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PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Effectiveness of Lufenuron (CGA-184699) and Diofenolan (CGA-59205) on the General Body Metabolism of the Red Palm Weevil, *Rhynchophorus ferrugineus* (Curculionidae: Coleoptera)

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Abstract: In the present study, prepupae of the red palm weevil *Rhynchophorus ferrugineus* were topically treated with a dose of 1.0, 0.1 or 0.01 μg Lufenuron or Diofenolan / insect. The main body metabolites, carbohydrates, proteins and lipids, were determined during the pupal stage. The lowest dose of Lufenuron or Diofenolan caused a gradual decrease in carbohydrate content, but the highest and medium doses induced a reciprocal V-shaped trend in this metabolite throughout the pupal life. The higher two dose-levels of Diofenolan and all dose-levels of Lufenuron, induced such trend in protein content of pupae. The available results indicated a greater reducing action on protein content of Lufenuron than of Diofenolan. In connection with the lipids, the higher two dose-levels of each acylurea suppressed and then stimulated, the content of this metabolite along the pupal stage. In other words, these IGRs reduced the lipid content at the two limits of the pupal stage, but enhanced the pupae to lipid increments at almostly the mid-stage.

Key words: *Rhynchophorus ferrugineus*, lufenuron, diofenolan, metabolism, carbohydrates, proteins, lipids

Introduction

All around the 1970s, a new approach to insect control was suggested depending on the use of compounds essential in the normal metabolism of the insect but applied at an abnormal period in the life cycle, the so-called "Third-generation insecticides" which are juvenile hormone analogues (JHAS) or juvenile hormone mimics (JHMs). In the late decades, many efforts have been made to synthesize and utilize a wide variety of JH-based insecticides against several insect species, originally advanced by Williams in 1976. All these compounds are called, also, insect growth regulators (IGRs). They are generally considered to be environmentally acceptable because they only affect systems unique to insects and certain other arthropods.

The benzoylphenyl ureas constitute a class of the IGRs that interfere with insect growth and development by inhibiting chitin synthesis in insects (Post and Vincent, 1973). Many institutions have engaged for searching about different derivatives of the optimum molecule of the benzoylphenyl ureas "diflubenzuron", which are considerably more potent than it on various agricultural pests (Ishaaya *et al.*, 1984, 1987; Ascher and Nemny, 1984).

The purpose of the present paper was to describe the possible effects of Lufenuron and Diofenolan (acylureas) on the main body metabolites of the red palm weevil *Rhynchophorus ferrugineus* which was reported as a serious pest of the date palms in Egypt.

Materials and Methods

The experimental insect: Red palm weevil, *Rhynchophorus ferrugineus*, is a serious pest for date palm in the Gulf States and Egypt (Cox, 1993). Transferring this pest from the infestation region (Ismailya and Sharqya Governorate) to other parts in Egypt was Legislatively. So, no culture could be established in the laboratory at Cairo. Chemically untreated, but infested, date trees were usually utilized for obtaining alive prepupae of *Rh. ferrugineus* for conducting the experimental work in the present study.

IGR treatments: Effects of the acylureas, Lufenuron (CGA-184699) and Diofenolan (CGA-59205) on the total contents of the main body metabolites were assessed. Three dose-levels: 1.00, 0.10 and 0.01 μg insect⁻¹ were prepared and topically applied through μl acetone onto the pronotum of prepupae. Control congeners received acetone only. All treated and control insects were kept at $27\pm 2^\circ\text{C}$ and $75\pm 5\%$ RH.

Preparation of samples: The developed pupae were used for achieving this purpose at three ages. 1-day old (early-aged pupae), 4-day old (mid-aged pupae) and 7-day old (late-aged pupae). Some pupae of each age were collected, weighed and freshly homogenized in 5 ml saline solution. Crude body tissues were separated by a centrifuge at 3000 r.p.m. for 5 minutes. Control samples were prepared by the

same manner for pupae developed from the acetone treated prepupae.

Determination of the main metabolites: The carbohydrate (as glycogen) content in the pupal body homogenate was quantitatively determined by using the anthrone reagent according to Singh and Sinha (1977) and utilizing the Spectrophotometer at 620 m μ . To estimate the protein content of the pupal homogenate, the Biuret reagent was used according to Garwall *et al.* (1949) using the Spectrophotometer at 550 m μ . Lipid extraction was carried out according to the technique of Folch *et al.* (1957) and the lipid estimation was taken place by phosphovanilin reagent depending on Knight *et al.* (1972) and using the Spectrophotometer at 520 m μ .

