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Differential Effects of Some Insect Growth Regulators on the Reproductive Potential of Lepidopteran Pest, *Spodoptera littoralis*

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Abstract

Laboratory experiments were conducted to evaluate the lethal and sublethal effects of novaluron, methoxyfenozide and chromafenozide on the fecundity and fertility of *Spodoptera littoralis* adults when applied on the newly molted 6th instar larvae. The effect of least concentration (LC_{10}) of tested IGRs on testes weight (mg), eupyrene and apyrene sperm numbers of treated male (48h-old) were also carried out. Different abnormalities of adults, deformed ovaries and testes of the treated *S. littoralis* females and males were clarified. There was no significant difference between the toxicity of the three IGR compounds with LC_{50} values 9.6, 7.6 and 11.8 mg L^{-1}, respectively, after 96 hrs post-treatment. Methoxyfenozide at LC_{50} value caused highly significant decrease in the average number of eggs laid per female (fecundity) and the percentages of hatched eggs (fertility) followed by novaluron and chromafenozide compared with LC_{25} and LC_{10} values. The hatchability percentages were 2.6, 7.8 and 15.2 % after the mating of treated female (TF) × treated male (TM), treated female (TF) × untreated male (UTM) and untreated female (UTF) × treated male (TM), respectively, for the treatment with LC_{50} value of methoxyfenozide. While, the hatchability percentages were 9.0, 17.4 and 26.8 % for LC_{50} value of novaluron and 45.8, 50.5 and 58.3 % for LC_{50} value of chromafenozide after the same previous mating possibilities, respectively, compared to 97.3 % in the control. The LC_{10} values of novaluron, methoxyfenozide and chromafenozide appeared to be dramatic when reducing the average number of eupyrene sperm to be 13074 (61.2%), 9335.67 (72.3%) and 19017.08 (43.55%), respectively, when compared to 33691.81 eupyrene sperm in the control. Furthermore, the LC_{10} of three tested IGRs decreased the average number of apyrene sperm by 1089257 (13.7%), 1005230.80 (20.37%), 1154880.35 (8.5%), respectively, when compared to 1262480.7 apyrene sperm in the control. Finally, the obtained results emphasized that novaluron, methoxyfenozide and chromafenozide are promising insecticides and suitable for IPM programs directed against lepidopteran pests.

Keywords: Lepidoptera, IGRs, ecdysone agonist, chitin synthesis inhibitor, reproduction.
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1. Introduction

The cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is one of the most harmful polyphagous insect pests throughout the world, causing great losses and threat not only for cotton plants but also for several cultivated crops, vegetables, ornamentals and orchard trees [1].

As a result of continuous and unwise uses of insecticides, insects began to develop high levels of resistance [2, 3]. In addition, the intensive use of broad-spectrum insecticides caused serious toxicological problems to humans and the environment [4]. For these reasons, it has become necessary to look for alternative means of pest control which may minimize the insecticides hazards and delay the resistance development in *S. littoralis* [5, 6].

During the last few decades, using the insect growth regulators (IGRs) is considered as one of the possible alternative ways for controlling *S. littoralis* [7]. In contrast to the classical insecticides, IGRs are not directly toxic, but act selectively on the development, metamorphosis or reproduction of the target insect species [8, 9].

The growth regulatory effects of IGRs are mostly concerned with its interference in the neuroendocrine system of the insects [10]. The main hormones involved in growth regulation in insects are ecdysone, 20-hydroxy-ecdysone (molting hormones) and juvenile hormone (JH). The compound 20-Hydroxyedysone (20 HE) is one of the most active insect ecdysteroid hormones, acting at every stage of the insect's growth to regulate molting and metamorphosis [11]. Depending on the mode of action, IGRs has been

grouped in chitin synthesis inhibitors (CSIs) and substances that interfere with the action of insect hormones (i.e. juvenile hormone analogues, and ecdysteriods) [12].

CSIs compounds are disrupting the moulting process of insect larvae by inhibiting chitin deposition in their cuticles during growth and development. This inhibition induces morphological disruption resulting in ecdysis failure, blackening, ruptured integuments and fluid loss, depending on the species [13]. Novaluron is a CSI benzoylphenylurea insecticide with good activity against several insects and low mammalian toxicity [14-16].

