	0.72	0.52	0.33

Means followed by the same letter in the same column have no significant differences.

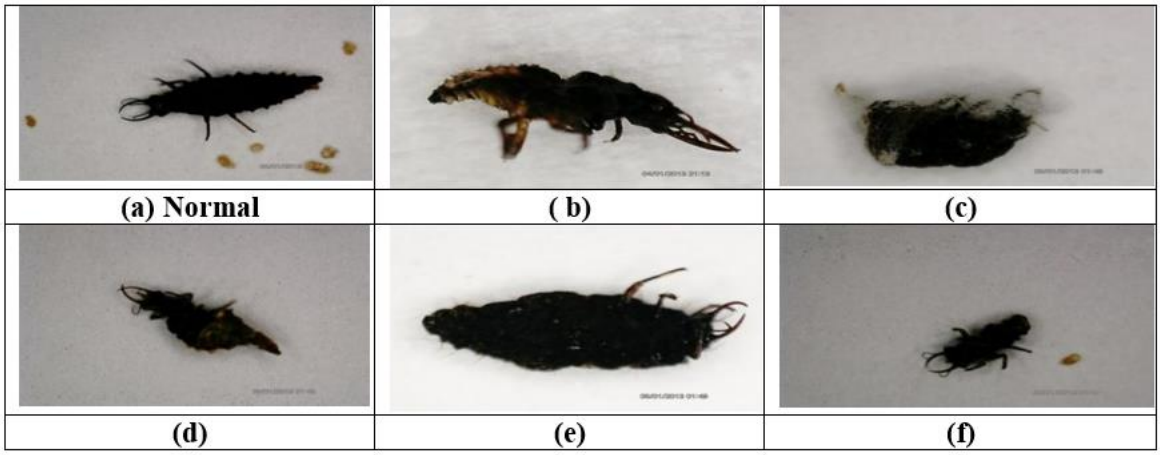

Fig. 1: Malformation occurred by tested IGRs (a) normal second instar, (b and c) malformed second instar treated with Tefelubenzeron, (d,e and f) malformed second instar treated with Lufenuron.

Table 2: Effect of Teflubenzeron and Lufenuronon some biochemical aspects of immature stages of *C. carnea*.

Biochemical parameters by contact technique						
Treatment	**LC50**	**Phenoloxidase (O.D. units/g.b.wt)**	**Total protein (mg/g.b.wt)**	**N- acytel- glucceamine (µg NAGA /g.b.wt)**	**Chitinase (µg NAGA x10^3/min/g.b.wt)**	**Free-amino acid (µg D, L-alanine/g.b.wt)**
Teteflubenzeron	43.17	**12. 0±0.28c**	**12.0±0.5 b**	**124.3±5.7 b**	**377.0±0.2a**	**269±1.2b**
Lufenuron	28.65	**15.7±0.5ab**	**11.6±0.7 b**	**87.6±3.4 c**	**644.0±1.34a**	**193.66±3.5c**
Control	-	**26.0±1.2a**	**23.4±1.1 a**	**264.0±3.5a**	**258.0±4.9c**	**286.33±2.5a**
F value	-	**21.421**	**22.356**	**322.114**	**4.612**	**532.441**
N	-	**3**	**3**	**3**	**3**	**3**
P-value	-	**0.0001*** **	**0.0013** **	**0.000*** **	**0.003** **	**0.022***
LSD 5%	-	**2.61**	**2.31**	**10.33**	**217**	**12.1**
Biochemical parameters by feeding technique						
Treatment	**LC50**	**Phenoloxidase (O.D. units/g.b. wt)**	**Total protein (mg/g.b. wt)**	**N- acytel- glucceamine (µg NAGA /g.b.wt)**	**Chitinase (µg NAGA x10^3/min/g.b.wt)**	**Free-amino acid (µg D, L-alanine/g.b.wt)**
Teteflubenzeron	**31.22**	**17.9±2.3 b**	**17.3±1.4b**	**108.3±6.4c**	**455.0±7.44 b**	**262.66±3.1b**
Lufenuron	**16.71**	**20.32± b**	**15.7±1.2c**	**136.2±1b**	**863.45±8.4 b**	**229±2.3 c**
Control	-	**26.0±1.2a**	**23.4±1.1 a**	**264.0±3.5a**	**258.0±4.9c**	**286.33±2.5a**
F value	-	**23.221**	**19.115**	**325.013**	**5.351**	**511.112**
N	-	**3**	**3**	**3**	**3**	**3**
P-value	-	**0.0031** **	**0.001*** **	**0.000*** **	**0.014***	**0.023***
LSD 5%	-	**2.31**	**2.44**	**10.45**	**218**	**11.87**

