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Metabolic activity of the chitin synthesis inhibitor, Flufenoxuron, on the desert locust *Schistocerca gregaria* (Orthoptera: Acrididae)

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ABSTRACT

After treatment of the newly moulted last (5th) instar nymphs of *Schistocerca gregaria* with a high (1000.0 ppm) or a low (62.5 ppm) concentration level of the chitin synthesis inhibitor, Flufenoxuron, the main metabolites were determined in two tissues: haemolymph and fat body of 5th instar nymphs and adult females. The effect of flufenoxuron on the haemolymph protein content of early- and mid-aged nymphs exhibited a reducing effect at the high concentration level but an inducing one at the lower concentration level. On the other hand, flufenoxuron prohibited the late-aged nymphs to gain normal protein content. Moreover, the inhibitory effect was extended to the adult stage. The proteins in fat body of late-aged nymphs were pronouncedly diminished, while proteins of mid- or late-aged nymphs varied depending upon the flufenoxuron concentration level. Slightly decreased content of proteins in fat body of adults (at low concentration level) was estimated. A little inducing effect of flufenoxuron, only at its low concentration level, on the lipid content in haemolymph and fat body of both early-aged nymphs and newly formed adults was detected. However, nymphs and adults of other ages suffered an inhibitory effect in both tissues. Only the early-aged nymphs and 1-day old adults had been subjected to an inhibitory effect of flufenoxuron on the carbohydrate content, in haemolymph. Other than these ages, all nymphs and adults were promoted to gain more carbohydrates. With regard to the fat body carbohydrate content, a dominant promoting action was recorded.

Keywords: Main metabolites, flufenoxuron, *Schistocerca gregaria*, haemolymph, fat body.

1. Introduction

The neurotoxic conventional insecticides have many disadvantages to various environmental aspects, including human health and economics. For avoiding the serious problems resulted from these synthetic insecticides, several research institutions have engaged in searching for safe alternatives in the field of pest control. The insect growth regulators (IGRs) seem promising control agents because of their specific mode of action on insects and their lower toxicity against non-target organisms than conventional insecticides [1, 2, 3, 4, 5]. The chitin synthesis inhibitors (CSIs) are usually classified in the IGRs because they are selectively suppress the growth and development of larvae and prohibit some reproductive potentials of adults. They affect the hormonal regulation of growth and development of insects [6]. Although flufenoxuron (Cascade) is classified in the CSIs, it caused some toxic effects on larvae of several insect species [7, 8, 9, 10]. Also, flufenoxuron has been found to be extremely active against wood borers [11] and it caused a disruption of the caste balance leading to collapse of the colony of termite *Microcerotermes diversus* [12]. A biomarker represents a biological response of the impact or presence of xenobiotic in the organism, and not the direct evidence of this one. This response must be measured in an organism or its products and indicate a change compared to the normal state. The energy reserves are considered as biomarkers of effect that give information on the health condition of the organism [13]. Therefore, the present study was planned and conducted for investigating possible effects of the chitin synthesis inhibitor Flufenoxuron on the general body metabolism of the economically dangerous insect, *Schistocerca gregaria*.

2. Materials and Methods

2.1 Experimental Insect:

Successive generations of the desert locust *S. gregaria* (Forsk.) were maintained for several years under the gregarious conditions in Department of Zoology, Faculty of Science, Al-Azhar university. It was originated by a sample provided from Locust and Grasshopper Res. Division, Plant Protection Research Institute, Giza, Egypt. The culture was raised and handled crowded breeding conditions described by [14]. The hoppers were reared in wooden cages with wire-gauze sides (40x40x60 cm) and small door in the upperside to allow the daily feeding and cleaning routine. Each cage was equipped internally with 60 W electric bulb for lightening (17:7 L: D) and warming (32±2 ºC). The relative humidity varied from 30-50% following the introduction of fresh food plant to 50-70% several hours later. Nymphs and adults were allowed to feed on fresh leaves of leguminous plant *Medicago sativa*. Daily routine of cleaning and monthly routine with an antiseptic agent had been carried out for all cages.

