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Effect of insect growth regulator, chromafenozide on the food consumption and metabolic efficiencies of the desert locust

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Abstract

The objective of the current investigation was to assess the food deterrence property of chromafenozide and its impact on different food metabolic parameters of the 5th instar nymphs of the desert locust *Schistocerca gregaria*. For this purpose, three doses (10, 100 and 150 µg/nymph) were topically applied (once) onto the newly molted last (5th) instar nymphs. chromafenozide exhibited contradictory effects on the feeding since it acted as a weak antifeedant against female nymphs only at the lowest dose but acted as phagostimulant against their congeners at the higher two doses. Furthermore, the compound exerted a general phagostimulant action on the male nymphs, regardless the dose. The food intake by the last nymphal instar females was slightly reduced only at only the lowest dose but remarkably increased at the higher doses. The male nymphs had been promoted to consume more food amount. Treated nymphs of both sexes attained slightly or significantly increasing relative weight gain and discharged more frass pellets. The treated nymphs of both sexes achieved slightly 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. Both the efficiency of conversion of ingested food into biomass (ECI) and efficiency of conversion of digested food into biomass (ECD) of the female nymphs slightly increased. ECI of male nymphs was enhanced but ECD was slightly inhibited. Depending on the present results, Chromafenozide cannot be recommended as a promising agent for controlling *S. gregaria*.

Keywords: approximate digestibility, biomass, consumption, conversion, frass, growth, instar, nymph.

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1. Introduction

The desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae), is a dangerous pest of crops, pastures and agricultural production in Egypt and other countries of North Africa and West Asia (Ammar *et al*., 2009; Lecoq and Mestre, 1988; Sanchez-Zapata *et al*., 2007). Current locust control measures are mainly based on organophosphorus pesticides as a result of the banning of organochlorines (Lecoq, 2001). The indiscriminate and excessive uses of such pesticides have dangerous problems, such as the development of insect resistance to insecticides, increased 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 (Bughio and Wilkins, 2004; Garriga and Caballero, 2011). For avoiding or limiting these problems, it is necessary to seek environmentally safe, convenient, and low-cost alternative control agents. In this regard, insect growth regulators (IGRs) come as promising agents among the various alternatives. They were manufactured to mimic, block or otherwise interact with the hormonal system of insects (Oetken *et al*., 2004). Moreover, IGRs 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). Depending on the mode of action, IGRs are grouped into juvenoids, antijuvenoids, ecdysteroids and chitin synthesis inhibitors as well as other related compounds (Mondal and Parween, 2000; Tunaz and Uygun, 2004). For the pest control, IGRs have been known to act as insect development inhibitors (Abdel-Aal, 2003; Farag, 2001; Seth *et al*., 2004). IGRs are "low risk" compounds with a minor harmful effect on the environment, rendering them important agents in IPM programs (Horowitz and Ishaaya, 2004). Ecdysteroids (Ecdysone agonists) had been found to disrupt the moulting process in insects (Schneider *et al*., 2003; 2008; Silhacek *et al*., 1990). In some detail, ecdysone agonists act more slowly than neurotoxic insecticides because they disrupt the hormonal system or disturb the physiological development of insects rather than directly killing them (Biddinger *et al*., 2006). In addition, they have short persistence in the environment making them promising compounds against many important agriculture and forest pests (Biddinger *et al*., 2006; Osorio *et al*., 2008; Smagghe *et al*., 2003; Sundaram *et al*., 2002). Chromafenozide (Virtu®) is a novel non-steroidal ecdysone agonist (Palli and Retnakaran, 2001; Smith, 2001; Yanagi *et al*., 2000). It was found very potent against Lepidoptera, but weak or inactive against other insect orders (Nakagawa *et al*., 2005). Also, the use of this ecdysone agonist, at recommended doses, did not pose any hazards to consumers under the open field conditions (Malhat *et al*., 2014). In insects, the physiological events that are linked to food consumption and utilization appear to be regulated by neural, endocrine and secretogogue mechanisms (Chapman, 1985). Hormones produced by the brain neurosecretory cells, the corpora cardiaca and corpora allata also control the digestive enzyme production (Prabhu and Sreekumar, 1994). 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). On the other hand, feeding and reproduction in insects are closely related to nutritional factors, the qualitative and quantitative aspects of which have impact on the growth, development and fecundity (Slansky and Scriber, 1985). Therefore, an understanding of the nutritional indices in relation to the effiencies of ingestion, digestion assimilation and conversion by the growing larvae would be useful (Scriber and Slansky, 1981). As defined by some authors (Isman, 2002; Lakshmanan *et al*., 2012; Pavunraj *et al*., 2012; Yasui *et al*., 1998), 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. 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. However, the actions of some juvenoids, ecdysteroids and chitin synthesis inhibitors on food consumption and utilization had been studied in various insect species (Bream *et al*., 1999; Farag, 1988, 1991; Ghoneim *et al*., 1998; Radwan *et al*., 1986). For these reasons, the present study was carried out for investigating the food deterrence property of Chromafenozide and its disruptive impact on the food consumption and metabolism in the last (5th) nymphal instar of *S. gregaria*.