Means followed by the same letter in the same row have no significant differences.

Data in Table (2) showed the mean values of Phenoloxidase, total protein, N- acytel-glucceamine, chitinase activity and Free-amino acids. The mean values for Phynoloxidase activity for Teflubenzuron and Lufenuron by contact technique were 12.0 and 15.7 (O.D.units/g.b.wt) respectively, compared with 26.0 (O.D.units/g.b.wt) for control.These mean values were 17.9 and 20.32 (O.D.units/g.b.wt) when feeding technique was used for Teflubenzuron and Lufenuron respectively, compared with 26.0 (O.D.units/g.b.wt) for control.

In addition, the mean values for N- acytel- glucceamine activity for Teflubenzuron and Lufenuron by contact technique were 87.6 and 124.3(µg NAGA /g.b. wt) respectively, compared with 264 (µg NAGA /g.b. wt) for control. These mean values were 108.3 and 136.2 (µg NAGA /g.b.wt) when the feeding technique was used for Teflubenzuron and Lufenuron, respectively compared with 264 (µg NAGA /g.b.wt) for control.

The same effect occurred with the level of total protein, the mean values for Teflubenzuron and Lufenuron by contact technique were 12.0 and 12.6 (mg/g.b. wt) respectively, compared with 23.4 (mg/g.b.wt)for control.These mean values were 15.7 and 17.3 (mg/g.b.wt) when feeding technique was used for Teflubenzuron and Lufenuron respectively ,compared with 23.4(mg/g.b.wt)for control.

The mean values for chitinase activity for Teflubenzuron and Lufenuron by contact technique were 377 and 644 (µg NAGA $x10^3$/min/g.b. wt) respectively compared with 258 for control. These mean values were 455 and 863 (µg NAGA $x10^3$/min/g.b. wt) when the feeding technique was used for Teflubenzuron and Lufenuron, respectively compared with 258 (µg NAGA $x10^3$/min/g.b. wt) for control. These values showed that the chitinase activity of full-grown larvae resulted from treated first instar larvae with two IGRs Teflubenzuron and Lufenuron caused a high increase.

Also, highly decreased occurred in Free-amino acids. The mean values for Teflubenzuron, Lufenuron and control by contact technique were 269, 193 and 286.33 (µg D,L- alanine/g.b.wt) ,respectively. The same effect occurred when the feeding technique was applied, the mean values were 262.66, 229 and 286.33 (µg D, L- alanine/g.b. wt) for Teflubenzuron , Lufenuron and control, respectively.

Data in Table (2) indicated that tested IGRs caused a significant high decrease in the level of Phenoloxidase, total protein, Free-amino acids and N- acytel-glucceamine which is necessary for chitin formation in the second larvae resulting from treated first instar larvae with Teflubenzuron, Lufenuron, respectively, compared with control, but significant-high increased occurred in Chitinase activity. (El-Sheikh *et al.,* 2013; Said *et al.,* 2017; El-Naggar, 2013; Bakr *et al.,* 2013and Tanani *et al.,* 2015).