2.2 Nymphal treatments with flufenoxuron:

The chitin synthesis inhibitor, Flufenoxuron (Cascade) 10%, is a benzoylphenylurea derivative with the generic name as

N-[[[4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-fluorophenyl]amino]carbonyl]-2,6-difluorobenzamide

Two concentration levels of flufenoxuron were prepared using the distilled water: 1000 and 62.5 ppm. The concentration range was chosen depending on some preliminary trials carried out on the present insect species. Feeding technique was applied using fresh clean clover leaves (*M. sativa*) after dipping for 3 minutes in the concentration level and then offered to the newly moulted last (5^{th}) instar nymphs. The control nymphs had been provided with fresh clean clover leaves after dipping in distilled water. Three replicates (10 nymphs/rep.) were carried out for each treatment or controls. Each individual nymph was kept in a suitable glass vial whose bottom covered with a thin layer of sterilized sand. All vials were carefully located in a cage provided with a suitable electric bulb for lightening and warming.

2.3 Determination of main metabolites in haemolymph and fat body:

Haemolymph (0.1ml) of 1-day old (early-aged), 4-day old (mid-aged) and 7-day old (late-aged) last instar nymphs was drawn out from the coxal joint into Eppendorf Pipetman containing few milligrams of phenoloxidase inhibitor (phenylthiourea) to prevent tanning or darkening and then diluted 5× with saline solution 0.7%. The haemolymph samples were then centrifuged at 2000 r.p.m. for 5 min, and only the supernatant fractions were used for assay directly or frozen until use. Three replicates were used and the haemolymph of two individuals were never mixed. The same nymphs (treated or control) have been dissected to collect their fat body (Visceral and parietal), then weighted and homogenized in a saline solution (the fat body of one insect / 1 ml saline solution 0.7 %) using a fine electric homogenizer, tissue grinder for 2 min. Homogenates were centrifuged at 4000 r.p.m. for 15 min. The supernatant was used directly or frozen until the use for the metabolites determination. Dealing with the adult females of 0-day old (newly emerged) and 4-day old, the same work for haemolymph and fat bodies was carried out.

Total protein content of haemolymph or fat body was conducted according to [15] and using a kit of Bioadwic company. The method depends on the protein forms a violet complex with cupric ions in alkaline medium, and then measured the absorbance at 550 nm using a spectrophotometer.

Total carbohydrate (as glycogen) content of haemolymph or fat body was quantitatively determined by using the anthrone reagent according to [16] and utilizing the Spectrophotometer at 620 nm.

Quantitative determination of the total lipid content of haemolymph or fat body was conducted according to the technique of [17] and lipid estimation was taken place by phosphovanillin reagent depending on [18] and using the Spectrophotometer at 520 nm.

2.4 Analysis of Data:

Data obtained were analyzed using the Student t-distribution and were refined by Bessel's correction [19] for testing the significance of difference between means.

3. Results

After treatment of the newly moulted last (5^{th}) instar nymphs of *Schistocerca gregaria* with a high (1000.0 ppm) or a low (62.5 ppm) concentration level of the chitin synthesis inhibitor, Flufenoxuron, the main metabolites were determined in two tissues: haemolymph and fat body of nymphs (of early-, mid- and late-aged, i.e: 1-, 4- and 7-day old) and adult females (of 0- and 1-day old).

3.1 Effect of Flufenoxuron on the protein content:

Data of total protein content are assorted in Table (1). As shown in the table, the effect of flufenoxuron on the haemolymph protein content of early- and mid-aged nymphs depended upon the concentration level because it exhibited a reducing effect at the high concentration level but an inducing one at the lower concentration level. On the other hand, flufenoxuron prohibited the late-aged nymphs to gain normal protein content in haemolymph, regardless of the concentration level (38.67±3.62 and 70.86±4.23 mg/ml at high and low concentration levels, respectively, in comparison with 78.46±7.11 mg/ml of control nymphs). Moreover, the inhibitory effect of flufenoxuron on the haemolymph proteins was extended to the adult stage because the newly formed adult females attained significantly (Change%: -71.90 at high concentration level) or non-significantly (Change%: -4.72 at low concentration level) depleted proteins.

