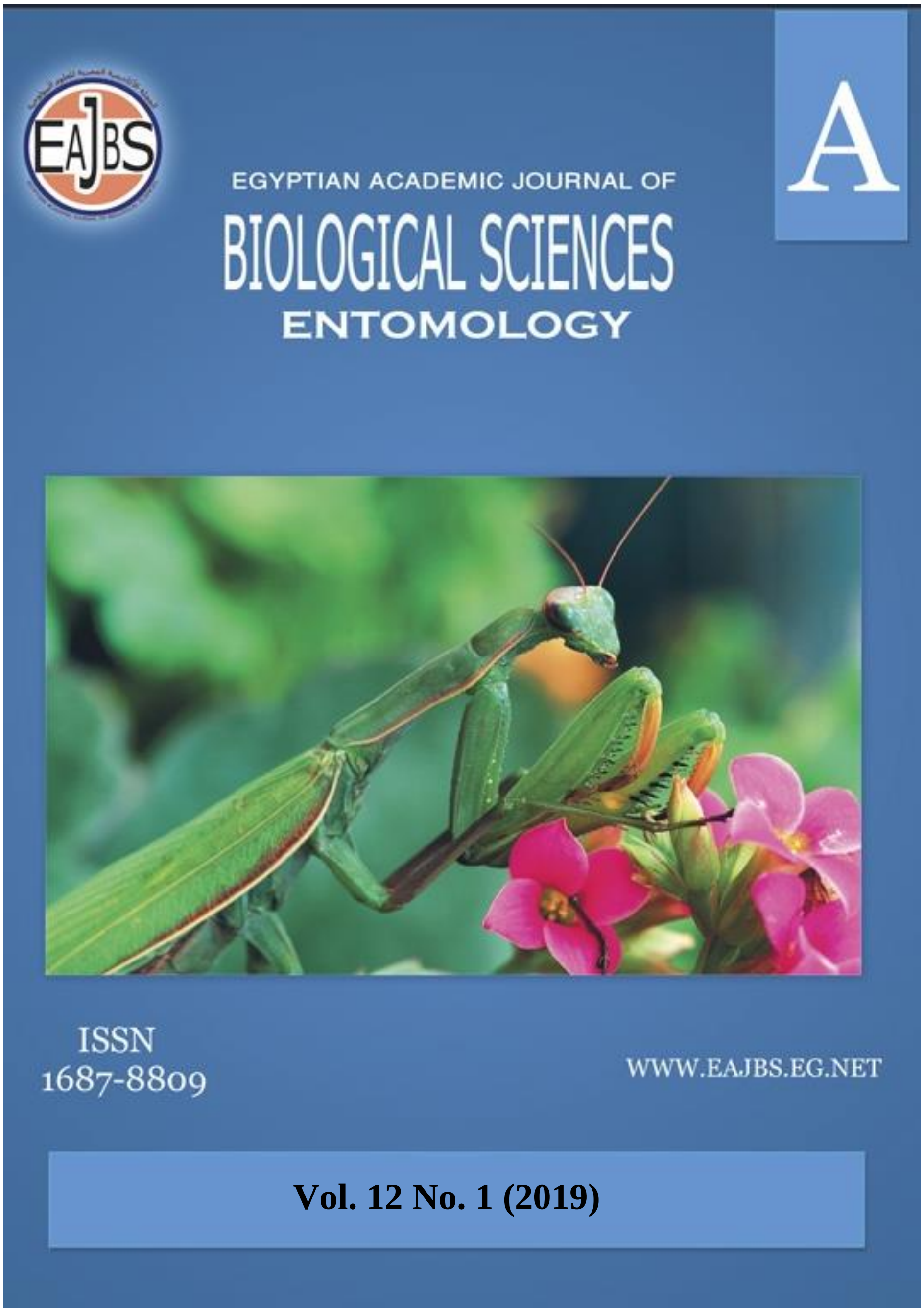

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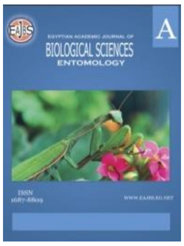

Assessment of the Inhibitory Impact of Novaluron, A Recent Insect Growth Regulator, on the Reproductive Potential of *Spodoptera littoralis* (Boisd.)(Lepidoptera: Noctuidae).