Methoxyfenozide and chromafenozide are belonging to a novel class of IGRs, the molting accelerating compounds or non-steroidal ecdysteroid agonists. These compounds mimic the mode of action of the natural insect molting hormones by true binding on the ecdysteroid receptors of the epidermal cells and inducing precocious molting [17]. They act more slowly than neurotoxin insecticides because they disrupt the hormonal system or the physiological development of insects rather than kill through direct toxic action [18]. The high effectiveness of IGRs on Lepidoptera reproduction has been widely recognized by researchers [19-23]. The effects of IGR compounds on reproduction can be grouped into many categories as reproductive behavior, oviposition, eggs hatchability and adult sterilization [24]. It is known that, ecdysteroids have essential functions in controlling the processes involved in insect reproduction, i.e., vitellogenesis, ovulation of matured eggs and spermatocyte growth [25].

Therefore, the present study is proposed to evaluate the lethal and sublethal effects of novaluron, methoxyfenozide and chromafenozide on certain reproductive parameters of *S. littoralis* adults when applied on the newly molted 6th instar larvae, because sexual maturation in this Lepidoptera species was implemented during pupae development.

2. Materials and Methods

a. Insect rearing:

A laboratory strain of *S. littoralis* was reared on a semi-artificial diet [26] at 25 ± 2ºC, 16 h light: 8 h dark photoperiod and 65 ± 5% relative humidity. Newly molted 6th instar larvae were used in these experiments.

b. Tested insecticides:

The selected insecticides common names, trade names, percentage of active ingredients, formulation types, manufacturer and IRAC mode of action are listed in Table (1).

Table (1): Details of the IGRs used against *S. littoralis* larvae.

Common name	Trade name	Manufacturer	IRAC MOA
Novaluron	Equo® 10% EC	Isagro Co., Italy.	Chitin biosynthesis inhibitors
Methoxyfenozide	Runner® 24% SC	Dow AgroSciences Co., England.	Ecdysone receptor agonists
Chromafenozide	Virto® 5% SC	Nippon kayaku Co., Japan.	Ecdysone receptor agonists

c. Laboratory bioassay test:

Six concentrations of each insecticide were prepared in distilled water (5, 10, 15, 20, 30 and 50 mg L^{-1}). About 2 mL of each concentration was added to 40 g of freshly prepared diet. This amount of the treated diet was divided into four replicates. Each one was poured into a Petri dish (12 cm diameter). Ten healthy larvae were transferred to the surface of the diet on each Petri dish. Similar numbers of larvae were transferred to untreated diet as a control treatment. Mortality percentages were recorded after 96 hrs post-treatment and subjected to probit analysis according to [27].

d. Lethal and sublethal effects of tested IGRs on the fecundity and fertility of *S. littoralis* adults:

Effect of novaluron, methoxyfenozide and chromafenozide at concentrations equivalent to LC_{50}, LC_{25} and LC_{10} values on the fecundity and fertility of *S. littoralis* adults was evaluated. Each concentration was mixed with freshly prepared diet and replicated five times. Twenty newly molted 6th instar larvae of *S. littoralis* per replicate were placed in glass jar (1 liter) and left to feed on treated diet. Untreated larvae were fed on diet mixed with distilled water only. Cotton ball immersed in sugar solution and folded sheet for egg laying were provided in each replicate. Untreated and treated females and males, which emerged in the same day, were identified. The mating efficiency was evaluated by implementation of the following crosses: Treated female × Treated male (TF × TM); Treated female × Untreated male (TF × UTM); Untreated female × Treated male (UTF × TM); Untreated female × Untreated male (UTF × UTM). The average number of eggs produced per female during the first 4 d after the onset of oviposition, and the percentages of hatched eggs from those collected in the first oviposition were used to evaluate the fecundity and the fertility, respectively.

e. Dissection of the reproductive tracts of both sexes' adults:

Treated and untreated females and males' adults were dissected. The bursa copulatrix were examined for the presence of spermatophores, which were counted and classified according to their abnormalities.

f. Counts of Spermatozoa:

The counts of spermatozoa were carried out in the treated and untreated males (48h-old). Both eupyrene (nucleated) and apyrene (anucleated) spermatozoa were present within

the testes in the form of sperm bundles from emerged male adult 48 hrs old. The testes were immersed in

1 ml of Hay's solution (9.0 g/l NaCl, 0.2 g/l KCl, 0.2 g/l CaCl, 0.1 g/l NaHCO3 at pH 8.5) according to the method modified from [28]. With the aid of a fine pair of needles, the testes were punctured and macerated to release their contents. The resulted mixture was thoroughly shaken before being further diluted by adding distilled water and one drop of Giemsa stain 10 % to show the nucleus in eupyrene bundles and to make 10 ml of a spermatozoa counting fluid. The sperm in a total volume of $25/10^5$ ml, of diluted fluid and were equally divided to be counted simultaneously by a haemocytometer as explained by [29]. The chamber of haemocytometer slide must be filled to insure the correct volume. The fluid not overloads to prevent the running down into the moats on either side. It was also necessary to prevent any air bubbles under the cover glass. Spermatozoa should be settled for 3 minutes before counting. Counting squares are arranged in groups of 16 with each group bounded by double lines. To obtain accurate count, five blocks each containing 16 of the smaller squares, each of smaller square in the chamber has dimensions of 0.05 by 0.1 mm, the total volume was $25/10^5$ ml, that was, 1/4000 of 1 ml. Therefore, the average number of spermatozoa per square could be multiplied by 4.000 to obtain the number of spermatozoa per cubic centimeter or milliliter [30]. The sperms may be so numerous that accurate counts cannot be made. So, the suspension had to dilute. Usually a dilution of 1 part sperm suspension to rather 9 parts water (1:9) would be sufficient to allow accurate counts. In this case, a dilution factor of l0 had to be included into the counting equation.

g. Statistical analysis:

Statistical analysis was fulfilled using (ANOVA) one-way F-test and calculated the LSD test statistically significant at $p \leq 0.05$ according to [31].

3. Results

3.1. Toxicity of the tested IGRs against the newly molted 6th instar larvae of *S. littoralis* after 96 hrs post-treatment:

Data presented in (Table 2) demonstrated the LC_{50}, LC_{25} and LC_{10} values, their confidence limits and slope ± SE for novaluron, methoxyfenozide and chromafenozide against the newly molted 6th instar larvae of *S. littoralis*. Results showed that, there was no significant difference between the toxicity of the three IGR compounds with LC_{50} values 9.6, 7.6 and 11.8 mg L^{-1}, respectively, after 96 hrs post-treatment. Also, LC_{25} values were 3.8, 2.6 and 5.3 mg L^{-1} and LC_{10} values were 1.3, 0.96 and 2.4 mg L^{-1} for novaluron, methoxyfenozide and chromafenozide, respectively (Table 2).

Table (2): Toxicity of the tested IGRs against the newly molted 6th instar larvae of *S. littoralis* after 96 hrs. post-treatment.

Insecticide	LC_{50} (mg L^{-1}) (95% CL)	LC_{25} (mg L^{-1}) (95% CL)	LC_{10} (mg L^{-1}) (95% CL)	Slope ± SE*
Novaluron	9.6 6.8-13.0	3.8 2.6-5.8	1.3 1.1-1.7	1.54 ± 0.29
Methoxyfenozide	7.6 5.8-9.7	2.6 1.6-3.8	0.96 0.82-1.2	1.59 ± 0.28
Chromafenozide	11.8 9.4-15.3	5.3 3.6-7.4	2.4 1.6-3.8	1.95 ± 0.31