The nymphal treatment with the high concentration level of flufenoxuron caused mortality of adults at a day after emergence. The protein content in haemolymph could not determine and, therefore, the effect of flufenoxuron could not be explore. At low concentration level, the 1-day old adults had only slightly decreased proteins in their haemolymph (Change%: -7.23).

Concerning the disturbance of protein content in fat body by Flufenoxuron, data of Table (1), also, show similar results to some extent. The proteins in fat body of late-aged nymphs were pronouncedly depleted, regardless of the concentration level (Change% s: -18.45 and -5.81, at high and low concentration levels, respectively) while the effect of flufenoxuron on proteins in fat body of 1- or 4-day old nymphs varied depending upon its concentration level. Flufenoxuron, at its high concentration level, exerted a prohibiting action on nymphs because decreasing protein content was measured (241.33±3.59 mg/g of 1-day old nymphs, vs. 245.61±6.33 mg/g; 233.5±5.82 mg/g of 4-day old nymphs, vs. 256.31±2.52 mg/g of control congeners).

Table 1: Protein content in the haemolymph and fat body of *Schistocerca gregaria* as influenced by Flufenoxuron after treatment of the newly moulted last instar nymphs.

Target tissue	Conc. (ppm)	5th nymphal instar (Age in days)						Adult stage (Age in days)			
		1-day old	Change %	4-day old	Change %	7-day old	Change %	0-day old	Change %	1-day old	Change %
Haemolymph (mg/ml ± S.D.)	1000.0	80.54 ± 3.85 a	- 6.72	59.34 ± 6.51 a	- 10.74	38.67 ± 3.62 d	- 50.71	23.17 ± 5.00 d	- 71.90	=	-
	62.5	94.44 ± 5.81 b	+9.38	71.85 ± 4.85 a	+8.08	70.86 ± 4.23 a	- 9.69	78.56 ± 7.33 a	- 4.72	91.41 ± 3.72 a	- 7.23
	Control	86.34 ± 2.56	-	66.48 ± 3.51	-	78.46 ± 7.11	-	82.45 ± 3.45	-	98.53 ± 5.33	-
Fat body (mg/g ± S.D.)	1000.0	241.33 ± 3.59 a	- 1.74	233.5 ± 5.82 d	- 8.89	198.80 ± 5.41 d	- 18.45	113.67 ± 8.33 d	- 57.18	=	-
	62.5	251.81 ± 7.33 a	+2.52	258.83 ± 6.45 a	+0.98	229.62 ± 5.48 c	- 5.81	255.5 ± 6.13 a	-3.74	262.85 ± 4.33 a	- 1.98
	Control	245.61 ± 6.33	-	256.31 ± 2.52	-	243.78 ± 4.26	-	265.44 ± 3.67	-	268.15 ± 4.50	-

Conc.: concentration, mean ± SD followed with the letter (a): is 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). =: died

Flufenoxuron exhibited a reducing effect on fat body proteins of adults in a dose-dependent manner (113.67±8.33 and 255.50±6.13 mg/g, at high and low concentration levels, respectively, as compared to 265.44±3.67 mg/g of control 0-day old adults). The proteins in fat body of 1-day old adults at low concentration level could be estimated in slightly decreased content (Chang%: -1.98).

3.2 Effect of Flufenoxuron on the lipid content:

As clearly seen in Table (2), data show a little inducing effect of Flufenoxuron, only at its low concentration level, on the lipid content in haemolymph and fat body of both 1-day old nymphs and 1-day old adults. At this low concentration level, lipid content increased in haemolymph (Chang%: +10.16) and fat body (Change%: +5.85) of 1-day old nymphs as well as lipid content increased in haemolymph (Change%: +15.80) and fat body (Change%: +3.86) of 1-day old adults. However, nymphs and adults of other ages suffered an inhibitory effect of flufenoxuron on their lipid contents in haemolymph and fat body.