2. Materials and methods

2.1 The insect culture

In the current study, the desert locust *Schistocerca gregaria* (Forskal) was used as an experimental insect. Its culture was originated by a sample of gregarious nymphs from Plant Protection Research Institute, Dokki, Giza, Egypt. As described by Hunter-Jones **(**1961) and improved by Ghoneim *et al*. (2009), desert locust was reared in wood cages (60 × 60 × 70 cm). The bottom of each cage was furnished with a layer of sterilized sand (20 cm depth) and provided with 10-15% humidity to be suitable for egg laying. An electric bulb (100 watt) was used to maintain a continuous photoperiod (12 L: 12 D) in each cage as well as in order to maintain an ambient temperature (32±2^{o}C). The culture was raised and handled under the crowded conditions. All insects were provided every day with fresh clean leaves of clover *Trifolium alexandrinum*.

2.2 Application of the ecdysone agonist

Chromafenozide (Virtu® 5%) is a non-steroidal agonist of the insect moulting hormone, ecdysone. It was purchased from Sankyo Co., Ltd. Japan. Chemically, it is 3,4-dihydro-5-methyl-2H-1-benzopyran-6-carboxylic acid 2-(3,5-dimethylbenzoyl)-2-(1,1-dimethylethyl) hydrazide. The last (5th) nymphal instar of *S. gregaria* was chosen in the present study. In a preliminary experiment on the same locust, the sublethal doses Chromafenozide were determined as 10, 100 and 150 µg/nymph. Chromafenozide 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 from both female and male were used as replicates for each treatment. The replicates were kept individually in 1 L glass jars for observing and determining the food consumption and utilization as described herein**.**

2.3 Feeding deterrence

Antifeedant index (AFI %) was calculated according to the equation of Ladhari *et al*. (2013) 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 Parameters of food consumption and utilization

In the present study, food consumption, digestion, absorption and conversion efficiencies were determined on a daily basis along the last nymphal instar. 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 food intake, 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 frass was estimated for each nymph during the last instar. Relative weight gain (RWG) = mg weight gain during the instar/ days (Johnson and Mundel, l987) with correction for a single instar. Feeding rate is the amount of food intake per instar along its feeding period; generally expressed on a "per day per unit body mass" basis (Slansky, 1993). Relative consumption rate was calculated according to Slansky (1985) as follows: RCR = mg consumed food/ g mean fresh body weight/ day. According to Waldbauer (1968), 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). Relative metabolic rate (RMR) was calculated according to Slansky (1980) 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 nutritional efficiencies which can affect on growth (Johnson and Mundel, 1987; Hinks *et al*., 1991). 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).

2.5 Data analysis

Data were analyzed by the Student's *t*-distribution, and refined by Bessel correction (Moroney, 1957) for the test significance of difference between means.