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ABSTRACT

The Egyptian cotton leafworm *Spodoptera littoralis* is one of the destructive pests of field crops in the tropical and subtropical areas of the world. The objective of the present study was to assess the impact of Novaluron on the reproductive potential of this pest. A series of concentrations (1.0, 0.1, 0.01, 0.001 & 0.0001ppm) was prepared and applied on the freshly molted penultimate instar larvae. Another series (0.1, 0.01, 0.001 & 0.0001 ppm) was prepared and applied on the newly molted last (6th) instar larvae. The results can be summarized as follows. The tested compound exhibited a predominantly inhibitory effect on the oviposition efficiency, regardless the concentration and time of larval treatment. Both fecundity and fertility had been drastically reduced, in a dose-dependent course, regardless the time of treatment. Irrespective of the concentration and time of treatment, Novaluron halted the embryonic development, since the incubation period of eggs was remarkably prolonged. In light of the present results, Novaluron acts as an antigonadotropic compound in *S. littoralis*.

INTRODUCTION

The Egyptian cotton leafworm *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) represents a destructive pest in different parts of the world (Hill, 1987). It attacks plants belonging to more than forty families of varying economic importance (Lal and Naji, 1990). As for examples, it damages the glasshouses crops and flower production in Southern Europe, various types of vegetables in North Africa and cotton in Egypt (El-Aswad *et al*., 2003; Roques *et al*., 2008). The intensive use of broad-spectrum pesticides against *S. littoralis* has led to different problems, such as the development of resistance to many pesticides making its control even more difficult (Smagghe *et al*., 1999; Miles and Lysandrou, 2002; Aydin and Gurkan, 2006). This insect received a great attention of research, much of which has been envisioned for finding new efficient measures to control (Hussain, 2012).

Over the late decades, the insect growth regulators (IGRs) have been considered as a possible alternative of the conventional insecticides for controlling this dangerous insect (Raslan, 2002). IGRs have novel modes of action which disrupt the development and some of the other physiological processes of the target pest. These compounds tend to be selective and less toxic to non-target organisms than conventional pesticides (Gurr *et al*., 1999). Depending on the mode of action, IGRs had been recently categorized in substances interfering with the functions of insect hormones and chitin synthesis inhibitors (CSIs)(Tunaz and Uygun, 2004). In insects, CSIs interfere with the chitin biosynthesis and thus prevent moulting, or produce deformed cuticle (Hammock and Quistad, 1981). CSIs had experimentally proved to act as effective inhibitors of the development of insect pests (Ishaaya *et al*., 2002, 2003; Kandil *et al*., 2012). CSIs also affect the hormonal balance in insects, thereby resulting in various physiological disturbances (Soltani *et al*., 1984).

As a relatively new benzoylphenyl urea CSI, Novaluron exhibits a remarkable activity against several insects, such as *Leptinotarsa decemlineata* (Cutler *et al*., 2007; Alyokhin *et al*., 2009), *Bemisia tabaci* and *Trialeurodes vaporariorum* (Kim *et al*., 2000) and *Helicoverpa* species (Ishaaya *et al*., 2001, 2002, 2003). According to many authors (Barazani, 2001; Ishaaya and Horowitz, 2002; Ishaaya *et al*., 2001, 2002), this CSI has probably a mild effect on the natural enemies and has no serious effect on parasitoids as well as has low mammalian toxicity. As recorded in Egypt by Malhata et al. (2014), Novaluron residues tend to dissipate with a half-life of 2.08 days and the safe use of it on tomato, and probably on other crops. The objective of the current study was to evaluate the disruptive effects of Novaluron on the most important reproductive criteria of *S. littoralis*.

