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Antifeedant activity and detrimental effects of ecdysteroid agonist Chromafenozide on the food metabolic parameters of *Schistocerca gregaria* (Forsk.) (Orthoptera: Acrididae)

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Abstract
The desert locust *S. gregaria* is a dangerous pest in Egypt and other countries of North Africa and West Asia. It can devastate the cultures and crops of these areas. The present study was conducted aiming to investigate the antifeedant activity of a novel ecdysone agonist, Chromafenozide, and its effects on the different nutritional parameters of last instar nymphs. Three dose levels (10, 100 & 150 µg/nymph) were topically applied (once) onto the newly moulted last instar nymphs. Chromafenozide exhibited contradictory effects on the feeding behaviour depending on the dose since it acted as a weak antifeedant against female nymphs only at the lowest dose but acted as phagostimulant against them at the higher two doses. Moreover, it exhibited a phagostimulant activity against the male nymphs, regardless the dose level. The amount of food eaten by females along the last nymphal instar slightly decreased only at the lowest dose, but considerably increased at the higher doses. The male nymphs had been enhanced by the IGR to consume more food. RCR was slightly elevated. Treated nymphs of both sexes attained slightly or remarkably increasing relative weight gain and discharged more fecal pellets. The treated female and male nymphs achieved insignificantly increasing approximate digestibility (AD), with two exceptions for the male nymphs which had a slightly decreased AD at the medium dose and un-affected AD at the highest dose level. Both the efficiency of conversion of ingested food into biomass (ECI) and efficiency of conversion of digested food into biomass (ECD) of females slightly increased. ECI of male nymphs was promoted by Chromafenozide but ECD was slightly inhibited. Also, the effects of Chromafenozide on assimilation rate, relative metabolic rate and growth rate had been recorded.

Keywords: assimilation, biomass, digestibility, consumption, conversion, faeces, growth

1. Introduction
The desert locust, *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae), is known as a very destructive insect pest in North Africa (Sanchez-Zapata *et al*., 2007; Ammar *et al*., 2009) [1, 2]. It is characterized by a phase polymorphism (Uvarov, 1966) [3] enabling the transition from a solitary phase to a gregarious one extremely dangerous for the agricultural productions and pastures. *S. gregaria* is perhaps the most dramatic and potentially devastating species, and can devastate the cultures of a whole continent (Lecoq and Mestre, 1988) [4]. Current locust control operations are mainly based on organophosphorus pesticides as a result of the banning of organochlorines (Lecoq, 2001) [5]. The indiscriminate use of such synthetic pesticides has considerable drawbacks, such as the development of insect resistance to insecticides, high costs, handling hazards, several adverse effects on food, soil, ground water and air, as well as carcinogenic, teratogenic and great threats to both human and environmental health (Lingk, 1991; Bughio and Wilkins, 2004; Garriga and Caballero, 2011) [6-8]. To overcome these problems, it is necessary to seek safe, convenient, environmental and low-cost alternative control methods for pest. Insect growth regulators (IGRs) come as important agents among the various alternatives. They were developed to mimic, block or otherwise interact with the hormonal system of insects (Oetken *et al*., 2004) [9]. On the basis of mode of action, IGRs are grouped into three categories: juvenile hormones and their analogues (Juvenoids); ecdysone agonists or ecdysteroids; and chitin synthesis inhibitors (CSIs) or moult inhibitors (Mondal and Parween, 2000; Tunaz and Uygun, 2004) [10, 11]. IGRs, *viz*., juvenoids, antijuvenoids, ecdysteroids and CSIs as well as other related compounds, have been reported to possess a specific activity spectrum with a novel mechanism not based on a neurotoxic action, like synthetic insecticides (Dhadialla *et al*., 2005) [12]. The use of IGRs in pest control is known as insect development inhibitors which inhibits or prevents normal metamorphosis of immature stages to the adult stage (Farag, 2001; Abdel-Aal, 2003; Seth *et al*., 2004) [13-15]. IGRs are "low risk" insecticides, which have a relatively minor detrimental effect on the environment and its inhabitants, rendering them important components in IPM programs (Horowitz and Ishaaya, 2004) [16].

Ecdysone agonists exhibit insecticidal activities on insects by disrupting the moulting process. They are highly selective against lepidopterous larvae, with the result that other insects are often less affected by them (Silhacek *et al*., 1990; Schneider *et al*., 2003, 2008) [17-19]. Like other IGRs, ecdysone agonists act more slowly than neurotoxic insecticides because they disrupt the hormonal system or the physiological development of insects rather than directly killing them (Biddinger *et al*., 2006) [20]. However, their narrow spectrum of activity, positive ecotoxicological profile, and short persistence in the environment make these compounds promising against many economically important agriculture and forest pests (Sundaram *et al*., 2002; Smagghe *et al*., 2003; Biddinger *et al*., 2006; Osorio *et al*., 2008)[20-23]. Chromafenozide (Virtu®) is a novel

non-steroidal ecdysone agonist (Yanagi *et al*., 2000; 2006; Palli and Retnakaran, 2001; Smith, 2001) [24-26]. It was found very potent against Lepidoptera, but weak or inactive against other insect orders such as Diptera and Coleoptera (Nakagawa *et al*., 2005) [27]. Also, the use of chromafenozide at recommended dose did not pose any hazards to consumers when applied in strawberry under open field conditions (Malhat *et al*., 2014) [28].