3. Results

3.1 Feeding deterrence property of chromafenozide against S. gregaria nymphs

As shown by data of Table (1), Chromafenozide exhibited contradictory effects on the feeding capacity of *S. gregaria* nymphs depending on its dose since it acted as antifeedant against female nymphs only at the lowest dose. Antifeedant index 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 general phagostimulating effect, regardless the dose level (-2.594, -0.914 and -4.656% at the dose levels 10, 100, 150 µg/nymph, respectively).

3.2 Affected food ingestion and consumption of S. gregaria nymphs by chromafenozide

With regard to the food eaten along the last nymphal instar of *S. gregaria* females, data of Table (2) exiguously revealed a slightly decreasing food consumption only at the lowest dose as determined in 4055.4±50.7 mg (*vs*. 4063.6±68.2 mg of control congeners) or expressed as reduced. 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 remarkably increased (4359.0±58.3 and 4370.0±187.2 mg, at 100 and 150 µg/nymph, respectively, *vs*. 4063.6±68.2 mg of controls) but RCR was slightly elevated. Concerning the male nymphs, data arranged 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 and 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 and 150 µg/nymph, respectively). Depending on the data of Table (2), treated female nymphs attained slightly or significantly 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).

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	---

Mean ± SD followed with the letter (a): 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). RWG: Relative weight gain. RCR: Relative consumption rate of food. (b): significantly different (P<0.05).

3.3 Impact of chromafenozide on food digestive, absorptive and conversion efficiencies of S. gregaria nymphs

Depending on the data of Table (4), female nymphs achieved slightly 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 enhanced AD was proportional to the increasing RCR achieved by treated nymphs and increasing of their fecal production throughout the last instar. In respect of AD of male nymphs, the present compound exhibited a diverse effect, depending its dose level, since AD slightly increased at the lowest dose but slightly decreased at the medium dose and un-affected at the highest dose (Table 5).

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	---

Mean ± SD followed with the letter (a): not significantly different (P>0.05). 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	---

Mean ± SD followed with the letter (a): not significantly different (P>0.05). AD: Approximate digestibility. ECI: Efficiency of conversion of ingested food. ECD: Efficiency of conversion of digested food.

According to data distributed in the same Table (4), efficiency of conversion of ingested food into biomass (ECI) and efficiency of conversion of digested food into biomass (ECD) of females slightly decreased. ECI increased in a reverse dose-dependent course and determined in 3.69, 3.51 and 3.17% (at 10, 100 & 150 µg/nymph, respectively) as well as ECD increased as estimated in 34.74 and 14.06 at the low and higher two doses. Similarly, but in no certain 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 slightly inhibited, in no précised trend (decrements: -19.37, -1.42 and -1.31% at 10, 100 and 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 Influenced food assimilation and growth of S. gregaria nymphs by chromafenozide

To study the food metabolism, two additional parameters (Assimilation Rate, AR, and Relative Metabolic Rate, RMR) may shed some light on the effect of Chromafenozide. As clearly shown by data of Table (6), AR values of both female and male nymphs increased, at the higher two doses, 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 some extent, RMR increased in a similar course, especially at the higher doses.

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

Mean ± SD followed with the letter (a): not significantly different (P>0.05), (b): significantly different (P<0.05), (c): highly significantly different (P<0.01). GR: Growth rate. AR: Assimilation rate. RMR: Relative metabolic rate.

Reviewing the data of Tables (2, 3, 4, 5 and 6) revealed a positive correlation of AR and RMR to RCR which was easily shown because these parameters were elevated by Chromafenozide, at the higher doses, indicating a high efficacy of nymphs to digest, absorb and assimilate the food which was eaten at high RCR. For investigating the interrelationship between growth and nutritional performance of *S. gregaria* nymphs as affected by chromafenozide, data arranged in Tables (2 and 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 clearly shown by data of Tables (3 & 6), a similar correlation of the relative growth rate with GR was determined for male nymphs. Also, GR of both sexes was generally promoted as both AR and RMR increased, with few exceptions.