*SE means standard error

3.2. Lethal and sublethal effects of tested IGRs on the fecundity and fertility of *S. littoralis* adults:

The effect of novaluron, methoxyfenozide and chromafenozide at concentrations equivalent to LC_{50}, LC_{25} and LC_{10} on the fecundity and fertility of *S. littoralis* adults when applied on the newly molted 6th instar larvae was evaluated and the results were shown in Tables (3, 4 and 5). Mating possibilities between males and females were carried out. Normal and deformed ovaries of *S. littoralis* females after treatment with LC_{10} values of novaluron and methoxyfenozide were clarified in photo (1). During the observation period, the three tested IGRs significantly suppressed the average number of eggs laid per female (fecundity) and the percentages of hatched eggs (fertility) compared with the control treatment. The average number of eggs laid per female was 1124.2, 1054.6 and 1132.8 eggs after mating of (TF × TM), (TF × UTM) and (UTF × TM), respectively, for the treatment with LC_{50} of novaluron. The average number of eggs laid per female for the LC_{50} of methoxyfenozide after the same previous mating possibilities was 1725.2, 877.3 and 1023.6 eggs, respectively. While, the average number of eggs laid per female for the LC_{50} of chromafenozide was 1203.5, 1652.5 and 1278.2 eggs, respectively, compared to 1807.3 eggs in the control (Table 3). The hatchability percentages were 9.0, 17.4 and 26.8 % produced from the mating after treatment with LC_{50} of novaluron followed by 2.6, 7.8 and 15.2% after treatment with LC_{50} of methoxyfenozide. Whereas, the hatchability percentages were 45.8, 50.5 and 58.3% after treatment with LC_{50} of chromafenozide, respectively, compared to 97.3 % in the control (Table 3).

Table (3): Effect of LC_{50} values of tested IGRs on the fecundity and fertility of *S. littoralis* adults when applied on the newly molted 6th instar larvae.

Treatments	Conc. (mg L^{-1})	Mating possibilities		Fecundity (Average no. eggs laid /female)	Fertility (% hatched eggs)
Control	-	UTF	UTM	1807.3[a]	97.3[a]
Novaluron	9.6	TF	TM	1124.2[e]	9.0[f]
		TF	UTM	1054.6[f]	17.4[e]
		UTF	TM	1132.8[e]	26.8[d]
Methoxyfenozide	7.6	TF	TM	1725.2[b]	2.6[g]
		TF	UTM	877.3[g]	7.8[f]
		UTF	TM	1023.6[f]	15.2[e]
Chromafenozide	11.8	TF	TM	1203.5[d]	45.8[c]
		TF	UTM	1652.5[c]	50.5[c]
		UTF	TM	1278.2[d]	58.3[b]

UTF: Untreated female; UTM: Untreated male; TF: Treated female; TM: Treated male. Within the same column, data followed by the same letter are not significantly different at $P = 0.05$.

Data in table (4) showed the effect of LC_{25} values of novaluron, methoxyfenozide and chromafenozide on the fecundity and fertility of *S. littoralis* adults when applied on the 6th instar larvae. The average number of eggs laid per female was 225.8, 256.4 and 326.3 eggs after mating of (TF × TM), (TF × UTM) and (UTF × TM), respectively, for the LC_{25} of novaluron. The average number of eggs laid per female for the LC_{25} of methoxyfenozide after the same previous mating possibilities was 162.5, 190.2 and 244.6 eggs, respectively. While, the average number of eggs laid per female for the LC_{25} of chromafenozide was 648.3, 896.5 and 1279.2 eggs, respectively, compared to 1682.7 eggs in the control (Table 4). The hatchability percentages were 17.9, 43.2 and 62.6 % for the eggs produced from the mating after treatment with LC_{25} of novaluron. The hatchability percentages after treatment with LC_{25} of methoxyfenozide were 8.2, 27.5 and 44.3 %. The hatchability percentages after treatment with LC_{25} of chromafenozide were 33.4, 57.8 and 78.5 % compared to 92.6 % in the control (Table 4).

Data in table (5) showed the average number of eggs laid per female was 256.2, 285.5 and 446.3 eggs after mating of (TF × TM), (TF × UTM) and (UTF × TM), respectively, for the LC_{10} of novaluron. The average number of eggs laid per female for the LC_{10} of methoxyfenozide after the same previous mating possibilities was 233.2, 278.4 and 383.7 eggs, respectively. While, the average number of eggs laid per female for the LC_{10} of chromafenozide was 986.3, 1045.2 and 1342.8 eggs, respectively, compared to 1734.6 eggs in the control (Table 5). The hatchability percentages were 21.6, 28.3 and 36.2 % for the eggs produced from the mating after treatment with LC_{10} of novaluron. The hatchability percentages after treatment with LC_{10} of methoxyfenozide were 26.4, 43.5 and 54.3 %. The hatchability percentages after treatment with LC_{10} of chromafenozide were 45.2, 63.8 and 84.5 % compared to 95.4 % in the control (Table 5).