Both feeding and reproduction in insects are very closely related to the nutritional factors, the qualitative and quantitative aspects of which have impact on the rate of growth, development and fecundity. The amount, rate and quality of food consumed by a larva influence its performance, growth rate, development time, final body weight and survival (Slansky and Scriber, 1985) [29]. Therefore, an understanding of the nutritional indices in relation to the rate of ingestion, digestion assimilation and conversion by the growing larvae would be useful (Scriber and Slansky, 1981) [30]. Also, reduction in the feeding activity of an insect may reduce the normal development, weight gain and fecundity, as well as increase its mortality (Van Duyn, 1971) [31].

It is important to point out that some of the natural products or synthetic chemicals disrupt the hormonal balance in insects by inhibiting the growth, metamorphosis and reproduction while, other chemicals affect the feeding behavior of insects and inhibit their feeding. As defined by some authors (Yasui *et al*., 1998; Isman, 2002; Lakshmanan *et al*., 2012; Pavunraj *et al*., 2012) [32-35], antifeedant is a chemical that inhibits the feeding without killing the insect pest directly, while it remains near the treated foliage and dies through starvation. Some insecticides, IGRs and botanicals have been found as appetite inhibitors for insects. Because deterrence is the act of preventing a particular act or behavior from happening, these compounds and products can be described as food deterrents, phagodeterrents or antifeedants against insects.

In insects, the physiological events that are linked to food consumption and utilization appear to be controlled by neural, endocrine and secretogogue mechanisms (Caldwell and Rankin, 1972; Chapman, 1985) [36, 37]. Hormones produced by the brain neurosecretory cells, corpora cardiaca and corpora allata also control the digestive enzyme production (Prabhu and Sreekumar, 1994) [38]. As for examples, in the last instar larvae of *Spodoptera mauritia*, feeding activity is maximum at high Juvenile hormone (JH) titer but when JH titer declines and the subsequent release of ecdysteroids, the feeding activity decreases (Balamani and Nair, 1992; Mona, 2001) [39, 40].

Besides their lethal action on insect immature stages and sterility in sexually mature adults, IGRs also inhibit the food consumption and growth of individuals which survive after sublethal treatments (Srivastava and Srivastava, 1990) [41]. IGRs are known to affect the digestion, utilization and other metabolic processes of ingested food (Ishaaya and Ascher, 1977) [42]. However, the action of some juvenoids, ecdysteroids and CSIs on food consumption and utilization was investigated in various insect species (Fytizus and Mourikis, 1979; Radwan *et al*., 1986; Farag, 1988, 1991; Ghoneim *et al*., 1998; Bream *et al*., 1999) [43-48]. Therefore, the present work was conducted aiming to investigate the antifeedant activity of the novel IGR Chromafenozide and its disruptive effects on the food consumption and utilization in the last nymphal instar of *S. gregaria*.

2. Materials and Methods

2.1 Experimental insect

The desert locust *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae) was used as an experimental insect in the present study. The present culture was originated by a sample of gregarious nymphs from Plant Protection Research Institute, Ministry of Agriculture, Giza. As designed by Hunter-Jones (1961) [49] and improved by Ghoneim *et al*. (2009) [50], insects were reared in wood formed cages (60 x 60 x 70 cm). The bottom was furnished with a sandy layer (20 cm depth) and provided with10-15% humidity to be suitable for egg laying. An electric bulb (100 watt) was adjusted to maintain a continuous photoperiod (12 L: 12 D) in each cage as well as in order to maintain an ambient temperature (32±2ºC). The insects were reared and handled under the crowded conditions. Fresh clean leaves of clover *Trifolium alexandrinum* were provided, as a food for insects, every day.

2.2 Chromafenozide application

Chromafenozide (Virtu® 5%) is a novel non-steroidal agonist of the insect moulting hormone 20-hydroxyecdysone. It was discovered and developed under cooperative works by Nippon Kayaku Co., Ltd. and Sankyo Co., Ltd. Japan from which it was purchased. Chemically, Chromafenozide is 3, 4-dihydro-5-methyl-2H-1-benzopyran-6-carboxylic acid 2-(3, 5-dimethylbenzoyl)-2-(1, 1-dimethylethyl) hydrazide.

Most of the total food consumption and growth usually occur during the penultimate and last larval instars and therefore performance values calculated for these instars tend to be representative of those calculated for the entire larval stage (Scriber and Slansky, 1981) [30]. Therefore, the last (5^{th}) nymphal instar was chosen in the present study. In a preliminary experiment on *S. gregaria*, the sublethal doses of Chromafenozide were found as 10, 100 & 150 µg/nymph. The present IGR was diluted with acetone for preparing these dose levels. Both of the newly moulted female and male nymphs of last instar were topically treated with 1 µl acetone containing Chromafenozide onto the prothoracic sternite using Hamilton microsyringe. Control nymphs were topically treated with acetone only. Ten nymphs were used as replicates for each treatment. The replicates were kept individually in 1 L glass jars for observing and determining the nutritional parameters as described herein.