MATERIALS AND METHODS

The Insect under Study:

A culture of a sensitive strain of *Spodoptera littoralis* (Lepidoptera: Noctuidae) was established at Faculty of Science, Al-Azhar University, Egypt, under controlled conditions (27±2ºC, 65±5% R.H., photoperiod 14 h L and 10 h D). This culture was originated by a sample of pupae kindly obtained from susceptible strain maintained for some generations in Plant Protection Research Institute, Doqqi, Giza, Egypt. The rearing procedure was achieved according to Ghoneim (1985) and improved according to Bakr *et al*. (2010). Every day, larvae had been provided with fresh castor bean leaves *Ricinus communis*. The emerged adults were supplemented with 10% honey solution on a cotton wick as a food source. Moths were allowed to lay eggs on *Oleander* branches, and the egg patches were collected daily and transferred into Petri dishes for another generation.

Novaluron and Larval Treatments:

The tested CSI, Novaluron (1-[3-chloro-4-(1, 1, 2-trißuoro-2-trißuoro- methoxy-ethoxy) phenyl]-3-(2,6-dißuorobenzoyl)urea) (Rimon, Chemtura Corporation, Middlebury, CT), was supplied by Sigma-Aldrich Chemicals (https://www.sigmaaldrich.com). A series of concentrations were prepared using distilled water. Freshly moulted 5th (penultimate) instar larvae were treated with the concentrations 1.0, 0.1, 0.01, 0.001 & 0.0001ppm, but the newly moulted 6th (last) instar larvae were treated with the concentrations 0.1, 0.01, 0.001 & 0.0001 ppm. The feeding larvae had been provided with fresh castor bean leaf discs after dipping in each concentration for 5 minutes. They were left to feed on the treated leaf discs for 24 hrs and then on untreated fresh leaf discs every day. Control larvae of the same age were allowed to feed on water-treated leaf discs. Ten replicates of treated and control larvae (one larva/replicate) were kept separately in glass vials. All larvae (control and treated) were carefully handled until the adult eclosion just after which all reproductive data were recorded.

Reproductive Criteria:

After 24 h of emergence, the treated adult females were kept separately in glass jars (1 L) and coupled with healthy adult males (1:2) of the same age obtained from the normal culture. Each jar was provided with sterilized cotton pieces soaked in 10% honey solution for feeding and fresh *Nerium oleander* branches as oviposition sites. During the oviposition period, egg-patches were collected daily and carefully transferred to Petri dishes to count eggs.

Oviposition rate was calculated as follows: Number of laid eggs per ♀/reproductive lifetime (in days). Eggs were counted for calculating the number of eggs per female **(**Fecundity**)**. The laid eggs were kept in Petri dishes under the same controlled conditions, as previously mentioned. Eggs were observed until hatching to calculate the incubation period (in days). The hatchability **(**Fertility**)** was usually expressed in hatching percentage of eggs. Sterility index was calculated according to Toppozada *et al.* (1966**)** as follows: Sterility Index = 100 – [(a b / A B) × 100]. Where: a: mean number of eggs laid per female in the treatment. b: percentage of hatching in the treatment. A: mean number of eggs laid per female in the controls. B: percentage of hatching in the controls.

Statistical Analysis of Data:

The present results were analyzed using the Student's *t*-distribution, and refined by Bessel correction (Moroney, 1956) for the test significance of the difference between means.

RESULTS

Effect of Novaluron on the Oviposition Efficiency of *S. littoralis*:

In the present study, penultimate (5th) or last (6th) instar larvae of *S. littoralis* were treated with Novaluron at a dose range 1.0-0.0001 ppm. Because the oviposition rate in insects can be used as an informative indicator of the oviposition efficiency, Data arranged in Table (1) clearly reveal a predominantly inhibitory effect of Novaluron on the oviposition efficiency, regardless the concentration applied on 5th instar larvae. However, the severely declined oviposition rate was determined at the higher two concentrations (24.63±1.37 and 16.14±2.04, respectively, *vs*. 192.64±4.33 of control females). A similar suppressing action of Novaluron on the oviposition rate was recorded after treatment of 6th instar larvae, regardless the concentration, as obviously demonstrated in Table (2). Depending on this table, the strongest inhibitory effect of Novaluron was exhibited at the highest concentration (108.33±4.17 *vs*. 192.64±4.33 of control adult females).