Statistical analysis: Data obtained were analyzed using the student's *t*-distribution and refined by Bessel correction (Moroney, 1956) for the test significance of different between means.

Results

Effect on the total body carbohydrate content: According to the results distributed in Table 1, the total body carbohydrate content was gradually decreased with the age of control pupae. A similar result was obtained for pupae at the lowest dose-level of Lufenuron or Diofenolan. On the other hand, treatment with the highest or medium dose-level caused a reciprocal V-shaped trend of carbohydrate content throughout the pupal life, i.e., the greatest amount of carbohydrates had been estimated at the mid-age of pupae.

To explore the possible action of Lufenuron and Diofenolan on this metabolite, data arranged in the same Table 1 evidently revealed the reducing effect on the newly formed pupae, regardless of the dose value (with an exception of the lowest dose of Diofenolan). In contrast, topical application of Lufenuron onto prepupae promoted the carbohydrate level among the mid-aged pupae (175.25 \pm 10.37, 160.06 \pm 8.35 and 156.54 \pm 18.45 at 1.0, 0.1 and 0.01 μ g prepupae⁻¹, respectively, vs 153.16 \pm 9.35 mg g⁻¹ carbohydrates of controls). A similar result was obtained after treatment with Diofenolan (188.36 \pm 5.26, 176.16 \pm 25.66 and 153.58 \pm 13.37 at 1.0, 0.1 and 0.01 μ g prepupae⁻¹, respectively, vs 153.16 \pm 9.33 mg g⁻¹ carbohydrates of controls). The same trend of effect on carbohydrate content of newly formed pupae was determined for the late-aged pupae and all estimates had been found lower than those of control congeners, whether the compound was Lufenuron or Diofenolan. Moreover, this reducing effect had been drastically detected by the higher two doses.

What is the stronger for reducing the carbohydrate content of pupae, Lufenuron or Diofenolan? To answer this question, data of Table 1 should be seen. In regard to the newly formed pupae, Diofenolan was stronger than Lufenuron only with the medium dose. After topical application onto prepupae with the highest or the lowest dose, Lufenuron was stronger. Thus, the dose value determines (or regulates) the efficiency of the IGR. To some extent, this conclusion could be obtained depending on the carbohydrate of mid- and late-aged pupae.

Effect on the total body protein content: As indicated in Table 2, the total body protein content was gradually decreased with the age of control pupae (695.82 \pm 15.33, 668.22 \pm 10.24 and 647.21 \pm 22.60 mg g⁻¹ proteins of early-, mid- and late-aged pupae, respectively). Dealing with the treated pupae, similar course of protein content throughout the pupal stage was detected by treating the prepupae with the lowest dose of Diofenolan (655.16 \pm 10.22, 646.22 \pm 18.65 and 595.18 \pm 11.16 mg g⁻¹ proteins of early-, mid- and late-aged pupae, respectively). The other two doses of this IGR and also all doses of Lufenuron, induced such metabolite content to take a reciprocal V-shaped course along the pupal life (for details, see the same Table 2).

The total body protein content of the newly formed treated pupae was more significantly depleted than that of their control congeners, whatever the IGR or its dose value. By moving to the mid age, such protein content raised up in the Lufenuron- treated pupae (681.64 \pm 11.35, 677.33 \pm 16.54 and 699.35 \pm 28.35 vs 668.22 \pm 10.24 mg g⁻¹ proteins of controls). Similarly, the total protein content was determined at the mid age of Diofenolan-treated pupae, especially with the higher two doses (699.65 \pm 9.88 and 685.15 \pm 10.12 vs 668.22 \pm 10.24 mg g⁻¹ proteins of controls). In the late-aged pupae, both IGRs caused detrimental reduction of protein content, irrespective of the dose-level. To compare the metabolic activity of Lufenuron and Diofenolan, data of the Table 2 show the greater reducing action of Lufenuron than of Diofenolan.