2.3 Antifeedant activity

Antifeedant index (AFI %) was calculated according to the equation of Ladhari *et al*. (2013) [51] as follows: AFI % = [(C-T)/(C +T)] x 100 Where C: amount of food eaten by the control insect. T: amount of food eaten by the treated insect.

2.4 Efficiencies of food metabolism

In the present work, food consumption, digestion, absorption and conversion efficiencies were determined on a daily basis along the last nymphal instar of *S. gregaria*. Body weight of both treated and control nymphs was recorded before and after feeding, fresh food leaves were weighed before introduction to the nymph, and then the fresh weight of remains was recorded after feeding every day. Each nymph was starved for 3 h before weighing to ensure an empty intestine. For calculating the corrected weight of consumed food, known weights of fresh food leaves were left without nymphs for 24 h, under the same

laboratory conditions, and re-weighed at the end of this interval. Weight of faeces is the amount of frass produced by the nymph during the last instar.

Relative weight gain (RWG) = mg weight gain during the instar/ days (Johnson and Mundel, l987) [52] with correction for a single instar.

Feeding rate is the amount of food consumed per instar along its feeding period; generally expressed on a "per day per unit body mass" basis (Slansky, 1993) [53]. Relative consumption rate was calculated according to Slansky (1985) [54] as follows: RCR = mg consumed food/ g mean fresh body weight/ day.

According to Waldbauer (1968) [55], the following parameters can be calculated. Approximate digestibility (AD) = [Weight of ingested food - Weight of faeces / Weight of ingested food] X 100. Efficiency of conversion of ingested food to body substance (ECI) = [Weight gain / Weight of ingested food] X 100. Efficiency of conversion of digested food to body substance (ECD): [Weight gain / Weight of ingested food - Weight of faeces] X 100.

Assimilation rate (AR) = RCR x AD (Scriber and Slansky, 1981) [30]. Relative metabolic rate (RMR) was calculated according to Slansky (1980) [56] but corrected for fresh weights and for a single nymphal instar as follows: RMR = (mg weight ingested food - weight of faeces) / g mean fresh body weight / day.

These parameters may help to clear the metabolic efficiencies which can affect growth (Johnson and Mundel, 1987; Hinks *et al*., 1991) [52, 57]. Growth rate (GR) can be calculated as follows: GR = fresh weight gain during feeding period / feeding period X mean fresh body weight of larvae during the feeding period (Waldbauer, 1968) [55].

2.5 Statistical analysis

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction (Moroney, 1956) [58] for the test significance of difference between means.

3. Results

3.1 Antifeedant activity of Chromafenozide against *S. gregaria* nymphs

According to data assorted in Table (1), Chromafenozide exhibited contradictory effects on the feeding behaviour of *S. gregaria* nymphs depending on its dose since it acted as antifeedant against female nymphs only at the lowest dose. Antifeedant index (AFI) was calculated in 0.101%. In contrast, it acted as phagostimulant against female nymphs at the higher two doses (- 3.507 and - 3.633%, respectively). In respect of the male nymphs, Chromafenozide exhibited a predominant phagostimulatory effect at all doses (-2.594, -0.914 and -4.656% at the dose levels 10, 100, 150 µg/nymph, respectively).

3.2 Effect of Chromafenozide on food ingestion and consumption of *S. gregaria* nymphs

On the basis of food eaten along the last nymphal instar of *S. gregaria* females, data arranged in Table (2) exiguously revealed slightly decreasing food consumption only at the lowest dose of Chromafenozide as determined in 4055.4±50.7 mg (*vs*. 4063.6±68.2 mg of control congeners) or expressed as Relative Consumption Rate (RCR) as 39.6±3.26 (compared to 43.2±4.79 of controls, with a change % of -8.33). At the higher two doses, the food consumption considerably increased (4359.0±58.3 and 4370.0±187.2 mg, at 100 & 150 µg/nymph, respectively, *vs*. 4063.6±68.2 mg of controls) but RCR was slightly elevated.

In respect of the male nymphs, data distributed in Table (3) revealed a significant or insignificant enhancing action of Chromafenozide on the food intake (3809.2±22.6, 3683.3±32.2 and 3969.8±23.3 mg at 10, 100 & 150 µg/nymph, respectively, compared to 3616.6±55.2 mg of control male nymphs). RCR was slightly promoted in a dose-dependent course (Change %: 2.68, 3.24 and 21.09 at 10, 100 & 150 µg/nymph, respectively). Recalling data of Table (2), treated female nymphs attained slightly or remarkably increasing relative weight gain (RWG) and discharged more fecal pellets. To some extent, similar increasing RWG and fecal production had been recorded for male nymphs (Table 3).