Based on previous results, Teflubenzuron and Lufenuron caused long-term inhibition effects on the second larvae, of *C. carnea*. In addition, tested IGRs caused a defect in the molting process and also caused malformations at different instars (Fig. 1). of the tested insect. It is noticeable that the tested compounds greatly affected insects, in addition to that, it was found that these compounds affect these insects by contact as well as by feeding, but the effect through feeding was greater. Thus, these compounds can be used, but at times other than the activity of this good predator, and therefore can be integrated into an integrated control program. Also, these compounds need future studies to show more physiological effects on the insect.

Conclusion

Effect of LC_{50} of Growth Regulators (IGR's) (Teflubenzuron, and Lufenuron) against green *Chrysopella carmea* under laboratory conditions was studied. Tested compounds were evaluated against fresh first instars by contact and feeding techniques. LC_{50s} were43.17 and 28.65 ppm, by contact technique for Teflubenzuron, and Lufenuron, respectively, but when the feeding technique was applied, the LC_{50s} were 31.22 and 16.71 ppm for Teflubenzuron, and Lufenuron, respectively. These compounds have biological and biochemical effects on larval and pupal periods resulting from treated first instar was studied. The obtained results showed prolongation in developmental periods. Also, previous compounds affected some biochemical parameters of second instar larvae resulting from treated fresh first instar larvae such as Phenoloxidase activity, total protein, N-acetyl-glucosamine significance, and chitinase activity. The results showed a significant reduction in all biochemical parameters but chitinase was significantly high. Generally, the tested compounds had significant biological and biochemical effects on immature stages and this work needs more physiological studies and how the previous compounds affect the different stages of *C. carnea.*

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ARABIC SUMMARY

تأثير تيفلوبينزرون ولوفينورون على بعض الجوانب البيولوجية للاطوار غير الكاملة لأسد المن (ستيفنس)

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1- قسم الحشرات الاقتصادية والحيوان الزراعي – كلية الزراعة – جامعة المنوفية

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تمت دراسة التأثيرات البيولوجية والكيميائية الحيوية لـ LC_{50} لمركبين من منظمات النمو (IGR's) Teflubenzuronو Lufenuron ضد اسد المن الاخضر (ستيفنس) تحت الظروف المعملية. تم تقييم المركبات المختبرة تجاه العمر الاول لليرقات حديثة الخروج وذلك من خلال تقنيتين هما: التلامس والتغذية على بيض *Corcyra cephalonica* معامل بالمركبات السابقة. تم تقدير LC_{50s} لهذه المركبات و كانت 43.17 و 28.65 جزء في المليون، عن طريق تقنية التلامس عند المعاملة بالمركبين Teflubenzuron ، و Lufenuron ، على التوالي ، ولكن عند تطبيق تقنية التغذية ، كانت LC_{50s} 31.22 و 16.71 جزء في المليون لتيفلوبنزورون و Lufenuron ، على التوالي. تمت دراسة التأثيرات البيولوجية لهذه المركبات على أطوار اليرقات والعذارى التي نتجت عن الطور الأول المعامل. أظهرت النتائج المتحصل عليها وجود استطالة في فترات نمو اليرقات والعذارى مقارنة مع مجموعة الكنترول الغير معاملة. تم دراسة التأثير التالي لهذه المركبات على بعض المتغيرات البيوكيميائية في يرقات العمر الثاني الناتجة عن يرقات الطور الأول المعاملة من حيث نشاط انزيم الفينولوكسيديز والبروتين الكلي ودلالة -N أسيتيل الجلوكوزامين ونشاط انزيم الكيتيناز. أظهرت النتائج انخفاضًا معنويًا في جميع المتغيرات البيوكيميائية ولكن كان انزيم الكيتيناز مرتفعًا بشكل معنوي مقارنة مع الكنترول. بشكل عام، كان للمركبات المختبرة تأثيرات بيولوجية وكيميائية حيوية على المراحل غير الكاملة من*C. carnea*