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Protein Disturbance in the Haemolymph and Fat Body of the Desert Locust *Schistocerca Gregaria* as a Response to Certain Insect Growth Regulators

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ABSTRACT

After treatment of the newly moulted last (5th) instar nymphs of the desert locust Schistocerca gregaria with 1000.0 or 62.5 ppm of the insect growth regulators (IGRs): pyriproxyfen (juvenoid), tebofenozide (ecdysone agonist) and lufenuron (chitin synthesis inhibitor), protein content was determined in the haemolymph and fat body of nymphs and adults. An inhibitory action on haemolymph proteins was generally exhibited by all IGRs along the nymphal stage with an exception of the day after treatment (1-day old nymphs) at which pyriproxyfen and lufenuron enhanced the nymphs to attain increasing proteins. Pyriproxyfen was comparatively the strongest IGR for preventing the mid- and late-aged nymphs to gain the normal protein content. In connection with the disturbed protein content in the fat body of nymphs, IGRs exhibited various degrees of reducing action with an exception of pyriproxyfen which slightly promoted the 1-day old nymphs to gain more proteins in fat body.
Depending on the present results, it can be concluded that all adult females had been subjected to serious inhibitory action of all IGRs on the haemolymph protein content. The most drastically depleted protein content was recorded after treatment with pyriproxyfen. Also, the adult females were deprived to gain proteins in their fat bodies as control congeners. The strongest reducing effect on the fat body protein content of 1-day old adults was exhibited by pyriproxyfen but of 4-day old adults was exhibited by lufenuron.
***Key Words*:** *Schistocerca gregaria*, Pyriproxyfen, Tebufenozide, Lufenorun, fat body, haemolymph, protein.

INTRODUCTION

Because the use of insecticides for controlling insect pests has several disadvantages to various environmental aspects, including human health and economics, numerous institutions have extensively searched alternatives such as insect growth regulators (IGRs) including juvenile hormone analogues (JHAs), chitin synthesis inhibitors (CSIs) and ecdysteroids [for reviews, see: 1, 2, 3, 4, 5, 6].

The JHAs interfere with important biochemical mechanisms such as the secretion and transportation of natural JHs from the secretory site to the target site, degradation, excretion and feedback control. Hence, their biological effects are very complex, and vary from one analogue to another. Also, the response to different compounds differs among the species [7].

Pyriproxyfen (S-31183) {2-[1-methyl-2-(4-phenoxyphenoxy)-ethoxy]-pyridine} has a juvenile hormone activity against different insect species. Its activity was studied against some lepidopterous pests [8, 9], houseflies and mosquitoes [10, 11, 12], cockroaches [13, 14], scale insects [15] and locusts [16, 17, 18].

Zooecdysteroids like Methoxyfenozide (RH-5849) and Tebufenozide (RH-5992) (produced by Rohm &Haas) are dibenzoyl hydrazines, possessing unique characteristics of mimicking insect moulting hormones [19, 20]. Ecdysone agonists, also, have chemosterilant activity on female insects [21]. They are effective at low doses, moderately persistent [22] and safe for non-target organisms [21].These compounds are both stomach and contact poisons against lepidopterans, coleopterans and dipterans [22]. However, [21] reported the compound RH-5992 to be specific against the lepidopterous larvae. The compound RH-5849, also, showed some systemic action in laboratory tests, causing paralysis and death, reducing oviposition and feeding in Coleoptera and Lepidoptera [23, 22].

Chitin synthesis inhibitors (CSIs) interfere with chitin biosynthesis in insects [24] and thus prevents moulting or produces an imperfect cuticle [25]. These compounds are effective suppressors of

development for the entire life cycle on insects [26]. However, these compounds, also, affect the hormonal balance in insects, thereby resulting in physiological disturbances, such as inhibition of DNA synthesis [27]; alteration of carbohydrates [28]; increase in phenyoxidase activity [29] cuticular lipids [30] and microsomal oxidase [31].

Although the first action of JHAs in particular, or IGRs in general, is the endocrine system, but many biochemical or physiological changes have been to reported to occur in different metabolism pathways [32, 33] Therefore, the present investigation aims to detect the protein changes in haemolymph and fat body of the destructive desert locust *S. gregaria* as responses to the IGRs: pyriproxyfen (a juvenoids), tebofenozide (an ecdysone agonist) and lufenuron (a chitin synthesis inhibitor).