3.3 Effect of Chromafenozide on food digestive, absorptive and conversion efficiencies of *S. gregaria* nymphs

According to data presented in Table (4), female nymphs achieved insignificantly increasing approximate digestibility (AD) in a dose-dependent course (increment %s: 1.97, 3.88 and 7.24, at 10, 100 & 150 µg/nymph, respectively). The AD enhancement was proportional to the increasing RCR achieved by treated nymphs and increasing of their fecal production throughout the last instar. With regard to AD of male nymphs, Chromafenozide exhibited a diverse effect depending its dose level since AD slightly increased at the lowest dose and slightly decreased at the medium dose but un-affected at the highest dose (Table 5).

Referring to the data of Table (4), both the efficiency of conversion of ingested food into biomass (ECI) and efficiency of conversion of digested food into biomass (ECD) of females slightly increased. ECI increased in a reverse dose-dependent course as estimated in 3.69, 3.51 and 3.17% (at 10, 100 & 150 µg/nymph, respectively) as well as ECD increased as calculated in 34.74 and 0.08 at the higher two doses. Similarly, but in no précised course, ECI of male nymphs was promoted by Chromafenozide (increment %s: 0.38, 17.65 and 2.17 at the increasing dose level, respectively, Table 5). On the contrary, ECD was insignificantly inhibited, in no certain trend, by the present IGR (decrements: -19.37, -1.42 and -1.31% at 10, 100 & 150 µg/nymph, respectively, Table 5). Depending on these data, there was a sexual difference in respect of ECD but similar difference had not been appeared in ECI.

3.4 Effect of Chromafenozide on the food assimilation and growth in *S. gregaria* nymphs

For investigating the food metabolism, two additional metabolic parameters (Assimilation Rate, AR, and Relative Metabolic Rate, RMR) may shed some light on the effect of Chromafenozide. As obviously seen in Table (6), AR of both female and male nymphs was subjected to an enhancing action of this IGR, at its higher two dose levels, to highly assimilate the absorbed food (33.85±3.24 and 38.83±5.11% of females, *vs*. 31.26±4.72% of controls, as well as 32.94±2.81 and 39.16±5.37% of males, *vs*. 32.34±5.12 % of controls). In general, AR increased in a dose-dependent fashion. To a great extent, RMR was promoted in a similar course, especially at the higher doses of Chromafenozide.

Looking deeply at data of Tables 2, 3, 4, 5 & 6 showed a positive correlation of AR and RMR to RCR which was easily detected because these parameters were elevated by

Chromafenozide, at the higher doses, indicating a high capacity of nymphs to digest, absorb and assimilate the food which was eaten at high RCR.

To investigate the interrelationship between growth and nutritional performance of *S. gregaria* nymphs as affected by chromafenozide, data arranged in Tables (2 & 6) show that the growth rate (GR) increased in no certain trend (8.4±0.9, 10.9±0.2 and 9.4±2.9%, at 10, 100 & 150 µg/nymph, respectively, *vs.* 9.3±0.8% of controls). As obviously seen in Tables (3 & 6), a similar correlation of the relative growth rate with GR was observed for male nymphs. Also, GR of both sexes was generally enhanced as both AR and RMR increased with few exceptions.

Table 1: Antifeedant index (%) of Chromafenozide against last instar nymphs of *S. gregaria*.

Dose (µg/nymph)	Females	Males
10	+ 0.101	-2.594
100	- 3.507	- 0.914
150	- 3.633	- 4.656
Control	---	---

Table 2: Effect of Chromafenozide on body weight gain, food consumption and faeces produced by females of last nymphal instar of *S. gregaria.*

Dose (µg/nymph)	RWG (mg±SD)	Food consumed (mg±SD)	Faeces produced (mg±SD)	RCR (x100) (mg±SD)	Change %
10	123.69±15.1 a	4055.4±50.7 a	1095.2±15.9 a	39.6±3.26 a	- 8.33
100	155.27±3.7 d	4359.0±58.3 d	1253.0±15.6 d	45.0±3.27 a	+4.17
150	138.86±31.4 a	4370.0±187.2 c	1204.3±12.5 d	50.0±5.52 a	+15.74
Control	119.49±11.75	4063.6±68.2	1048.6±50.5	43.2±4.79	---

Mean ± SD followed with the letter (a):: not significantly different (P>0.05), (c): highly significantly different (P<0.01), (d): very highly significantly different (P<0.001). RWG: Relative weight gain. RCR: Relative consumption rate of food.

Table (3): Effect of Chromafenozide on body weight gain, food consumption and faeces produced by males of last nymphal instar of *S. gregaria.*

Dose (µg/nymph)	RWG (mg±SD)	Food consumed (mg±SD)	Faeces produced (mg±SD)	RCR (x100) (mg±SD)	Change %
10	127.0±27.61 a	3809.2±22.6 d	867.0±89.6 a	48.6±5.54 a	+2.68
100	97.55±15.17 a	3683.3±32.2 a	889.0±67.4 b	46.25±2.77 a	+3.24
150	88.23±15.66 a	3969.8±23.3 d	879.6±100.3 b	54.25±7.89 a	+21.09
Control	96.0±24.21	3616.6±55.2	786.0±32.4	44.8±6.68	---
a, d, RWG, RCR: see footnote of Table (2). (b): significantly different (P<0.05).					