Table 2: Lipid content in the haemolymph and fat body of *Schistocerca gregaria* as influenced by Flufenoxuron after treatment of the newly moulted last instar nymphs.

Target tissue	Conc. (ppm)	5th nymphal instar (Age in days)						Adult stage (Age in days)			
		1-day old	Change%	4-day old	Change%	7-day old	Change %	0-day old	Change %	1-day old	Change %
Haemolymph (mg/ml ± S.D)	1000.0	13.21 ± 2.41 c	- 40.63	12.66 ± 2.54 a	- 31.01	11.59 ± 1.63 c	- 43.02	17.71 ± 2.48 a	- 20.97	=	-
	62.5	24.51 ± 2.46 a	+10.16	17.34 ± 2.54 a	- 5.50	19.51 ± 2.43 a	- 4.08	25.42 ± 3.11 a	+13.43	32.61 ± 5.66 a	+15.80
	Control	22.25 ± 3.21	-	18.35 ± 4.32	-	20.34 ± 3.42	-	22.41 ± 5.48	-	28.16 ± 5.11	-
Fat body (mg/g ± S.D)	1000.0	80.52 ± 3.21 d	- 8.77	53.41 ± 5.44 d	- 42.86	38.61 ± 2.55 d	- 48.64	58.63 ± 2.45 d	- 39.76	=	-
	62.5	93.42 ± 5.42 a	+5.85	86.22 ± 2.52 c	- 7.77	72.51 ± 5.60 a	- 3.54	88.81 ± 7.66 b	- 8.75	90.62 ± 3.57 a	+3.86
	Control	88.26 ± 4.22	-	93.48 ± 4.85	-	75.17 ± 5.12	-	97.33 ± 5.24	-	87.25 ± 2.42	-

Conc., a, b, c, d, =: See footnote of Table (1).

3.3 Effect of Flufenoxuron on the carbohydrate content:

Data of carbohydrate content in haemolymph and fat body of nymphs and adults as affected by flufenoxuron are summarized in Table (3). Only the 1-day old nymphs and 1-day old adults had been subjected to an inhibitory effect of flufenoxuron on the carbohydrate content, in hemolymph tissue. The strongest inhibitory effect was achieved on carbohydrate content in haemolymph of 1-day old nymphs at the low concentration level (31.51±2.67 mg/ml, in a change%: -15.41, as compared to 37.25±2.34 mg/ml of control correspondings).

Other than 1-day old nymphs and 1-day old adults, all nymphs and adults were promoted to gain more carbohydrates in haemolymph. The strongest promoting effect of flufenoxuron was exhibited on the newly formed adults (Change%: +18.37 at the high concentration level).

With regard to the fat body carbohydrate content, data distributed in Table (3) unambiguously show that all nymphs and adults had been enhanced to gain excess carbohydrates in their fat bodies. For some details, the greatest inducing effect of flufenoxuron appeared as increased fat body carbohydrates of mid-aged nymphs (Change%: +36.59, at the low concentration level) while the least inducing effect was exhibited in fat body carbohydrates of newly emerged adults (Chang%: +4.65, at the low concentration level).

Table 3: Carbohydrate content in the haemolymph and fat body of *Schistocerca gregaria* as influenced by Flufenoxuron after treatment of the newly moulted last instar nymphs.