Effect on the total body lipid content: After topical application with different dose values of Lufenuron and Diofenolan onto the prepupae, total lipid contents of the resulted pupae had been estimated and then arranged in Table 2. Firstly, it should be overlooked that lipid content of control pupae decreasingly runs along the stage (443.15 \pm 16.80, 425.66 \pm 13.45 and 417.37 \pm 14.30 mg g⁻¹ lipids of early-, mid- and late-aged pupae, respectively). The same tendency was detected in the pupae after prepupal treatment with the lowest dose (0.01 μ g prepupae⁻¹) of Lufenuron (421.50 \pm 28.60, 420.28 \pm 26.61 and 342.80 \pm 13.40

Table 1: Determination of total carbohydrate content (mg/g ± SD) of the red palm weevil *Rhynchophorus ferrugineus* pupae as affected by certain IGRs applied topically onto the prepupae

Age	Control	Lufenuron (µg/insect)			Diofenolan (µg/insect)		
		1.0	0.1	0.01	1.0	0.1	0.01
Early-aged	170.69±5.6a	96.35±13.5d	135.25±8.7c	156.6±9.2b	105.23±8.5 d	124.44±13.1b	172.17±12.6a
Mid-aged	153.16±9.33a	175.25±10.37b	160.06±8.35c	156.54±18.45a	188.36±5.26 c	176.16±25.66b	153.58±13.37a
Late-aged	129.21±8.6a	86.52±7.6c	85.16±13.8c	123.05±14.3a	77.05±8.9 c	90.18±9.0b	121.07±8.3a

Early-aged: 1 – day old pupae, Mid – aged: 4 – day old pupae, Late – aged: 7 – day old pupae. Eight prepupae were used as replicates for each treatment and for controls. Means ± SD followed with the same letter (a) are not significantly different (P > 0.05), (b): significantly different (P < 0.05), (c): highly significantly different (P < 0.01), (d): very highly significantly different (P < 0.001).

Table 2: Determination of total protein content (mg/g ± SD) of the red palm weevil *Rhynchophorus ferrugineus* pupae as affected by certain IGRs applied topically onto the prepupae

Age	Control	Lufenuron (µg/insect)			Diofenolan (µg/insect)		
		1.0	0.1	0.01	1.0	0.1	0.01
Early-aged	695.82±15.33a	605.16±12.17c	623.80±19.25c	632.14±18.06b	631.05±16.18b	636.00±20.18b	655.16±10.22b
Mid-aged	668.22±10.24a	681.64±11.35b	677.33±16.54a	669.35±28.35a	699.65±9.88c	685.15±10.12b	646.22±18.65a
Late-aged	647.21±22.60a	535.11±11.12c	550.05±15.27c	587.11±12.08b	530.12±13.5c	572.26±14.17c	595.18±11.16b

Early-aged, Mid-aged, Late-aged pupae; a, b, c, d: see the footnote of Table (1). Eight prepupae were used as replicates for each treatment and for controls

mg g⁻¹ lipids of early-, mid- and late-aged pupae, respectively) and of Diofenolan (431.50±16.20, 428.12±25.36 and 411.18±19.30 mg g⁻¹ lipids of early-, mid- and late-aged pupae, respectively). The higher two doses of Lufenuron or Diofenolan suppressed and then stimulated the lipids throughout the pupal life in a reciprocal V-shaped course (Table 3).

Data of the same table clearly revealed the diminishing of total lipid content in the treated newly formed pupae when compared to that of control congeners at the same age. Such diminishing effect was profoundly noticed after the prepupal treatment with both highest and medium dose-levels of Lufenuron or Diofenolan. A similar result was obtained by the treated late-aged pupae. On contrary, the treated mid-aged pupae attained lipid in more amounts than those of controls, whatever the IGR or its dose-level. It was concluded that the present IGRs reduced the lipid content at two limits of the pupal stage, but induced to its increments at almostly the mid-stage.

Discussion

The results in the present study, on *Rhynchophorus ferrugineus*, evidently revealed the reducing action of Lufenuron and Diofenolan, especially at the highest and medium dose-levels (1.0 and 0.1 µg insect⁻¹, respectively), on the carbohydrate content in the newly formed pupae and also in late-aged ones. In contrast, each of these IGRs promoted the carbohydrate content among pupae at their mid-age. Literature contains various effects of several IGRs on this metabolite in different insect species, appeared sometimes in increasing content and sometimes in decreasing one. Also, the effect varies during the developmental stage, as occurred in the present study.