Table 4: Effect of Chromafenozide on the food digestion, absorption and conversion efficiencies of females of last nymphal instar of *S. gregaria*.

Dose (µg/nymph)	AD (mg±SD)	Change %	ECI (mg±SD)	Change %	ECD (mg±SD)	Change %
10	73.84±2.49 a	+1.97	26.14±5.17 a	+3.69	35.64±7.77 a	+34.74
100	75.22±0.52 a	+3.88	19.77±1.95 a	+3.51	26.47±2.34 a	+0.08
150	77.65±1.83 a	+7.24	19.73±1.95 a	+3.17	22.73±2.45 a	+14.06
Control	72.41±5.21	---	19.1±5.0	---	26.45±7.42	---

a : see footnote of Table (2). AD: Approximate digestibility. ECI: Efficiency of conversion of ingested food. ECD: Efficiency of conversion of digested food.

Table 5: Effect of Chromafenozide on the food digestion, absorption and conversion efficiencies of males of last nymphal instar of *S. gregaria*.

Dose (µg/nymph)	AD (mg±SD)	Change %	ECI (mg±SD)	Change %	ECD (mg±SD)	Change %
10	72.57±2.16 a	+0.53	21.80±4.43 a	+0.38	24.56±2.44 a	-19.37
100	71.23±0.70 a	-1.33	25.46±0.48 a	+17.65	30.05±1.20 a	-1.42
150	72.19±3.61 a	0.00	22.11±3.42 a	+2.17	30.06±4.57 a	-1.31
Control	72.19±3.61	---	21.64±4.27	---	30.46±6.94	---

a: see footnote of Table (2). AD, ECI, ECD: see footnote of Table (4).

Table 6: The correlation of GR (x100) to AR (x100) and RMR (x100) as affected by Chromafenozide along the last nymphal instar of *S. gregaria*.

Dose (µg/nymph)	GR		AR		RMR	
	Females	Males	Females	Males	Females	Males
10	8.4±0.9 a	13.2±4.0 a	29.24±4.55 a	31.61±3.66 a	29.2±3.0 a	32.3±5.0 a
100	10.9±0.2 c	9.6±0.1 a	33.85±3.24 a	32.94±2.81 a	31.6±2.0 a	34.4±3.0 a
150	9.4±2.9 a	10.0±2.3 a	38.83±5.11 b	39.16±5.37 a	36.8±4.0 a	42.3±6.0 b
Control	9.3±0.8	9.5±1.2	31.26±4.72	32.34±5.12	31.1±5.0	32.6±7.0

a, c: see footnote of Table (2). b: see footnote of Table (3). GR: Growth rate. AR: Assimilation rate. RMR: Relative metabolic rate.

4. Discussion

Food utilization efficiencies are useful for measuring the growth rate and development of the consumer (Scriber and Slansky, 1981; Slansky and Scriber, 1985) [30, 29]. Several metabolic parameters were suggested and usually used to determine the food utilization. However, the common three efficiencies are: approximate digestibility (AD), efficiency of conversion of ingested food to biomass (ECI) and efficiency of conversion of digested food to biomass (ECD) (Waldbauer, 1968; Woodring *et al*., 1979; Slansky and Scriber, 1985; Slansky, 1993) [55, 59, 29, 53]. As described by Senthil-Nathan et al. (2005) [60], ECI is an overall measure of an insect's ability to utilize the food that it ingests for growth and development and ECD is a measure of the efficiency of conversion of digested food into growth. ECD is sometimes called "Net growth efficiency" or "Metabolic efficiency" (Slansky and Scriber, 1985)[29]. Food metabolic efficiencies vary widely with the insect species. As for example, ECI and ECD of lepidopterous larvae are about double those of orthopterous larvae, while the AD being about the same. The efficiencies of food utilization vary, also, with age (both within and between instars) and sex as well as with different environmental factors.

4.1 Antifeedant activity of Chromafenozide against *S. gregaria* nymphs

Some authors did not determine the food deterrence or antifeedant activity of insecticides or IGRs against the target insect pests but used the food consumption as an informative indicator for it. On the other hand, few researchers recorded antifeedant or deterrence index, as reported in the available literature. The insecticides osthole and pregnenolone showed significant antifeedant activities against larvae of *Spodoptera litura* (Kalpana, 2005) [61]. Among seventeen monoterpenoids assessed against *Pieris brassicae* 4th instar larvae, the strongest deterrent effect was exhibited by α-phellandrene- and β-ionone but (S)-(+)-carvone exhibited a slight antifeedant effect (Kordan and Gabryś, 2013)[62]. The antifeedant activities of chlorpyrifos and deltamethrin, individually and in combination, were observed on the *Atractomorpha crenulata* 4th instar nymphs (Rani and Sanjayan, 2014) [63]. With special reference to *Spodoptera littoralis*, chlorantraniliprole, thiamethoxam and novaluron exhibited feeding deterrent actions against 4th instar larvae. Moreover, Chlorantraniliprole exhibited the strongest deterrent action (Barrania, 2013) [64]. On feeding of the 4th instar larvae of the same insect on castor bean leaves treated with emamectin benzoate, rynaxypyr, indoxacarb, spinetorm and spinosad for 24 hrs, the highest inhibition of feeding and antifeedant indices were recorded for indoxacarb and rynaxypyr (Rashwan, 2013) [65].