Target tissue	Conc. (ppm)	5th nymphal instar (Age in days)						Adult stage (Age in days)			
		1-day old	Change %	4-day old	Change %	7-day old	Change %	0-day old	Change %	1-day old	Change %
Haemolymph (mg/ml ± S.D)	1000.0	36.71 ± 2.13 a	- 1.54	52.34 ± 1.57 a	+8.57	65.23 ± 2.65 b	+9.70	88.35 ± 1.25 d	+18.37	=	-
	62.5	31.51 ± 2.67 b	- 15.41	49.21 ± 2.48 a	+2.07	62.31 ± 4.51 a	+4.79	78.63 ± 3.87 a	+5.36	68.36 ± 5.44 a	- 1.23
	Control	37.25 ± 2.34	-	48.21 ± 2.56	-	59.46 ± 3.52	-	74.64 ± 2.5	-	69.21 ± 1.89	-
Fat body (mg/g ± S.D)	1000.0	22.15 ± 1.29 a	+19.60	31.21 ± 1.85 c	+36.59	38.71 ± 2.57 a	+13.72	46.43 ± 4.78 c	+22.67	=	-
	62.5	20.67 ± 2.31 a	+11.61	25.61 ± 5.42 a	+12.08	36.61 ± 5.41 a	+7.55	39.61 ± 2.70 a	+4.65	38.74 ± 1.58 a	+9.65
	Control	18.52 ± 2.56	-	22.85 ± 3.44	-	34.04 ± 2.81	-	37.85 ± 2.57	-	35.33 ± 1.84	-

Conc., a, b, c, d, =: See footnote of Table (1).

4. Discussion

Protein synthesis is necessary for the maintenance of body growth and reproduction. Many factors had been implicated in the control of protein synthesis [20]. Proteins enter at various reactions such as the hormonal regulation and they integrated in the cell as a structural element at the same time as the carbohydrates and the lipids [21, 22].

In the present study, newly moulted last (5th) instar nymphs of *Schistocerca gregaria* were treated with a high (1000.0 ppm) or a low (62.5 ppm) concentration level of the chitin synthesis inhibitor, Flufenoxuron for assessment its effect on the main metabolites in two tissues: haemolymph and fat body of nymphs (of 1-, 4- and 7-day old, as expressed in early-, mid- and late-aged nymphs) and adult females (of 0- and 1-day old).

4.1 Disturbed protein content of *S. gregaria* by Flufenoxuron:

The haemolymph protein content in late-aged last instar nymphs of *S. gregaria* was suppressed by the juvenoids fenoxycarb [23]. In the same orthopteran species, [24] estimated depleted protein content for the last instar nymphs after treatment with chlorfluazuron (IKI-7899); pyriproxyfen prevent the mid and late-aged nymphs (5th) of *S. gregaria* to gain the normal hemolymph protein content [25]. For the dipteran *Musca domestica*, [26] recorded an inhibitory effect of diflubenzuron (Dimilin), triflumuron (Bay Sir-8514) and methoprene (Altosid) on the total protein content during the larval stage. More or less, similar inhibition of protein content was reported for the lepidopteran *Spodoptera littoralis* by chlorfluazuron [27] and flufenoxuron (Cascade) and chlorfluazuron [28]. Significant reduction in the total protein after treatment with pyriproxyfen was recorded for *Spodoptera litura* [29]. In *Tenebrio molitor*, the application of halofenozide (RH- 0345) [30] resulted in significantly depleted protein level of the larval haemolymph. In the silkworm *Bombyx mori*, pyriproxyfen caused an inhibition of larval haemolymph protein after exposure of 5th instar larvae to pyriproxyfen residue [31] but the protein band pattern in the haemolymph of treated larvae did not affected [32]. In the mosquito *Culiseta longiareolata*, treatment of 4th instar larvae with the chitin synthesis inhibitor Novaluron led to pronouncedly decreased protein content of the whole body at the days 5 and 7 post-treatment [5]. Also, some IGRs caused various degrees of inhibition in the protein content of the lepidopteran *T. molitor* [33], the coleopteran *Callosobruchus maculatus* [34], the Indian meal moth *Plodia interpunctella* by 20-hydroxyecdysone and azadirachtin [35] and the hemipteran *Eurygaster integriceps* by pyriproxyfen [36, 37].