MATERIALS AND METHODS

Culturing and maintenance of *Schistocerca gregaria*:

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 [34]. The hoppers were reared in wooden cages with wire-gauze sides (40x40x60 cm) and small door in the upper side to allow the daily feeding and cleaning routine. Each cage was equipped internally with 60 W electric bulb for lightening (17:7 LD) and warming (32±2 C.). 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.

Nymphal treatments with IGRs:

Pyriproxyfen (S-31183) is a product of Sumitomo Chemical Co. Ltd., Pesticides Division, Osaka, Japan, with the chemical formula: 2-{1-methyl-2-(4-phenoxy-phenoxy) ethyl} pyridine. A technical grade of **Tebofenozide** (RH-5992) was used. Its chemical name is 1-N-t-butyl-1 (3, 5-dimethyl benzoyl)-2-(4-ethylbenzoyl) hydrazine (Rohm and Haas Company, Philadelphia, PA). **Lufenuron** (Match, CGA-184699) was used. Its chemical formula is: N-{{{ 2,5-dichloro-4-(1,1,2,3,3-hexafluoro-propoxyl)-phenyl} amino}-2,6-difluorobenzamide (CA)}}}.

Two concentration levels of each IGR 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. Each individual nymph was kept in a suitable glass vial whose bottom was 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.

Biochemical Assay:

Haemolymph 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 Eppendorff 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 (1 nymph/replicate) 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) and then 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 enzymatic determination. Three replicates were used (1 nymph/replicate) and the fat bodies from two individuals were

avoided to be mixed. 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.

Quantitative determination of the total protein content of haemolymph or fat body was conducted according to [35] and using a kit of Bioadwic company. The method depends on the protein forms a violet complex with cupric ions in alkaline medium, then measured the absorbance at 550 nm using a spectrophotometer.

Analysis of Data:

Data obtained were analyzed using the Student t-distribution and were refined by Bessel's correction [36] for testing the significance of difference between means.

RESULTS

(A) Quantitative changes in the protein content of nymphs:

After treatment of the newly moulted last (5^{th}) instar nymphs of the desert locust Schistocerca gregaria with 1000.0 or 62.5 ppm of each of the IGRs: pyriproxyfen, tebofenozide and lufenuron, data of the haemolymph protein content were arranged in Table (1). An inhibitory action on the haemolymph protein content was generally exhibited by all IGRs along the nymphal stage with an exception of the day after treatment at which only tebofenuzide considerably (Change%: -9.03, at the high concentration level) or slightly (Change%: -6.53, at the low concentration level) prohibited this main metabolite whereas pyriproxyfen and lufenuron enhanced those nymphs to attain increasing proteins. In other words, tebofenuzide remarkably prohibited the haemolymph protein content allover the nymphal life but the other two IGRs pronouncedly suppressed it for the mid-aged (4-day old) and late-aged (7-day old) nymphs.

Depending on the data of the same table, lufenuron exerted the strongest inducing action on the haemolymph protein content for the 1-day old nymphs (98.55±3.25 mg/gm at the high concentration level in comparison with 86.34±2.56 mg/gm of control nymphs).

However, the nymphs of the age other than 1-day old suffered from an inhibitory action of all IGRs on the haemolymph protein content. Pyriproxyfen was comparatively the strongest IGR for preventing nymphs to gain normal protein content (Change% s: -47.72 and -16.8 at high and low concentration levels, respectively, in the mid-aged nymphs, as well as -70.07 and -13.48 at the same concentration levels, respectively, in the late-aged nymphs).

In connection with the disturbed protein content in the fat bodies of nymphs, data of Table (2) obviously show various degrees of the prohibiting effects of the present IGRs except pyriproxyfen which slightly promoted such main metabolite in the fat bodies of 1-day old nymphs only (250.53±3.48 and 248.99±5.66 mg/gm at the high and low concentration levels, respectively, vs. 241.61±6.33 mg/gm of control congeners). Also, the present results unambiguously indicate a higher suppressing action of each IGR at the high concentration level (1000 ppm). The most dangerous effect on the fat body protein content of 1-day old nymphs was exhibited by lufenuron (Change%: -4.07 at the high concentration level) but the most reducing effect in the 4-day old nymphs was exhibited by tebofenuzide (Change%: -25.98 at the high concentration level) and in the 7-day old nymphs was exhibited by pyriproxyfen (Change%: -39.2 at the high concentration level).