Table (4): Effect of LC_{25} values of tested IGRs on the fecundity and fertility of *S. littoralis* adults when applied on the newly molted 6th instar larvae.

Treatments	Conc. (mg L^{-1})	Mating possibilities		Fecundity (Average no. eggs laid /female)	Fertility (% hatched eggs)
Control	-	UTF	UTM	1682.7[a]	92.6[a]
Novaluron	3.8	TF	TM	225.8[f]	17.9[h]
		TF	UTM	256.4[ef]	43.2[e]
		UTF	TM	326.3[e]	62.6[c]
Methoxyfenozide	2.6	TF	TM	162.5[f]	8.2[i]
		TF	UTM	190.2[f]	27.5[g]
		UTF	TM	244.6[ef]	44.3[e]
Chromafenozide	5.3	TF	TM	648.3[d]	33.4[f]
		TF	UTM	896.5[c]	57.8[d]
		UTF	TM	1279.2[b]	78.5[b]

UTF: Untreated female; UTM: Untreated male; TF: Treated female; TM: Treated male. Within the same column, data followed by the same letter are not significantly different at $P = 0.05$.

Table (5): Effect of LC_{10} values of tested IGRs on the fecundity and fertility of *S. littoralis* adults when applied on the newly molted 6th instar larvae.

Treatments	Conc. (mg L^{-1})	Mating possibilities		Fecundity (Average no. eggs laid /female)	Fertility (% hatched eggs)
Control	-	UTF	UTM	1734.6^{a}	95.4^{a}
Novaluron	1.3	TF	TM	256.2^{h}	21.6^{h}
		TF	UTM	285.5^{g}	28.3^{g}
		UTF	TM	446.3^{e}	36.2^{f}
Methoxyfenozide	0.96	TF	TM	233.2^{i}	26.4^{g}
		TF	UTM	278.4^{g}	43.5^{e}
		UTF	TM	383.7^{f}	54.3^{d}
Chromafenozide	2.4	TF	TM	986.3^{d}	45.2^{e}
		TF	UTM	1045.2^{c}	63.8^{c}
		UTF	TM	1342.8^{b}	84.5^{b}

UTF: Untreated female; UTM: Untreated male; TF: Treated female; TM: Treated male. Within the same column, data followed by the same letter are not significantly different at $P = 0.05$.

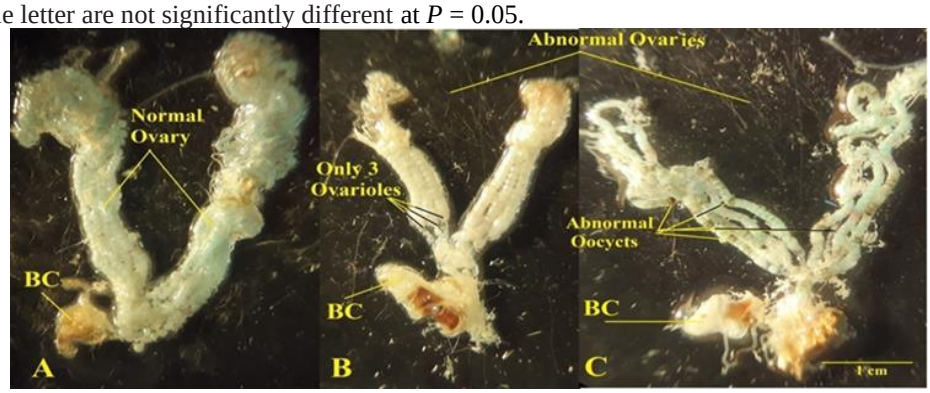

Photo (1): A: Normal ovary of female resulted from untreated larva, B and C: deformed ovaries of the *S. littoralis* females when the 6th instar larvae were treated with LC_{10} values of novaluron and methoxyfenozide. BC; Bursa Copulatrix