4. Discussion

Several metabolic parameters had been suggested for measuring the growth rate and development of the consumer and usually used to determine the food utilization (Scriber and Slansky, 1981; Slansky and Scriber, 1985). 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) (Senthil-Nathan *et al*., 2005; Slansky, 1993; Waldbauer, 1968). Food metabolic efficiencies vary widely with the insect species, age (both within and between instars) and sex as well as with different environmental factors.

4.1 Food deterrence property of chromafenozide against S. gregaria nymphs

Few researchers recorded antifeedant activity or deterrence index of insecticides, insect growth regulators (IGRs) or other compounds against the target insect pests. For examples, osthole and pregnenolone showed significant antifeedant activity against larvae of *Spodoptera litura* (Kalpana, 2005). Among seventeen monoterpenoids assessed against *Pieris* *brassicae* 4th instar larvae, the strongest deterrent effect was exhibited by α-phellandrene- and β-ionone (Kordan and Gabryś, 2013). For more examples, see Rani and Sanjayan (2014) for the *Atractomorpha crenulata* 4th instar nymphs; Barrania (2013) and Rashwan (2013) for *Spodoptera littoralis* 4th instar larvae. On comparison with those reported results, chromafenozide unexceptionally exhibited contradictory effects on the feeding of the desert locust *Schistocerca gregaria* nymphs, in the current investigation, depending on its dose since it acted as a weak antifeedant against female nymphs only at the lowest dose (10 µg/nymph) but acted as phagostimulant against them at the higher two doses (100 and 150 µg/nymph). Moreover, chromafenozide exhibited a phagostimulant activity against the male nymphs, regardless the dose level. To understand the weak antifeedant activity of chromafenozide in the present study, it may stimulate specific 'deterrent' cells in chemoreceptors and also block the firing of 'sugar' receptor cells, which normally stimulate feeding (Blaney *et al*., 1990; Simmonds *et al*., 1990). This feeding inhibition might lead to starvation and death of the insect by feeding deterrence alone (Koul and Wahab, 2004). On the other hand, the phagostimulatory action of the present IGR cannot be explicated right now.

4.2 Food consumption by S. gregaria nymphs as affected by chromafenozide

Depending on the reported results, food consumption had been significantly reduced in several insect species by various insecticides or IGRs and IGR-related compounds. For examples, a considerable reduction in the food consumption was determined for *Leptinotarsa decemlineata* larvae by Flucycloxuron (Szczepanik, 1998), for 5-day-old adults of *S. gregaria* by fenitrothion (Ouali-N'goran *et al*., 2003) and for *Callosobruchus muculatus* larvae by Cyromazine (Al-Mekhlafi *et al*., 2012). With regard to *S. littoralis*, feeding of larvae on castor bean leaves treated with Mancozeb, bromoxynil and profenofos (Marzouk *et al*., 2012), Pyriban (Chlorpyrifos) (Ebeid and Gesraha, 2012), chlorantraniliprole, thiamethoxam and novaluron (Barrania, 2013), rynaxypyr and indoxacarb (Rashwan, 2013), Flufenoxuron and triflumuron (El-Naggar, 2013), chlorfenapyr (Ebeid *et al*., 2015) or Diazinon and flufenoxuron (El-Helaly and El-bendary, 2015) resulted in significantly reduced food consumption of larvae. In the current study, results of food consumption of last instar nymphs of *S. gregaria* disagreed with those reported results because Chromafenozide exhibited a general promoting action and thus food consumption generally increased. In the present investigation, also, treated nymphs of both sexes attained slightly or remarkably increasing relative weight gain and discharged more fecal pellets. The increasing food consumption could indicate the action of chromafenozide as a digestive attractant, as previously suggested by Piechowicz *et al*. (2012) for deltamethrin and pyriproxy-fen against the adults of Spanish slug *Arion lusitanicus*. On the other hand, an exceptional case of 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 might be acceptable since Masih and Vaishya (2014) reported that IGR disturbs the process of chitin synthesis or/and deposition during ecdysis as well as leads to the insect failure to feed, due to displacement of mandibles and labrum or blockage of the gut.