Disrupted Reproductive Capacity of *S. littoralis* by Novaluron:

In the light of data assorted in Table (1), treatment of 5th instar larvae of *S. littoralis* with Novaluron resulted in seriously inhibited fecundity, in a dose-dependent manner. With regard to the higher two concentrations, fecundity dropped (78.00±22.14 and 100.00±11.00 eggs/treated ♀, respectively, *vs*. 1733.40±57.23 eggs/control ♀). Also, strongly reducing action of Novaluron on the female fecundity was exerted after treatment of last instar larvae, in a dose-dependent course. The most reducing the action was exerted at the highest concentration (787.50±109.60 eggs/treated ♀ vs. 1733.40±57.23 eggs/ control ♀, table 2).

Data contained in Table (1) unexceptionally reveal a deteriorating action of Novaluron on fertility (egg viability) after treatment of 5th instar larvae. Although the reduction of fertility could not be detected in certain trend, the most potent reducing action of Novaluron was exerted at 1.00 and 0.01 ppm (37.23±0.00 and

44.70±4.24%, respectively, in comparison with 97.80±0.78% hatching eggs of controls). Moreover, the sterility index was found in a dose-dependent course. A similar fertility reduction was, also, determined after treatment of 6th instar larvae with Novaluron. In addition, the highest sterility index was calculated in 86.90 at the highest concentration (for detail, see table 2).

Table 1: Oviposition efficiency and reproductive potential of *S. littoralis* as affected by the treatment of newly moulted penultimate instar larvae with Novaluron.

Conc. (ppm)	Oviposition rate	Fecundity (mean eggs±SD)	Fertility (%)	Sterility Index	Incubation period (mean days±SD)
1.00	024.63±1.37 d	078.00±22.14 d	37.23±0.00 d	98.33	4.00±0.00 d
0.10	016.14±2.04 d	100.00±11.00 d	72.00±0.00 d	95.83	4.00±0.00 d
0.01	140.08±4.12 d	721.00±18.39 d	44.70±4.24 d	81.00	4.00±0.00 d
0.001	132.58±3.67 d	945.00±76.88 d	65.60±0.57 d	63.45	3.00±1.00 d
0.0001	168.57±3.25 c	1128.85±62.94 d	84.20±1.94 d	43.91	3.43±0.53 d
Control	192.64±4.33	1733.40±57.23	97.80±0.78	---	2.20±0.45

Conc.: concentration level.. Mean ± SD followed with the same letter (a): insignificantly different (P >0.01), (b): significantly different (P<0.05), (c): highly significantly different (P<0.01), (d): very highly significantly different (P<0.001).

Table 2: Oviposition efficiency and reproductive potential of *S. littoralis* as affected by the treatment of newly moulted last instar larvae with Novaluron.

Conc. (ppm)	Oviposition rate	Fecundity (mean eggs±SD)	Fertility (%)	Sterility Index	Incubation period (mean days±SD)
0.10	108.33±4.17 d	787.50±109.60 d	66.85±1.91 d	86.90	4.00±0.00 d
0.01	150.79±5.67 c	1251.67±76.54 d	75.43±17.67 d	44.38	3.67± 0.58 d
0.001	173.39±4.51 b	1378.75±124.19 d	84.88±2.18 d	30.90	3.25±0.50 d
0.0001	152.81±3.97 c	1149.17±135.63 d	82.53±1.14 d	44.13	3.33±0.52 d
Control	192.64±4.33	1733.40±57.23	97.80±0.78	---	2.20±0.45

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

Disturbed embryonic development of *S. littoralis* by Novaluron:

The incubation period of insect eggs can be used as a good indicator of the embryonic developmental rate, i.e., the shorter period usually denotes faster developmental rate and *vice versa*. After treatment of 5th instar larvae with Novaluron, data of the incubation period were distributed in Table (1). The embryonic development in *S. littoralis* had been generally subjected to a prolonged inhibitory effect of the tested compound since the incubation period was remarkably prolonged. At the highest concentration, the longest period was estimated (4.00±0.00 days, compared to 2.20±0.45 days of control eggs. Also, similar retarding action of Novaluron on the embryonic development was exerted after treatment of last instar larvae since the incubation period was significantly lengthened, regardless the concentration (see Table 2).