3.3. Counts of apyrene and eupyrene sperm:

Testes weight, eupyrene and apyrene sperms numbers of the treated male (48h-old) resulting from treatment of the 6th instar larvae of *S. littoralis* were calculated in another experiment. Furthermore, testes of the untreated and treated *S. littoralis* males with LC_{10} value of methoxyfenozide were clarified in photo (2). With the aid of the information obtained from Table (6), the least concentration (LC_{10}) of novaluron, methoxyfenozide and chromafenozide were selected to conduct this experiment especially when a considerable number of resulted adults were available. Ten males (48h-old) resulted from each treatment. Averages of the fused testes weight in freshly emerged adult insects were 3.56, 2.1, 3.03 and 3.87 mg for the LC_{10} of novaluron, methoxyfenozide, chromafenozide and the control, respectively. The testes weight of treated male of the LC_{10} of methoxyfenozide approximately recorded half the weight of the testes in the control (45.74%). It was noticed that the LC_{10} of chromafenozide reduced the tested weight by 21.7% (3.03 mg) while the LC_{10} of novaluron reduced the testes weight by 8% (3.56 mg) when compared with control (3.87 mg). The counts of eupyrene sperm were 13074, 9335.67, 19017.08 and 33691.81 for the LC_{10} of novaluron, methoxyfenozide, chromafenozide and the control, respectively. Whereas, the counts of apyrene sperm recorded 1089257, 1005230.80, 1154880.35 and 1262480.7 for the previous insecticides and the control, respectively (Table 6). It was cleared that the LC_{10} of novaluron, methoxyfenozide and chromafenozide appeared to be dramatic when reducing the average number of eupyrene sperm to be 13074 (61.2%), 9335.67 (72.3%) and 19017.08 (43.55%), respectively, when compared to 33691.81 eupyrene sperm in the control. Furthermore, the LC_{10} of novaluron, methoxyfenozide and chromafenozide decreased the average number of apyrene sperms by 1089257 (13.7%), 1005230.80 (20.37%), 1154880.35 (8.5%), respectively, when compared to 1262480.7 apyrene sperms in the control (Table 6).

Table (6): Effect of LC_{10} values of tested IGRs on the testes weight, eupyrene and apyrene sperms numbers of treated male (48h-old) of *S. littoralis*:

Treatments	Testes weight (mg) (mean ± SD)	Eupyrene sperm (no.) (mean ± SD)	Reduction percentages of eupyrene sperm (%)	Apyrene sperm (no.) (mean ± SD)	Reduction percentages of apyrene sperm (%)
Control	3.87 ± 1.85^{a}	33691.81 ± 15385.42^{a}	0	1262480.7 ± 118235.24^{a}	0
Novaluron	3.56 ± 1.55^{a}	13074.00 ± 1804.52^{bc}	61.2	1089257.00 ± 47607.08^{ab}	13.7
Methoxyfenozide	2.1 ± 1.06^{b}	9335.67 ± 752.45^{c}	72.3	1005230.80 ± 73771. 47^{b}	20.37
Chromafenozide	3.03 ± 2.12^{ab}	19017.08 ± 16496.14^{b}	43.55	1154880.35 ± 122514.17^{a}	8.5

No. of male used from each treatment = 10. Within the same column, data followed by the same letter are not significantly different at $P = 0.05$.

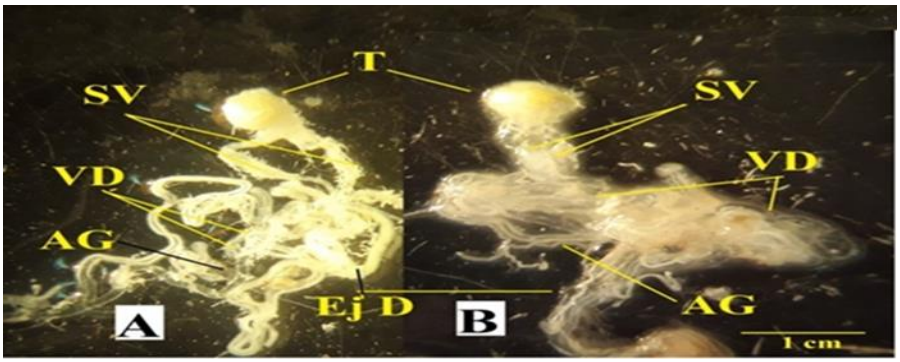

Photo (2): Male reproductive tracts (48-old) (A): Testes of the treated male with LC_{10} value of methoxyfenozide (B): Testes of untreated male (control). SV: Seminal Vesicle; VD: Vas Deferens; AG: Accessory Glands; Ej D: Ejaculatory Ducts