In the present study, Chromafenozide unexceptionally exhibited diverse effects on the feeding behaviour of *S. gregaria* nymphs, depending on its dose, since it acted as a weak antifeedant against female nymphs, only at the lowest dose (10µg/nymph), but acted as phagostimulant against them, at the higher two doses (100 & 150 µg/nymph). Moreover, it exhibited a phagostimulant activity against the male nymphs, regardless the dose. On comparison with those reported results, the present diverse results can be due to the different method of treatment since the nymphs themselves had been topically treated and allowed to feed on normal fresh food leaves. However, to understand the weak antifeedant activity of the present IGR, it may stimulate specific 'deterrent' cells in chemoreceptors and also block the firing of 'sugar' receptor cells, which normally stimulate the feeding (Blaney *et al*., 1990; Simmonds *et al*., 1990) [66, 67]. This result in the feeding inhibition, culminating in the starvation and death of the insect by feeding deterrence alone (Koul and Wahab, 2004) [68]. On the other hand, the phagostimulatory action of Chromafenozide cannot be interpreted right now!!

4.2 Food ingestion and consumption by *S. gregaria* nymphs as influenced by Chromafenozide

Depending on the reported results in the available literature, food consumption had been significantly reduced in several insect species by various insecticides or IGRs and IGR-related compounds. A considerable reduction in the food consumption was determined for *Leptinotarsa decemlineata* larvae by Flucycloxuron (Szczepanik, 1998) [69], for 5 day-old adults of *S. gregaria* by fenitrothion (Ouali-N'goran *et al*., 2003) [70] and for *Callosobruchus muculatus* larvae by Cyromazine (Al-Mekhlafi *et al*., 2012) [71]. With regard to *S. littoralis*, feeding of larvae on castor bean leaves treated with Mancozeb, bromoxynil and profenofos (Marzouk *et al*., 2012) [72], Pyriban (Chlorpyrifos) (Ebeid and Gesraha, 2012) [73], chlorantraniliprole, thiamethoxam and novaluron (Barrania, 2013) [64], rynaxypyr and indoxacarb (Rashwan, 2013) [65], Flufenoxuron and triflumuron (El-Naggar, 2013) [74], or chlorfenapyr (Ebeid *et al*., 2015) [75] resulted in significantly reduced food consumption of larvae. Also, LC_{50} of Diazinon and flufenoxuron were applied on the 2nd instar larvae and reduction of food consumption had been recorded (El-Helaly and El-bendary, 2015) [76].

In the current work, results of food consumption of last instar nymphs of *S. gregaria* disagree with those reported results because Chromafenozide exhibited a prevalent promoting action and thus food consumption generally increased. For some detail, the food consumption considerably increased at the higher two doses (100 & 150 µg/nymph), while RCR was slightly elevated. Also, the male nymphs had been enhanced by the IGR to consume significantly or insignificantly increasing amount of food. RCR was slightly promoted in a dose-dependent course. In the present investigation, also, treated nymphs of both sexes attained slightly or remarkably increasing relative weight gain (RWG) and discharged more fecal pellets. The increasing food consumption can indicate the action of Chromafenozide as digestive attractants, as suggested by Piechowicz *et al*. (2012) [77] for the insecticide deltamethrin and IGR pyriproxyfen against the adults of Spanish slug *Arion lusitanicus*. Another conceivable interpretation cannot, unfortunately, be provided right now!

On the other hand, an exceptional case of slightly decreased food consumption was observed in the current investigation for female nymphs of *S. gregaria*, only at the lowest dose (10 µg/nymph) of Chromafenozide. However, this case of decreasing food consumption can be partly interpreted by the antifeedant activity of this IGR at the lowest dose. Another suggestion may be acceptable since Masih and Vaishya (2014) [78] reported that the bioefficacy of IGR is noticed during ecdysis, as it disturbs the process of chitin synthesis or/and deposition due to which the insect dies. It leads, also, to the insect failure to feed, owing to displacement of mandibles and labrum or blockage of the gut.

4.3 Food digestive and absorptive capacities of *S. gregaria* nymphs as influenced by Chromafenozide

Special attention should be paid to another important nutritional parameter, AD, which expresses the digestion and absorption capacity of the insect. AD in insects is based on differences between the weight of food ingested and the weight of the faeces actually represents the food which is stored or metabolized. Therefore, the AD estimates the percentage of ingested food that is digested and assimilated (Slansky and Scriber, 1985) [29].

In the present study, Chromafenozide-treated female nymphs of *S. gregaria* achieved insignificantly increasing AD in a dose-dependent course. Chromafenozide exhibited a diverse effect on AD of male nymphs since they had a slightly increased AD at the lowest dose and a slightly decreased AD at the medium dose but un-affected AD at the highest dose. The present results of female and partially of males are, to some extent, in agreement with those reported results of significantly increased AD by some authors (Abou El- Ghar *et al*., 1996; Bream *et al*., 1999; Garside *et al*., 2000) [79, 48, 80]. Also, after topical application of different doses of Hydroprene onto the last (6^{th}) instar larvae of *Spodoptera mauritia*, AD increased (Sindhu and Nair, 2004) [81]. AD of 4^{th} instar larvae of *S. littoralis* was enhanced by chlorfenapyr, at 0.25% concentration (Ebeid *et al*., 2015) [75].