DISCUSSION

The insect growth regulators (IGRs)-treated larvae may develop as deformed adults who would be non-viable or with the least reproductive capacity (Williams and Amos 1974). This result may be due to sterility or reduced fecundity in the treated insects (Ghoneim *et al*., 2014). However, disruptive impacts of IGRs on the reproduction in insects can be categorized into: i) deteriorated reproductive behaviour, ii) inhibited oviposition, iii) reduced hatchability of eggs, and iv) adult sterilization (Mondal and Parween, 2000).

Inhibited Oviposition Efficiency of *S. littoralis* by Novaluron:

In insects, the oviposition efficiency can be indicated by the oviposition rate. In the current study, Novaluron exerted a predominant inhibitory action on the oviposition efficiency of *S. littoralis*, irrespective of the time of larval treatment or concentration. This result has coincided with the reported inhibition of oviposition efficiency of the pink bollworm *Pectinophora gossypiella* after treatment of newly hatched and full-grown larvae with novaluron (Hassan *et al*., 2017) as well as reported results for other insects, such as *S. littoralis* by Tebufenozide (Bakr *et al*., 2005), and flufenoxuron (Bakr *et al*., 2010); the desert locust *Schistocerca gregaria* by flufenoxuron and lufenuron (Soltani-Mazouni and Soltani, 1994) or tebufenozide (Al-Dali *et al*., 2008); the Indianmeal moth *Plodia interpunctella* by the ecdysteroid agonist RH-5849 (Smagghe and Degheele, 1994) and the cowpea weevil *Callosobruchas maculates* by cyromazine (Al-Mekhlafi *et al*., 2011). This result was also in agreement with those reported results of inhibited oviposition of the olive leaf moth *Palpita unionalis* after treatment of newly moulted last instar larvae with methoxyfenozide (Hamadah *et al*., 2017) and *P. gossypiella* after treatment of 1-day old eggs with Noviflumuron or Novaluron (Tanani and Ghoneim, 2018). In contrast, the current result disagreed with the enhanced oviposition of the field cricket *Gryllus bimaculatus* by some ecdysteroids (Behrens and Hoffmann, 1983). The suppressed oviposition, in the current study, may be understood by the inhibition of ovarian DNA synthesis or interference of the tested compound with vitellogenesis *via* certain biochemical processes. However, this IGR might exert a reverse action to those exhibited by the ecdysteroids that induce the neurosecretory cells to release a myotropic ovulation hormone (Smagghe *et al*., 1996; Parween *et al*., 2001).

Perturbation of the Reproductive Capacity of *S. littoralis* by Novaluron:

1. Reduced Fecundity:

There are many reported results of reduced fecundity of *S. littoralis* after treatment of larvae with various IGRs, such as diflubenzuron (Aref *et al*., 2010), methoxyfenozide (Pineda *et al*., 2009) and lufenuron (Abdel-Rahman *et al*., 2007; Gaaboub *et al*., 2012). Also, fecundity of other insects was reduced by many IGRs, such as the Mediterranean flour moth *Ephestia kuehniella* by tebufenozide (Khebbeb *et al*., 2008); the European grapevine moth *Lobesia botrana* (Saenz-de-Cabezon *et al*., 2005) and the tobacco cutworm *Spodoptera litura* (Shahout *et al*., 2011) by methoxyfenozide; the Colorado potato beetle *Leptinotarsa decemlineata* (Farinos *et al*., 1999) and the mealworm beetle *Tenebrio molitor* (Taibi *et al*., 2003) by the halofenozide; *S. litura* by chlorfluazuron (Perveen and Miyata, 2000), the house fly *Musca domestica* by lufenuron (Hamadah, 2003), the red cotton stainer *Dysdercus koenigii* by flufenoxuron (Khan and Qamar, 2011); *E. kuehniella* by diflubenzuron and hexaflumuron (Ashouri *et al*., 2014); the diamondback moth *Plutella xylostella* by pyriproxyfen (Mahmoudvand *et al*., 2015); the red flour beetle *Tribolium castaneum* (Gado *et al*., 2015) and the onion fly *Delia antique* (Zhou *et al*., 2016) by lufenuron; the rice moth *Corcyra cephalonica* by fenoxycarb (Begum and Qamar, 2016); *P. unionalis* by Methoxyfenozide (Hamadah *et al*., 2017) and *P. gossypiella* by Noviflumuron or Novaluron (Tanani and Ghoneim, 2018). Results of the present study were in accordance with these reported results because treatment of penultimate instar larvae of *S. littoralis* with **novaluron** resulted in drastically reduced fecundity in a dose-dependent manner. A similar suppressing action on fecundity was exerted after treatment of last instar larvae with this IGR.