3.4. Effect of tested IGRs on the adult's formation:

S. littoralis adults resulting after treatment of the 6th instar larvae indicated different types of pupal-adult intermediates or adult deformities due to partial incomplete molting of the emerged moths. Abnormal adults, showing shortened crumpled fore and hind wings, shortened wings not totally covering the abdomen of the deformed moths due to the partially or incompletely stretched and unfolded wings; besides the abnormal thoracic legs and mouth parts Abnormal adults were failed to emerge from pupa stage, different abnormalities of adults formed, males and females adults were failed to separate after mating when the 6th instar larvae of *S. littoralis* were treated with LC_{10} values of novaluron and methoxyfenozide were clarified in photos 3, 4 and 5.

Photo (3): Malformation adults of *S. littoralis* were failed to emerge from pupa stage when the 6th instar larvae were treated with LC_{10} values of novaluron and methoxyfenozide.

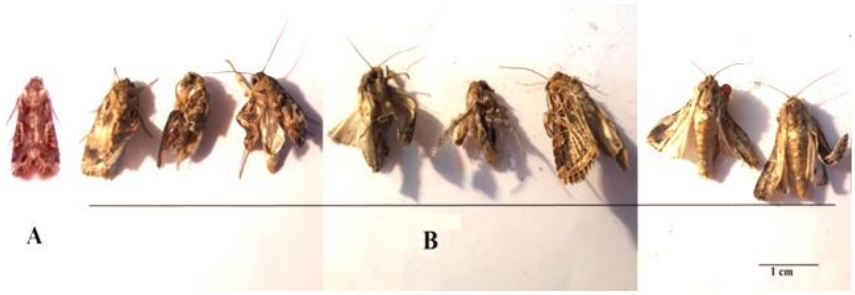

Photo (4): A: Normal adult; B: Abnormal adults formed when the 6th instar larvae of *S. littoralis* were treated with LC_{10} values of novaluron and methoxyfenozide.

Photo **(5):** The males and females' adults of *S. littoralis* were failed to separate after mating, produced from treated larvae by LC_{10} values of novaluron and methoxyfenozide.

4. Discussion

Molting and metamorphosis are two critical physiological events in the life of insects. These two events are regulated by the steroid 20-hydroxyecdysone, and the sesquiterpenoid juvenile hormone [32]. The use of IGR compounds in insect control is known as insect development inhibition, which inhibits or prevents normal metamorphosis of immature stages to the adult's stage.

In the present study, the reduction in fecundity and fertility of *S. littoralis* adults after treatment with novaluron, methoxyfenozide and chromafenozide was recorded. These results agree with those obtained by [33] where they reported that treatment of penultimate or last instar larvae of *S. littoralis* with novaluron resulted in drastically reduced fecundity in a dose-dependent course. A reducing action of novaluron was exerted also on fertility after treatment of larvae with different concentration levels, regardless the time of treatment. Similarly, the methoxyfenozide reduced in a dose-dependent manner the fecundity and fertility of *S. littoralis* adults [34, 35]. An explanation about the chemosterilizing effect on female Lepidoptera by interfering with ovulation and oviposition have suggested by [36, 37]. Their studies indicated that the reduction in egg laying is a result of inhibition of new oöcyte formation and induction of oöcyte desorption. In addition, the halofenozide caused degeneration of the ovaries, reduced oöcyte growth and inhibited vitellogenin synthesis in the beetle *Leptinotarsa decemlineata* [38]. Also, the ecdysteroids play a role in the regulation of oogenesis of lepidopteran. It can be expected that ecdysone agonists influence ovarian development after adult exclusion. It was observed in the codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae), where application of tebufenozide and methoxyfenozide to adults resulted reduction in fecundity [39]. The present data is also, in accordance with [40] when they found a decline in fertility of treated male, *Heliothis zea* when crossed with normal or treated females. A reduction in fecundity was found in matings of codling moth, *C. pomonella*, in which both sexes were treated with tebufenozide [41]. The effects of flufenoxuron on the fecundity and fertility of *S. littoralis* adults resulting from the treatment of the sixth instar larvae by topical application [42]. Adults resulted from three flufenoxuron dosages only (1, 5 and 10 *η*g/larva) were used to accomplish mating possibilities. The mating done for each dosage was: TF × TM, TF × UTM and UTF × TM. The control was expressed by UTF ×UTM. The female resulted from treated larva with the dosage of 1 *η*g/larva flufenoxuron produced 1356.8 eggs with 11.48% hatchability when mated with male resulted from treated larva, while it produced 1076 eggs with 15.71% hatchability when mated with UTM. On the other hand, the UTF produced 1887 eggs with 15.1% hatchability when mated with male resulted from treated larva. About the dosage of 5 *η*g/larva flufenoxuron, the treated female produced 906 eggs with 6.4% hatchability when mated with TM. Also, the TF laid 784.4 eggs with 8.16% hatchability when mated with UTM. The UTF laid 1288 eggs with zero% hatchability when mated with treated male. Finally, the dosage of 10 *η*g/ larva caused no hatchability (zero %) when the males produced from the treated larvae were used in mating.