To some extent, the inhibitory effect of flufenoxuron on the haemolymph protein content of *S. gregaria*, in the present study,

run in accordance with the previously reported results for other insect species. The effect of flufenoxuron on the haemolymph protein content of early- and mid-aged nymphs depended upon the concentration level because it exhibited a reducing effect at the high concentration level but an inducing one at the lower concentration level. On the other hand, flufenoxuron prohibited the late-aged nymphs to gain normal protein content in haemolymph, regardless of the concentration level. Moreover, the inhibitory effect of flufenoxuron on the haemolymph proteins was extended to the adult stage because the newly formed adult females attained, significantly or non-significantly, depleted proteins. The nymphal treatment with the high concentration level caused mortality of adults at a day after emergence and therefore the protein content in haemolymph could not be determined. At low concentration level, the 1-day old adults had only slightly decreased proteins in haemolymph.

Concerning the disturbing effect of Flufenoxuron on the protein content in fat body of *S. gregaria*, in the present study, the fat body proteins of late-aged nymphs were pronouncedly diminished, regardless of the concentration level while the effect of flufenoxuron on fat body proteins of early- or mid-aged nymphs varied depending upon its concentration level because at the high concentration level, it exerted a prohibiting action on nymphs as decreasing protein content was measured. Because the adult females died a day after emergence at the high concentration level of Flufenoxuron, only the proteins in fat body of adults (at low concentration level) could be estimated in slightly decreased content.

In spite of the overall inhibitory effect of flufenoxuron on fat body proteins of *S. gregaria*, various insect growth regulators (IGRs) enhanced other insects to gain excess proteins as reported herein. [38] determined increasing protein content in the fat body of adult females of *Locusta migratoria* after nymphal treatment with a JH compound; [39] estimated increasing haemolymph protein content during the first 6 days of last nymphal instar of *S. gregaria* after treatment with fenoxycarb; [40] recorded increasing haemolymph proteins during the last larval instar of *S. littoralis* after treatment with methoprene, hydroprene or kinoprene; [27] observed remarkable rise of protein level in newly formed and mid-aged pupae of the same species as a response to the action of Chlorfluazuron; [41] reported an inducing action of lufenuron on the late-aged pupae of *M. domestica* to gain more proteins; [42] recorded increasing proteins in the fly *Bactrocera cucurbitae* as a response to the JHA, methoprene; [5] estimated protein increments in the whole body of mosquito *Culiseta longiareolata* at day 3 post-treatment of 4th instar larvae with LC_{50} (0.91µg/L) or LC_{90} (4.30 µg/L) Novaluron; and pyriproxyfen and lufenuron enhanced the nymphs (1-day old 5th) of *S. gregaria* to attain increasing proteins [25] etc.

However, the general inhibition of total protein content in the haemolymph or fat body of last instar nymphs of *S. gregaria* may be interpreted in the light of some acceptable suggestions as follows. The disturbance in the total protein content of larval haemolymph may partially correlated with the temporal increase in the endogenous titer of ecdysone because the exogenous ecdysone and/ or JH and JHAs have been shown to regulate the concentration of stage specific proteins in the haemolymph [43]. Also, the change in total protein content after treatment the nymphs with IGRs (ecdysteroids) may be due to the inhibition of DNA synthesis and metabolism or to the interference of the ecdysone analogues with the protein synthesis [44, 45]. With regard to foreign compounds, proteins help insects to synthesize the microsomal detoxifying enzymes [46], i.e. proteins can bind with foreign compounds and therefore the decrease in proteins may reflect the decrease in activity of these enzymes [47, 48, 49, 50] reported that different stresses on the silkworm *B. mori* can inhibit the total protein in haemolymph. This could be due to the breakdown of protein into amino acids, so with the entrance of these amino acids to TCA cycle as a keto acid, they will help to supply energy for the insect. So, protein depletion in tissues may constitute a physiological mechanism and might play a role in compensatory mechanisms under insecticidal stress, to provide intermediates to the krebs cycle, by retaining free amino acid content in haemolymph [51]. Also, the reduction in proteins may be understood in the light of decreasing enzyme constituents (especially the glutamic-pyruvic transaminase and glutamic-oxaloacetic transaminase [52, 53] or in the light of a direct effect IGRs on the nutritional requirements as suggested for *B. mori* [53, 54].