In contrast, the recorded result in the current study disagreed with the failure of some IGRs to halt the fecundity of some insects, such as fenoxycarb against the honey bee *Apis mellifera* (Thompson *et al*., 2005), methoxyfenozide against the beet armyworm *Spodoptera exigua* (Christian-Lius and Pineda, 2010) and Novaluron and Diflubenzuron against the brown marmorated stink bug *Halyomorpha halys* (Kamminga *et al*., 2012). Furthermore, the feeding of larvae on leaves treated with methoxyfenozide enhanced the fecundity of *S. littoralis* (Ishaaya *et al*., 1995). These diverse effects could be attributed to the different modes of action of IGRs, different insect susceptibilities, time of treatment and some of the other factors.

The drastic reduction of fecundity in *S. littoralis*, after larval treatment with novaluron in the present study, might be due to its interference with some processes, from the development of ovarian follicle to egg maturation. However, some scenarios can be discussed in this context. First, Novaluron might induce some disorders in the developing ovarioles during the immature stages (Davey, 1993), including cell death in the germarium, oocyte resorption in the pre-vitellarium and vitellarium before oviposition (Zhou *et al*., 2016), and proliferation of follicle cells (Lucantoni *et al*., 2006; Khan *et al*., 2007). Second, Novaluron might inhibit ovarian growth during the oogenesis (Smagghe *et al*., 1996; Salem *et al*., 1997). Third, it might disturb the production and/or function of the gonadotropic hormone responsible for the synthesis of vitellogenins and regulation of vitellogenesis (Di Ilio *et al*., 1999). Also, it might disturb the ecdysone activity, the threshold of which is crucial for the normal oogenesis (Terashima *et al*., 2005). Fourth, eggs may develop normally in ovaries, but they could not be lay, because of the seriously deformed ovipositors of females or to the reduced mechanical strength (Moreno *et al*., 1994). Fifth, the prohibited fecundity of *S. littoralis*, in the current study, might be due to an inhibitory effect of the tested IGR on the synthesis of both DNA and RNA, suboptimal nutrition because of reduced feeding, altered copulation behaviour as a result of sublethal intoxication against the tested compound, or a combination of factors.

2. Inhibited Fertility:

Fertility is a principal parameter of the reproductive capacity in insects. In the current study, novaluron exhibited a potent reducing effect on the fertility of *S. littoralis*, regardless of the concentration or the time of larval treatment. To a great extent, this result was in agreement with many reported results of drastically reduced fertility of the same pest by several IGRs, such as chlorfluazuron (Sammour *et al*., 2008), methoxyfenozide (Pineda *et al*., 2009), diflubenzuron (Aref *et al*., 2010), lufenuron (Gaaboub *et al*., 2012), triflumuron (El-Naggar, 2013), as well as remarkably reduced fertility of *P. gossypiella* after treatment of 1-day old eggs with hexaflumuron (El-Barkey *et al*., 2009), lufenuron, chlorfluazuron or chromafenozide (Kandil *et al*., 2012), chlorfluazuron or hexaflumuron (Kandil *et al*., 2013), chromafenozide or diflubenzuron (Salem, 2015), noviflumuron or novaluron (Tanani and Ghoneim, 2018), or after treatment of newly hatched larvae with novaluron (Hassan *et al*., 2017). Moreover, the present result was in accordance, to some extent, with several reported results of inhibited fertility of other insect species by various IGRs, such as *S. litura* (Perveen and Miyata, 2000), *M. domestica* by diofenolan (Hamadah, 2003), *T. molitor* by Halofenozide (Taibi *et al*., 2003), *T. castaneum* by novaluron (Kostyukovsky and Trostanelsky, 2004), *D. koenigi* by flufenoxuron (Khan and Qamar, 2011), *C. maculates* by cyromazine (Al-Mekhlafi *et al*., 2011), *E. kuehniella* by tebufenozide (Khebbeb *et al*., 2008), diflubenzuron or hexaflumuron (Ashouri *et al*., 2014), methoxyfenozide (Bouzera and Soltani-Mazouni, 2014), *S. exigua* (Christian-Lius *et al*., 2010), *S. litura* (Shahout *et al*., 2011), by methoxyfenozide as well as *P. unionalis* methoxyfenozide (Hamadah *et al*., 2017).