During postembryonal development of males of *S. littoralis* the paired four-follicular larval testes undergo fusion and torsion, forming in the prepupal stage one gonad composed of eight testicular follicles. From the 6th larval till early pupal stage, the interior of the testicular follicles is divided into the following zones: 1) germarium with apical complex (an apical cell and two kinds of spermatogonia); 2) a zone, in which the single spermatogonia become surrounded by somatic cells, thus forming spermatogonial cysts; 3) a zone in which the spermatogonia inside the cysts undergo six incomplete mitotic divisions to form a syncytium of 64 spermatocytes (eupyrene spermatocytes with spherical nuclei or apyrene ones with polymorphic nuclei); 4) a zone, in which the spermatocytes transform into eupyrene or apyrene spermatids (256 per one cyst). In the mid-period of pupal stage two events occur: the apical cell in germarium degenerates and the eupyrene spermatogenesis ends. The apyrene spermatogenesis starts in the 6th larval instar and ends in the late pupa. In the late pupal and young imago testis, apyrene spermatozoa cysts form a compact layer under the gonadal wall, whereas the eupyrene cysts are loosely scattered in the central region of testicular follicles. In seminal follicles the apyrene spermatozoa acquire a thick coating exhibiting periodic structure [43]. The testes of the emerged adult of *Spodoptera litura* treated insects were significantly reduced in size in a dose-dependent way. The IGR, tebufenozide, reduced the testicular volume by 11.5% (0.5 μg/ larva) and 28.5% (2.0 μg/ larva) [44]. According to [45], the Lepidopteran produces two types of sperm cells, nucleated eupyrene sperm and smaller anucleated apyrene sperm which were cleared in the present results. Both types are transferred to the female during copulation via the spermatophore and both reach the site of sperm storage, the spermatheca. Apyrene sperm bundles dissociate and become motile prior to male ejaculation while eupyrene sperm remain in bundles [46-48]. Until recently, the function of apyrene sperm was unknown although several hypotheses have been postulated. The function of the apyrene spermatozoa that have been proposed are: 1- They provide nutrients for the eupyrene spermatozoa within the female genital tract [49]. 2- The DNA of their discarded nuclei is metabolized and serves as substrate for glycogen biosynthesis [50]. 3- They facilitate acquisition of motility by the eupyrene spermatozoa within the female tract [51]. 4- In polyandrous species, they play a role in the competition among the spermatozoa of the different inseminating males within the female genital tract [52]. 5- They help in transporting the eupyrene spermatozoa within the female genital duct [53]. 6- They make way for the migration of the eupyrene spermatozoa across the testicular basement membrane [54].

5. Conclusion

It is concluded that the three tested IGRs, regardless their toxic effect, they disruptively affected the reproduction of *S. littoralis*. These effects are very important because offspring can then be reduced and as a consequence, the insect population can be maintained below a level of economic loss.

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