On the contrary, the present results of predominantly enhancing action of Chromafenozide on AD of *S. gregaria* nymphs had not been consistent with some reported results of inhibited AD in some insects by various IGRs and insecticides. Significantly reduced AD was recorded for *S. gregaria* nymphs after treatment with Fenoxycarb (Ismail, 1995) [82] and for *S. littoralis* larvae after treatment with Teflubenzuron (Abdel-Aal and Abdel-Khalek, 2006) [83], Sumialfa (El-Malla and Radwan, 2008) [84] or rynaxypyr and indoxacarb (Rashwan, 2013) [65]. However, the exceptional case of slightly inhibited AD of male nymphs, in the present study, may agree with these reported results. In addition, the exceptional case of unaffected AD of male nymphs (at the highest dose of Chromafenozide) came in agreement with those reported results of unchanged AD of few insects by certain compounds, such as *H. virescens* larvae after feeding on tobacco plants, expressing potato proteinase inhibitors (PIN-2) (Brito *et al*., 2001) [85] and the 4^{th} instar larvae of *S. littoralis* after feeding on food treated with LC_{50} of Flufenoxuron or Triflumuron (El-Naggar, 2013) [74].

The predominantly enhancing action of Chromafenozide on AD of *S. gregaria* nymphs, in the present study, can be interpreted as earlier suggested as attempts made by the insect to compensate for the reduced consumption and utilization of food in order to maintain growth rate (Reese and Beck, 1976) [86]. Generally, increasing AD could be understood in the light of increasing RCR as well as increasing amounts of food eaten by treated nymphs in the current work. The odd datum of slightly inhibited AD of male nymphs, in the present study, should not be neglected because inhibition or reduction of AD may be due to the toxicity of a chemical on the digestive cells causing a damage of the gut epithelium, and so treated insects are unable to digest food properly (Meyer and Lamberts, 1965) [87] and the food absorption capacity may be impaired (Baghban *et al*., 2014; Abu ElEla and ElSayed, 2015) [88, 89].

4.4 Food conversion efficiencies of *S. gregaria* nymphs as influenced by Chromafenozide

From the metabolic view of point, the most important efficiencies of food conversion are ECI and ECD into the biomass. In other words, ECI measures the overall ability of insect to convert ingested food into the body tissues while ECD is the conversion capacity of digested food into biomass. According to the reported results in literature, ECI and ECD of various insects had been considerably or slightly reduced by different insecticides and IGRs, such as fenarimol (Farag, I991) [46], Tebufenozide (Bream *et al*., 1999) [48], Sumialfa (El-Malla and Radwan, 2008) [84], Flufenoxuron and Triflumuron (El-Naggar, 2013) [74] or Rynaxypyr and Indoxacarb (Rashwan, 2013) [65] against *S. littoralis* larvae. Recently, treatment of *S. littoralis* larvae with LC_{50} of Diazinon and Flufenoxuron (El-Helaly and El-bendary, 2015) [76] or the low concentration of Chlorfenapyr (Ebeid *et al*., 2015) [75] resulted in remarkably reduced ECI and ECD. In contrast, enhanced ECI had been reported for few insects by some IGRs and other chemicals since significantly increasing ECI was recorded in the last instar larvae of *S. mauritia* after topical application with Hydroprene (Sindhu and Nair, 2004) [81] and in the last instar larvae of *S. littoralis* after treatment with Cadmium (Abu ElEla and El-Sayed, 2015) [89].

In the present study, both ECI and ECD of female nymphs of *S. gregaria* were slightly enhanced by Chromafenozide. Also, ECI of male nymphs was promoted while ECD was slightly inhibited, in no certain trend. Thus, there was a sexual difference in respect of ECD but similar sexual difference had not been appeared in ECI. However, the enhanced ECI and ECD may be attributed to the fact that the insect requires a lot of energy to deal with the used chemical toxicity as suggested by Emre *et al*. (2013) [90] for *Galleria mellonella*, Baghban *et al*. (2014) [88] for *H. armigera* and Abu El-Ela and El-Sayed (2015) [89] for *S. littoralis*. On the other hand, the inhibited ECD may indicate that the ingested IGR does exhibit some chronic toxicity against the insect (Wheeler and Isman, 2001) [91]. In this respect, El-Shazly (1993) [92] indicated that ECI will vary with the digestibility of food and proportional amount of the digestible portion of food which is converted to body substance and metabolized for energy to maintain life.

4.5 Food assimilation and metabolism in *S. gregaria* nymphs as influenced by Chromafenozide

It is interesting to mention that some other additional nutritional parameters had been reported in this area of study, *viz.* Assimilation rate (AR) and Relative metabolic rate (RMR). These parameters may help to clear the metabolic efficiencies which can affect the growth (Hinks *et al*., 1991) [57].