(B) Quantitative changes in the protein content of adults:

Data of the total protein in the haemolymph of the adult females after treatments of newly moulted last instar nymphs with two concentration levels of pyriproxyfen, tebofenozide or lufenuron have been summarized in Table (3). Just a look at these data, it can be concluded that all adult females had been subjected to serious inhibitory effects of all IGRs on the haemolymph total proteins. Excluding the adult deaths as a response to the lethal action of tebofenuzide (at the day after emergence) or pyriproxyfen (at the 4^{th} day after emergence), at the high concentration level of each, the most drastically depleted protein content was recorded after treatment with pyriproxyfen (24.91±2.77 mg/gm at the high concentration level in comparison with 82.45±3.45 mg/gm of control adults) as well as with lufenuron (25.26±3.68 mg/gm at the high concentration level of control correspondings).

In addition to the loose tissue, haemolymph, the protein content was determined in the storage tissue, fat body. As easily seen in Table (4), adult females were deprived to gain proteins as control congeners because of the prohibiting action of the present IGRs. The most reducing effect on the fat body protein content of 1-day old adults (Change%: -53.71) was exhibited by pyriproxyfen (at its high concentration level) but on the fat body protein content of 4-day old adults (Change%: -52.69) was exhibited by lufenuron (at its high concentration level).

DISCUSSION

Protein synthesis is necessary for the maintenance of body growth and reproduction. Many factors had been implicated in the control of protein synthesis [37]. In addition, proteins in all viable cells, as nucleoproteins, are essential to the cell division and as enzymes and hormones are essential to control many chemical reactions in the cell metabolism [38]. In other words, 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 [39, 40].

In the present study, the newly moulted last (5^{th}) instar nymphs of the desert locust *Schistocerca gregaria* were treated (*via* fresh plant food) with 1000.0 or 62.5 ppm of the IGRs: pyriproxyfen (juvenoids), tebofenozide (ecdysone agonist) and lufenuron (chitin synthesis inhibitor). An inhibitory action on the haemolymph protein content was generally exhibited by all IGRs along the nymphal instar with an exception of the day after treatment (1-day old nymphs) at which pyriproxyfen and lufenuron enhanced the nymphs to attain elevated protein level.

Excluding the odd exception for each of pyriproxyfen and lufenuron at the day after treatment, the present results are in agreement with similar results obtained for various insect species after treatment with different IGRs. The haemolymph protein content in late-aged last instar nymphs of *S. gregaria* was suppressed by the juvenoids fenoxycarb [41]. In the same orthopteran species, [42] estimated depleted protein content for the last instar nymphs after treatment with chlorfluazuron (IKI- 7899) For the dipteran *Musca domestica*, [43] 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 [44] and flufenoxuron (Cascade) and chlorfluazurn [45]. Significant reduction in the total protein after treatment with pyriproxyfen was recorded for *Spodoptera litura* [46]. In *Tenebrio molitor*, the application of halofenozide (RH- 0345) [47] 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 5^{th} instar larvae to pyriproxyfen residue [48] but the protein band pattern in the haemolymph of treated larvae did not affected [49]. In the mosquito *Culiseta longiarealata*, treatment of 4^{th} 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 [50]. Also, some IGRs caused various degrees of inhibition in the protein content of the lepidopteran *Tenebrio molitor* [51], the coleopteran *Callosobruchus maculatus* [52], the Indian meal moth *Plodia interpunctella* by 20-hydroxyecdysone and azadirachtin [53] and the hemipteran *Eurgaster integriceps* by pyriproxyfen [54]. In addition, some IGRs and various pesticides had been reported as protein inhibitors in ovaries and testes of several insects [55-62].

In the present study, pyriproxyfen, tebofenozide and lufenuron treatments led to significant inhibition of haemolymph protein content. Moreover, pyriproxyfen was comparatively the strongest IGR for preventing the mid- and late-aged nymphs to gain normal protein content. In addition to this loose tissue, the present study focused also on a storage tissue, fat body which was subjected to a prohibiting action of each of these IGRs on the protein content with an exception of early-aged nymphs in which pyriproxyfen only enhanced to increasing proteins.

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 [63]. 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 [27, 64]. With regard to foreign compounds, proteins help insects to synthesize the microsomal detoxifying enzymes [65], i.e. proteins can bind with foreign compounds and therefore the decrease in proteins may reflect the decrease in activity of these enzymes [66, 67]. [68, 69] 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 [70].

Table (1): Total protein content (mg/ml±SD) in the haemolymph of the desert locust, *Schistocerca gregaria*, nymphs after treatment of the early last instar nymphs with some IGRs.