4.2 Lipid perturbation in *S. gregaria* by Flufenoxuron:

Lipids are necessary as a source of energy in the alive creatures such as insects. Insects obtain lipids from the food sources or synthesize them from within the bodies [55]. It has been reported that the lipid accumulation is more likely to be related to a lack of juvenile hormone [56]. Hence, the lipid turnover in insects is regulated by neuroendocrine-controlled feedback loops [57].

A little inducing effect of flufenoxuron, only at its low concentration level, on the lipid content in haemolymph and fat body of both early-aged nymphs and newly formed adults was detected. However, nymphs and adults of other ages suffered an inhibitory effect of flufenoxuron on their lipid contents in haemolymph and fat body since significantly or non-significantly diminished lipids in haemolymph and fat body of mid- and late-aged nymphs and of the newly formed adults were estimated. Excluding the few exceptional cases of increasing lipids, the reduced lipid content of *S. gregaria*, in the present study, agree with those results reported by some authors for different insect by various IGRs, e.g. Decreased lipid content was recorded for the rice moth *Corcyra cephalonica* by the action of pyriproxyfen [58], for the cotton leafworm *Spodoptera littoralis* by (Bay Sir-8514) [59] and for the same insect by pyriproxyfen [60]; lipid levels in the haemolymph and fat body of 6th instar larvae of the spruce budworm *Choristoneura fumiferana* were severely depleted as a result of fenoxycarb treatments [61]; lipid content decrease in the pupae of *S. littoralis* was estimated after larval treatment with mevalonic acid [27]; lufenuron and diofenolan treatments resulted in decreasing lipids at the start and end days of pupae of the red palm weevil *Rhynchophorus ferrugineus* but increasing lipids were estimated for mid-aged pupae [62]. In addition, diofenolan treatments remarkably reduced the lipid content along the pupal stage of the house fly *Musca domestica* except the last day at which pupae gained more lipids [63]; lipid levels in the haemolymph of silkworm *Bombyx mori* larvae were declined throughout the experimental period but were elevated initially in the fat body and then lowered [31]; lipid content in the whole body of *Plodia interpunctella* larvae was reduced as a response to the action of pyriproxyfen [64]; pyriproxyfen treatments resulted in decreasing lipid content in haemolymph and fat body of the sunn pest *Eurygaster integriceps* nymphs [36]; and a predominant inhibitory effect of IGRs (pyriproxyfen, tebufenozoide and lufenuron) was detected in the fat bodies of nymphs of *S. gregaria* [65] etc.

Instead of the inhibition of lipid content by flufenoxuron, activation of this metabolite was recorded in the present study, as exceptional cases of the early-aged nymphs and newly formed adults of S. gregaria. However, similar induced lipid content was reported by some authors using different IGRs against various insect species, e.g. mevalonic acid treatments against last instar larvae of *S. littoralis* resulted in significantly increased lipids in haemolymph and fat body [66]; induced lipid content in haemolymph of larvae and prepupae of *S. littoralis* after treatment with chlorfluazuron (IKI-7899) [27]; diflubenzuron treatments against the pupae or adult females of the mealworm *Tenebrio molitor* resulted in increasing lipid concentration [67]; novaluron treatments against the 4th instar larvae of the mosquito *Culiseta longiareolata* resulted in increasing lipids in the whole body starting from day 5 [5]; and hemolymph lipid content of the early aged nymphs of *S. gregaria* had been subjected to a reducing effect after treatment with high concentration of IGRs (pyriproxyfen, tebufenozoide and lufenuron) [65] etc.