In the present study, AR of both female and male nymphs of *S. gregaria* was subjected to an enhancing action of Chromafenozide, at the higher two dose levels, to highly assimilate the absorbed food. AR increased in a dose-dependent fashion. RMR was promoted in a similar course, especially at the higher doses. A positive correlation of AR and RMR to RCR was easily detected. These results are in contrast to several reported results of regressed AR and RMR in larvae of various insect species by the action of some IGRs, such as *Agrotis ipsilon* (Reese and Beck, 1976) [86], *Manduca sexta*

(Dahlaman, 1977) [93], *S. litura* (Sundaramurthy, 1977) [94], *S. littoralis* (Bream *et al*., 1999) [48] and *S. gregaria* (Ismail, 1995) [82]. These differences may be due to the difference of IGRs, method of treatment and the response of the insect.

4.6 Interrelationship between growth and nutritional performance of *S. gregaria* nymphs under stress of Chromafenozide

As clearly reported in the literature, relative weight gain **(**RWG**)** or/and relative growth rate **(**RGR**)** of many insects had been declined by several insecticides or IGRs and IGR-related compounds. Concerning *S. littoralis*, RGR of 4th instar larvae was significantly reduced by some IGRs (El-Basyouni and Sharaf, 2002) [95]. Significantly deceasing RGR of 4th instar larvae was determined after feeding on leaves treated with Chlorantraniliprole, Thiamethoxam and Novaluron (Barrania, 2013) [64], Flufenoxuron and Triflumuron (El-Naggar, 2013) [74] or Rynaxypyr and Indoxacarb (Rashwan, 2013) [65]. In *A. crenulata*, Chlorpyrifos showed stronger growth inhibitory action than Deltamethrin (Rani and Sanjayan, 2014) [63]. Recently, feeding of the 2nd instar larvae of *S. littoralis* on plant leaves treated with Diazinon and Flufenoxuron resulted in reduction of RGR (El-Helaly and El-bendary, 2015) [76].

In contrast, the growth rate (GR) of both sexes of *S. gregaria* nymphs, in the present study, was generally enhanced by Chromafenozide since both AR and RMR increased, with few exceptions. Also, GR was positively correlated with RWG. These results are, to a great extent, in congruence with the enhancement of larval growth by IGRs as reported in the literature since topical treatment of the last instar larvae of *S. mauritia* with different doses of Hydroprene resulted in an increase of RGR (Sindhu and Nair, 2004) [81]. Novaluron exhibited a reversible effect on the larval RWG and GR of *S. littoralis*, depending on the concentration (Ghoneim *et al*., 2015) [96].

However, the interpretation of inhibited growth of various insects by IGRs or IGR-related compounds has been available in the literature (Martinez and van Emden, 1999; Marie *et al*., 2009; Mehrkhou, 2013; Abu El-Ela and El-Sayed, 2015; Giongo *et al*., 2015) [97-99, 89, 100]. In this respect, Woodering *et al*. (1978) [101] and Sundaramurthy (1977) [94] indicated that the amount of growth reduction was proportional in general to reduced food consumption. No acceptable interpretation of the enhanced growth of *S. gregaria* nymphs, as seen in the present study after treatment with Chromafenozide, is available right now!!

In respect of the fecal production by larvae, it is important to point out that feeding is necessary for the stimulation of digestive enzyme activities (Smirle *et al*., 1996) [102] and may have interfered with the enzyme-substrate complex thus affecting the peristaltic movement of the gut (Broadway, and Duffey, 1988) [103]. Some IGRs prohibited the fecal production by insects since *S. littoralis* larvae produced remarkably reduced faeces after treatment with Fenarimol or Naurimol (Farag, 1991) [46], Tebufenozide (Bream *et al*., 1999) [48] or Lufenuron (Adel, 2012) [104]. Also, reduction of faecal production was recorded for *S. gregaria* after treatment with Fenoxycarb (Ismail, 1995) [82] and for *S. mauritia* after treatment with Diflubenzuron (Jagannadh and Nair, 1997) [105]. Dissimilar to those reported results, the present results of *S. gregaria* show increasing fecal production by nymphs and their RCR had been proportional to the enhanced AD throughout the last instar after topical treatment with Chromafenozide. We are unable right now to provide a conceivable explanation to the increasing fecal production and the matter is still obscure!! Likewise, the increasing fecal production may indicate higher capacity of *S. gregaria* nymphs to digest, absorb and assimilate the ingested food as a response to the tested IGR Chromafenozide.

5. Conclusion

As clearly shown in the present study, Chromafenozide exhibited a weak antifeedant activity, only at the lowest dose level, against the last instar nymphs of *S. gregaria*. At its dose level, this IGR exerted a slight inhibitory action of the food consumption but enhanced the nymphs to eat more food amounts, gained increased somatic weight and discharged more fecal pellets. Also, it failed to pronouncedly inhibit the food digestibility, conversion and assimilation capacities of the treated nymphs, at the higher dose levels. Depending on these results, Chromafenozide cannot be considered as a promising agent for controlling this pest.

6. References

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