As for examples, treatment of *Musca domestica* larvae with Altosid (ZR-515) led to decreased carbohydrates in

1-day old pupae but some increments in 3-day and 5-day old pupae (Abou El-Ela *et al.*, 1990). Increased carbohydrate content in different times during the pupal stage was estimated by Ghoneim (1994) after larval treatment with the chitin inhibitor IKI-7899 and mevalonic acid, separately or combined. On the other hand, Abou El-Ela *et al.* (1993) determined great reduction in carbohydrates during the pupal stage of the fly *Synthesomyia nudiseta* after larval treatment with some IGRs. As well as, significant increases of carbohydrate content were observed in larvae of *Spodoptera littoralis* by the JHA (Isopropyl 3, 7, 11-triethyl-2, 4-dodacadiote) (Ismail, 1980); of *Schistocerca gregaria* by fenoxycarb (El-Gammal *et al.*, 1989) and of *S. littoralis* by Kinoprene (Fouda and Amer, 1990).

The varied effect of Lufenuron and Diofenolan, in the present study, may be due to their hormonal actions on the carbohydrate metabolism because each type of hormonally conditioned developmental cycles can be characterized by the determined pattern in the course of the total body metabolism (Slama, 1965; Sehna and Slama, 1966; Slama and Hodkova, 1975). Moreover, synthesis of the main body metabolites, such as carbohydrates, is affected by JH (Orr, 1964; Price, 1973; Imboden and Luscher, 1976; Rutz *et al.*, 1976).

Protein synthesis is necessary for the maintenance of body growth and reproduction. Many factors have been implicated in the control of protein synthesis (Carlisle *et al.*, 1987). In the present study on *Rh. Ferrugineus*, each of Lufenuron and Diofenolan caused significant reductions in the protein of the newly formed and late-aged pupae, irrespective of the dose-level. Lufenuron was found more effective than Diofenolan for reducing this metabolite during the pupal stage. More or less, similar effect was observed on the total protein content of *M.*

domestica as a response to larval treatments with Dimilin, Bay SIR-8514 and Altosid (Bakr *et al.*, 1991).

On the contrast, Amer (1990) determined increasing protein content in pupae of *S. littoralis* after larval treatment with mevalonic acid. The same compound, separately or combined with the chitin inhibitor IKI-7899 caused, almostly, similar effect on the same lepidopteran (Ghoneim, 1994). Also, Basiouny (2000) estimated considerable increments of protein throughout different developmental stages of *Muscina stabulans* by the chitin inhibitors IKI-7899 and XRD-473.

The protein increments may be due to the failure in the insect tissues to uptake the produced and released proteins from fat body and haemolymph during the pupal or adult apolysis (Yin *et al.*, 1989). On the other hand, the protein reductions, as obtained in the present study, suggested a hormonal activity of Lufenuron and Diofenolan during the pupal stage. This suggestion was given, also, in the light of the results of some authors (Orr, 1964; Price, 1973; Rutz *et al.*, 1976; Ueno *et al.*, 1983). Also, Dimilin, or its analogues, interferes with the insect endocrine system (Yu and Terriers, 1975; Richards, 1981) causing a hormonal imbalance (Hajjar and Casida, 1979) and affecting the insect metabolism (De Mark and Bennett, 1989).

Needless to say that the lipids are important source of energy for insects. Lipid turnover in insects is regulated by neuroendocrine-controlled feedback loops (Downer, 1985). In the present study, the two higher dose-levels of Lufenuron and Diofenolan suppressed and then stimulated, the lipids in a reciprocal V-shaped course along the pupal life. In the other words, the used IGRs reduced the lipid content at the two limits of pupal stage, but induced to lipid increment at almostly the mid-age. Another IGR, fenoxycarb, affected the lipid synthesis in the fat body during the 6-day experimental period of *Choristoneura fumiferana* (Mulye and Gordan, 1993). On the contrary, Ghoneim (1994) determined significant increments of lipid content throughout the pupal stage of *S. littoralis* by the larval treatments with mevalonic acid or IKI-7899. The hormonal activity of Lufenuron and Diofenolan, *de novo*, is acceptable on the lipid content during the pupal stage of *Rh. Ferrugineus*, in the present study. This can be substantiated by results and suggestion of Stephen and Gilbert (1970) for *Hyalophora ciroppia* and Doan (1973) for *Drosophila melanogaster*.

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