IGRs	Conc. (ppm)	Last instar nymphs (Age in days)					
		1-day old	Change %	4-day old	Change %	7-day old	Change %
Pyriproxyfen	1000.0	90.34 ± 5.22 a	4.65	34.75 ± 4.17 d	-47.72	23.48 ± 5.41 d	-70.07
	62.5	87.51 ± 4.01 a	1.35	55.33 ± 5.22 b	-16.8	67.88 ± 4.82 b	-13.48
	Controls	86.34 ± 2.56	_	66.48 ± 3.51	_	78.46 ± 7.11	_
Tebufenozide	1000.0	78.56 ± 4.31 b	-9.03	34.15 ± 5.41 d	-48.56	33.45 ± 6.80 c	-57.36
	62.5	80.66 ± 4.28 a	-6.53	55.34 ± 6.2 b	-16.75	70.34 ± 5.22 a	-10.34
	Controls	86.34 ± 2.56	_	66.48 ± 3.51	_	78.46 ± 7.11	_
Lufenuron	1000.0	98.55 ± 3.25 c	13.95	55.47 ± 3.78 c	-16.61	38.16 ± 6.45 d	-51.4
	62.5	90.15 ± 3.48 a	4.15	64.75 ± 7.42 a	-2.56	75.54 ± 3.53 a	-2.55
	Controls	86.34 ± 2.56	_	66.48 ± 3.51	_	78.46 ± 7.11	_

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).

Table (2): Total protein content (mg/g±SD) in the fat body of the desert locust *Schistocerca*

IGRs	Conc. (ppm)	Last instar nymphs (Age in days)					
		1-day old	Change %	4-day old	Change %	7-day old	Change %
Pyriproxyfen	1000.0	250.53 ± 3.48 a	2.04	205.6 ± 4.81 d	-19.78	148.35 ± 2.88 d	-39.2
	62.5	248.99 ± 5.66 a	1.22	250.97 ± 7.48 a	-2.07	155.41 ± 3.22 a	-36.25
	Controls	245.61 ± 6.33	_	256.31 ± 2.52	_	243.78 ± 4.26	_
Tebufenozide	1000.0	241.42 ± 3.62 a	-1.63	189.76 ± 7.11 d	-25.98	175.43 ± 3.22 d	-28.02
	62.5	239.48 ± 6.42 a	-3.33	250.33 ± 4.83 a	-2.34	240.66 ± 3.11 a	-1.23
	Controls	245.61 ± 6.33	_	256.31 ± 2.52	_	243.78 ± 4.26	_

Lufenuron	1000.0	235.63 ± 4.88 a	-4.07	215.71 ± 8.57 d	-15.84	174.50 ± 3.66 d	-28.39
	62.5	241.77 ± 6.05 a	-1.63	253.51 ± 6.71 a	-1.17	236.89 ± 6.60 a	-2.88
	Controls	245.61 ± 6.33	_	256.31 ± 2.52	_	243.78 ± 4.26	_

Conc., a and d: See footnote of Table (1).

Table (3): Total protein content (mg/ml±SD) in the haemolymph of the desert locust *Schistocerca gregaria* adults after treatment of the early last instar nymphs with some IGRs.

IGRs	Conc. (ppm)	Adult stage (Age in days)			
		1-day old	Change %	4-day old	Change %
Pyriproxyfen	1000.0	24.91 ± 2.77 d	-69.79	=	_
	62.5	79.57 ± 3.41 a	-3.49	75.77 ± 4.22 d	-23.09
	Controls	82.45 ± 3.45	_	98.53 ± 5.33	_
Tebufenozide	1000.0	=	_	=	_
	62.5	79.77 ± 8.51 a	-3.25	77.61 ± 4.86 c	-21.2
	Controls	82.45 ± 3.45	_	98.53 ± 5.33	_
Lufenuron	1000.0	25.26 ± 3.68 d	-69.53	27.45 ± 5.20 d	-26.4
	62.5	76.78 ± 4.15 b	-19.05	90.53 ± 5.42 a	-8.12
	Controls	82.45 ± 3.45	_	98.53 ± 5.33	_

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

Table (4): Total protein content (mg/g±SD) in the fat body of the desert locust *Schistocerca gregaria* adults after treatment of the early last instar nymphs with some IGRs.