It is well known that the egg maturation in insects depends on the vitellogenins, including proteins, carbohydrates and lipids, all of which are prerequisite metabolites for the embryonic development (Soltani and Mazouni, 1992; Chapman, 1998). These materials are synthesized principally by the fat body during the immature stages (Telfer, 2009) or by the ovary *in situ* (Indrasith *et al*., 1988). For explicating the reduced fertility of *S. littoralis*, after larval treatment with novaluron, in the present investigation, it might disturb the production and/or accumulation of vitellogenins in the adult females leading to reduction of fertility (for some detail, see Taibi *et al*., 2003; Pineda *et al*., 2006; Osorio *et al*., 2008). On the other hand, the tested IGR might indirectly affect the fertility *via* its adverse action on "potency" of intracellular spaces in the follicular epithelium or generally prohibited the role of the gonadotropic hormone responsible for the vitellogenin deposition into oocytes (Davey and Gordon, 1996). Also, the fertility reduction might be due to the penetration of novaluron residues through mothers into eggs and disturbed the synthesis of the embryonic cuticle. So, the fully mature embryos had poorly chitinized mouth parts which were insufficiently rigid to perforate the surrounding vitellin membrane for egg hatching (Marco and Vinuela, 1994; Sallam, 1999; Sammour *et al*., 2008). Another suggestion for the fertility reduction after treatment of *S. littoralis* larvae with novaluron, in the current study, can be provided herein. This compound might adversely affect the survival of developing embryos at certain stages resulting in decreasing hatching percentage. On the basis of some molecular studies, some IGRs affect the insect reproduction *via* the perturbation of gene expression in the hierarchy cascade of vitellogenesis and/or choriogenesis (Sun *et al*., 2003), the tested IGR might interfere with the gene expression resulting in a reduction of the developing embryos in *S. littoralis*.

Retarded Embryogenesis of *S. littoralis* by Novaluron:

In insects, the egg incubation period usually indicates the rate of embryonic developmental, i.e., the shorter period usually denotes faster rate and *vice versa*. In the present study, treatment of *S. littoralis* larvae with novaluron resulted generally in retarded embryonic development; since the incubation period was significantly prolonged, irrespective of the concentration or time of treatment. This result corroborated with little reported results recording a similar retarding action of some IGRs on the embryonic development of *P. gossypiella*, after treatment of eggs with hexaflumuron (El-Barkey *et al*., 2009) or noviflumuron (Tanani and Ghoneim, 2018), and after treatment of newly hatched larvae with LC_{50} values of lufenuron, chlorfluazuron or chromafenozide (Kandil *et al*., 2012), novaluron (Hassan *et al*., 2017). Also, such period was prolonged after treatment of *C. maculates* larvae with cyromazine (Al-Mekhlafi *et al*., 2011) and after treatment of *P. unionalis* last instar larvae with methoxyfenozide (Hamadah *et al*., 2017). The retarded embryonic development in *S. littoralis* after treatment of 5th or 6th instar larvae with novaluron, in the present study, might be attributed to its effect on the ecdysteroids responsible for the regulation of certain stages of embryogenesis, especially those ecdysteroids originating from the ovary *in situ* (Chapman, 1998).

Conclusion:

In the light of the present results, novaluron acts as an antigonadotropic compound in *S. littoralis*, reducing the female fecundity, prohibiting the fertility and retarding the embryonic development. Therefore, this IGR can be used as an effective agent in the integrated pest management of this dangerous pest because it has developed resistance to the majority of conventional insecticides.

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