Therefore, the exceptional cases of increasing lipid content in nymphs and adults after nymphal treatment with flufenoxuron may indicate its pronounced interference with not only the synthesis of lipids but also their mobilization as promoted to convert into other metabolites or fatty acids. This suggestion may be supported by the increasing cholesterol in the mid gut brush border membrane of silkworm *B. mori* larva after treatment with fenoxycarb (JHA) [68] or in the haemolymph of 120h post-treatment of silkworm larvae with pyriproxyfen (JHA) [31].

4.3 Defused carbohydrate mobilization in *S. gregaria* by Flufenoxuron:

Carbohydrates, also, play an important role for the structure and functions of all tissues during metamorphosis as well as for the normal functioning of the male and female reproductive organs and embryonic development [69]. Some authors estimated increasing carbohydrate content in some insect species as a response to the action of different insect growth regulators (IGRs) while others reported reversed results. These contradictory findings may be attributed to the differences in species sensitivity, the potency of the IGRs, or the developmental stage.

Increasing carbohydrate content was obtained by several insects after treatment with different IGRs, e.g. kinoprene significantly induced the carbohydrate content in the last instar larvae of *S. littoralis* [40]; chlorfluazuron and mevalonic acid (separately or combined) promoted the last instar larvae and pupae of *S. littoralis* to gain various increments of carbohydrate in haemolymph and fat body [27]; increasing carbohydrate content of *S. gregaria* was triggered by chlorfluazuron [70]; diflubenzuron-applied pupae or adult females of *Tenebrio molitor* had excessive carbohydrate[71, 67]; the mid-aged pupae of *Rh. ferrugineus* were fostered to accumulate excess carbohydrates after treatment of prepupae with lufenuron and diofenolan [62]; topical application of lufenuron or diofenolan onto the late last (3rd) instar larvae of *M. domestica* led to increasing carbohydrates allover the pupal life, with few exceptions [72]; novaluron-treated 4th instar larvae of the mosquito *Culiseta longiareolata* attained increasing carbohydrate content starting from the day 5 post-treatment [5]; tebufenozoide induced the nymphs of *S. gregaria* to gain more carbohydrates [73] etc. In agreement with these reported results, flufenoxuron promoted all nymphs and adults of *S. gregaria*, in the present study, to gain more carbohydrate content in fat bodies than their control congeners. Similar increasing carbohydrate content was determined in haemolymph of all nymphs other than the early-aged nymphs, as well as of 1-day old adults.

On the contrast, only the early-aged nymphs and 1-day old adults of *S. gregaria* had been subjected to an inhibitory effect of flufenoxuron on the carbohydrate content in haemolymph. These cases of reduced carbohydrate content are in accordance with some reported results because various juvenoids (JHAs), and IGRs in general, caused depleted carbohydrate content in some insects, e.g. *Spodoptera littoralis* by the JHA isopropyl 3,7,11-triethyl-2,4-dodecadiote [74]; *Schistocerca gregaria* by fenoxycarb [23]; *Musca domestica* by methoprene [75]; *Synthesiomyia nudiseta* by some IGRs [76]; the newly formed and late-aged pupae of *Rhynchophorus ferrugineus* by lufenuron and diofenolan [62], *M. domestica* by lufenuron and diofenolan [72]; *S. gregaria* nymphs by pyriproxyfen [73] etc.

However, the varied effects of flufenoxuron on the carbohydrate content in haemolymph or fat bodies of nymphs, in the present study on *S. gregaria*, 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 metabolites, such as carbohydrates, is affected by JH [77, 78, 79]. Also, the production or utilization of the main body metabolites, such as carbohydrates, under the control of JH (or IGRs, in general) was suggested by several authors [80, 81, 82, 22]. Also, the disturbance in carbohydrate content of *S. gregaria* nymphs and adults, as evidently recorded by the current results after treatment with flufenoxuron, can be understood in the light of the ability of the organism to modify the synthesis of certain metabolite and disrupt the functionality of the organism [83].

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