4.3 Food digestion and absorption capacity of S. gregaria nymphs as affected by chromafenozide

Another important nutritional parameter is AD, which expresses the digestion and absorption capacity of the insect. AD estimates the percentage of ingested food that is digested and assimilated (Slansky and Scriber, 1985). In the present study, Chromafenozide-treated female nymphs of *S. gregaria* achieved slightly increasing AD, in a dose-dependent course. Chromafenozide exhibited a diverse effect on AD of male nymphs, depending on the concentration. The male nymphs achieved a slightly increased AD at the lowest dose but a slightly decreased AD at the medium dose and un-affected AD at the highest dose. The present results of females and partially of males were, to some extent, in agreement with those reported results of remarkably increased AD (Abou El- Ghar *et al*., 1996; Bream *et al*., 1999; Garside *et al*., 2000). Also, after topical application of different doses of Hydroprene onto the last (6th) instar larvae of *Spodoptera mauritia*, AD increased (Sindhu and Nair, 2004). AD of 4th instar larvae of *S. littoralis* was enhanced by chlorfenapyr, at 0.25% concentration (Ebeid *et al*., 2015). On the contrary, the present results of the 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. Fore examples, pronouncedly reduced AD was recorded for *S. gregaria* nymphs after treatment with fenoxycarb (Ismail, 1995) and for *S. littoralis* larvae after treatment with teflubenzuron (Abdel-Aal and Abdel-Khalek, 2006), Sumialfa (El-Malla and Radwan, 2008) or rynaxypyr and indoxacarb (Rashwan, 2013). However, the present results had shown an exceptional case of un-affected AD of male nymphs (at the highest dose of Chromafenozide). This result 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) and the 4th instar larvae of *S. littoralis* after feeding on food treated with LC_{50} of flufenoxuron or Triflumuron (El-Naggar, 2013). The prevalent enhancing action of Chromafenozide on AD of *S. gregaria* nymphs, in the current study, could 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). 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 exceptional result of inhibited AD of male nymphs, in the present study, should not be neglected because inhibition or reduction of AD might be due to the toxicity of chromafenozide 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) and the food absorption capacity might be impaired (Abu ElEla and ElSayed, 2015; Baghban *et al*., 2014).

4.4 Influenced food conversion efficiencies of S. gregaria nymphs by chromafenozide

In insects, 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. In the present study, ECIs of both female and male nymphs of *S. gregaria* had been enhanced by Chromafenozide. Similarly, ECD of female nymphs had been enhanced. These results were, to some extent, in agreement with reported results of enhanced ECI in few insects, since significantly increasing ECI was recorded in the last instar larvae of *S. mauritia* after topical application with hydroprene (Sindhu and Nair, 2004) and in the last instar larvae of *S. littoralis* after treatment with Cadmium (Abu ElEla and ElSayed, 2015). In contrast, chromafenozide exhibited an inhibitory effect on ECD in the male nymphs only, in the current work. However, ECI and ECD of various insect species had been considerably or slightly inhibited by different insecticides and IGRs, such as fenarimol (Farag, I991), tebufenozide (Bream *et al*., 1999), Sumialfa (El-Malla and Radwan, 2008), flufenoxuron and triflumuron (El-Naggar, 2013) or rynaxypyr and indoxacarb (Rashwan, 2013) against *S. littoralis* larvae. Also, treatment of *S. littoralis* larvae with LC_{50} of Diazinon and flufenoxuron (El-Helaly and El-bendary, 2015) or the low concentration of chlorfenapyr (Ebeid *et al*., 2015) resulted in remarkably inhibited ECI and ECD. In the present study, enhanced ECI and ECD of *S. gregaria* nymphs, after treatment with Chromafenozide, might be understood because the insect requires a lot of energy to deal with the used chemical toxicity, as suggested by Emre *et al*. (2013) for *Galleria mellonella*, Baghban *et al*. (2014) for *H. armigera* and Abu ElEla and ElSayed (2015) for *S. littoralis*. On the other hand, the inhibited ECD might indicate that the ingested Chromafenozide exhibited some chronic toxicity against the insect (Wheeler and Isman, 2001). In this respect, El-Shazly (1993) 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 Influenced food assimilation in S. gregaria nymphs by chromafenozide