IGRs	Conc. (ppm)	Adult stage (Age in days)			
		1-day old	Change %	4-day old	Change %
Pyriproxyfen	1000.0	122.86 ± 5.62 a	-53.71	=	_
	62.5	236.42 ± 3.37 a	-10.92	198.15 ± 4.57 d	26.1
	Controls	265.44 ± 3.67	_	268.15 ± 4.50	_
Tebufenozide	1000.0	=	_	=	_
	62.5	247.51 ± 6.33 c	-6.74	242.31 ± 5.66 d	-9.6
	Controls	265.44 ± 3.67	_	268.15 ± 4.50	_
Lufenuron	1000.0	177.15 ± 3.40 d	-33.2	110.74 ± 6.01 d	-52.69
	62.5	205.74 ± 7.62 d	-22.37	237.48 ± 4.60 d	-31
	Controls	265.44 ± 3.67	_	268.15 ± 4.50	_

Conc., a, c and d: See footnote of Table (1). =: see footnote of Table (3).

On the contrast, several IGRs caused some increments of the haemolymph or fat body protein contents as recorded by [71] who determined increased protein content in the fat body of adult females of *Locusta migratoria* after nymphal treatment with a JH compound, [72] who estimated increased haemolymph protein content during the first 6 days of last nymphal instar of *S. gregaria* after treatment with fenoxycarb, [73] who estimated increasing haemolymph proteins during the last larval instar of *S. littoralis* after treatment with methoprene, hydroprene or kinoprene, [44] who 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, [74] who reported an inducing action of lufenuron on the late-aged pupae of *M. domestica* to gain more proteins, [75] who recorded increasing proteins in the fly *Bactrocera cucurbitae* as a response to the JHA, methoprene and [50] who estimated protein increments in the whole body of mosquito *C. longiarealata* at day 3 post-treatment of 4th instar larvae with LC_{50} $(0.91\mu g/L)$ or LC_{90} $(4.30\ \mu g/L)$ Novaluron.

Although the general (or dominant) action of pyriproxyfen, tebofenozide and lufenuron was inhibitory on the protein content in nymphs of *S. gregaria* in the present study, few exceptions of the increased proteins had been estimated in the haemolymph at a day after treatment (1-day old nymphs) as a response to the enhancing effect of pyriproxyfen and lufenuron. These increasing proteins may be attributed to the transport of proteins in the haemolymph, released from the mobilization of the reserves, destined for the synthesis of the new cuticle [76]. On the other hand, the increasing protein content of the fat bodies of the same nymphs as a response to a promoting effect of pyriproxyfen only are still needed to be interpreted, but unfortunately we have no acceptable interpretation right now!!

Depending on the available results in the present study on *S. gregaria*, it can be concluded that all adult females suffered a tremendous inhibitory action of all IGRs (pyriproxyfen, tebofenozide and lufenuron) because the protein content of haemolymph and fat bodies was drastically depleted. Pyriproxyfen exhibited the strongest prohibiting effect on the proteins in haemolymph and fat body while lufenuron exhibited the strongest prohibiting effect on this main metabolite in fat body especially for the 4-day old adults. More or less, similar effect was detected in the adult ovaries of the Indian meal moth *Plodia interpunctella* after treatment of larvae with pyriproxyfen [77] and in adult testicles of the Mediterranean flour moth *Ephestia kuehniella* after treatment of newly formed pupae with tebufenozide [78], whereas [79] estimated significantly increased protein content in the adult females of mealybug *Ferrisia virgata* at the day 4 post-treatment but remarkably decreased content after the day 10, as a response to JHA pyriproxyfen. However, the depleted protein in haemolymph or fat body of adult *S. gregaria*, in the present study, could be due to major mobilization of this metabolite as well as reduction of its synthesis. Also, the reduction in proteins may be understood in the light of decreasing enzyme constituents (especially the glutamic-pyruvic transaminase and glutamic-oxaloacetic transaminase [80] or in the light of a direct effect IGRs on the nutritional requirements as suggested for *B. mori* [81,82]. In spite of these suggestions, [83] concluded that the IGRs had no effects on the egg production in the citrus mealybug, *Planococcus citri*.

In conclusion, results of the present study on the desert locust *S. gregaria* obviously indicated the disturbing action of IGRs (pyriproxyfen, tebufenozide and lufenuron) on the proteins, and may by an interrupted the hormonal balance or enzymatic hierarchy, in nymphs and adult females which can explain their effects on growth, development, morphogenesis reproduction and ultrastructural constituents as reported in previous works on this destructive pest [84, 17, 18, 85, 86, 87, 88, 89, 90, 91].

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