From the metabolic view of point, additional nutritional parameters had been reported, *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). In the current study, AR of both female and male nymphs of *S. gregaria* increased after treatment with Chromafenozide, especially with the higher two doses, to highly assimilate the absorbed food. RMR was promoted, especially at the higher doses. A positive correlation of AR and RMR to RCR was easily detected for *S. gregaria* nymphs. These results were 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), *Manduca sexta* (Dahlman, 1977), *S. litura* (Sundaramurthy, 1977), *S. littoralis* (Bream *et al*., 1999) and *S. gregaria* (Ismail, 1995). These differences might be due to the difference of IGRs, method of treatment and the response of the insect.

4.6 Growth in relation to the nutritional efficiencies of S. gregaria nymphs as affected by chromafenozide

As reported for many insects, relative weight gain (RWG) or/and growth rate (GR) had been reduced by several insecticides or IGRs and IGR-related compounds. As for examples, GR of *S. littoralis* 4th instar larvae was significantly reduced after treatment with some IGRs (El-Basyouni and Sharaf, 2002), or chlorantraniliprole, thiamethoxam and Novaluron (Barrania, 2013), or flufenoxuron and Triflumuron (El-Naggar, 2013), or rynaxypyr and indoxacarb (Rashwan, 2013), or Diazinon and flufenoxuron (El-Helaly and El-bendary, 2015). Also, Chlorpyrifos showed stronger growth inhibitory action than deltamethrin in *A. crenulata* (Rani and Sanjayan, 2014). In contrast to these reported results of inhibited GR, 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. However, our results were, to a great extent, in agreement with the enhancement of larval growth by IGRs as reported in some insects since topical treatment of the last instar larvae of *S. mauritia* with different doses of Hydroprene resulted in an increase of GR (Sindhu and Nair, 2004) while Novaluron exhibited a reversible effect on the larval RWG and GR of *S. littoralis*, depending on the concentration (Ghoneim *et al*., 2015). The interpretation of inhibited growth of various insects after treatment with IGRs or IGR-related compounds had been available in the literature (Martinez and van Emden, 1999; Marie *et al*., 2009; Mehrkhou, 2013; Abu ElEla and ElSayed, 2015; Giongo *et al*., 2015). Unfortunately, an acceptable interpretation of the enhanced growth of *S. gregaria* nymphs, as recorded in the present study after treatment with Chromafenozide, could not be provided right now.

4.7 Frass production by S. gregaria as influenced by chromafenozide

It is important to point out that the feeding is necessary for the stimulation of digestive enzyme activities in insects (Smirle *et al*., 1996) and may have interfered with the enzyme-substrate complex thus affecting the peristaltic movement of the alimentary canal (Broadway, and Duffey, 1988). Some IGRs prohibited the fecal production by insects. For examples, the *S. littoralis* larvae produced remarkably reduced frass amount after treatment with fenarimol or naurimol (Farag, 1991), tebufenozide (Bream *et al*., 1999) or Lufenuron (Adel, 2012). Also, reduction of frass production was recorded for *S. gregaria* after treatment with fenoxycarb (Ismail, 1995) and for *S. mauritia* after treatment with diflubenzuron (Jagannadh and Nair, 1997). The present results disagreed with those reported results because the fecal production by chromafenozide-treated nymphs of *S. gregaria* increased and had been proportional to their increased RCR the enhanced AD. We are unable right now to provide a conceivable explanation to the increasing fecal production and the matter is still obscure. However, the increasing fecal production might indicate higher capacity of *S. gregaria* nymphs to digest, absorb and assimilate the ingested food as a response to 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.

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