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Effectiveness of Novaluron, Chitin Synthesis Inhibitor, on the Adult Performance of Egyptian cotton Leafworm, *Spodoptera littoralis* (Boisd.)(Lepidoptera: Noctuidae)

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Abstract: *For evaluating the effects of Novaluron on the adult performance of Spodoptera littoralis females, a concentration range of 1.00-0.0001ppm was applied on the newly molted penultimate (5^{th}) instar larvae and a concentration range of 0.10-0.0001ppm was applied on the newly molted last instar larvae. Novaluron failed to affect the adult survival except at the higher concentration levels, regardless the time of treatment. Treatment of last instar larvae resulted in some features of impaired morphogenesis only at the higher two concentrations. On the contrary, no adult deformities had been produced after treatment of penultimate instar larvae. The total adult longevity was significantly shortened, regardless the time of larval treatment. Treatment of penultimate instar larvae with Novaluron resulted in remarkably accelerated the ovarian maturation, at all concentrations except the highest one. On the other hand, a diverse effect was detected after treatment of last instar larvae because Novaluron prohibited this vital process at the higher two concentrations, but remarkably enhanced it at the lower two concentrations. Novaluron exhibited a prevalent stimulatory effect on the reproductive life-time (oviposition period) of adult females to quickly lay eggs during a very short interval, regardless the time of larval treatment.*

Keywords: *longevity, morphogenesis, oviposition period, pre-oviposition period, survival.*

1. INTRODUCTION

The Egyptian cotton leafworm, *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae), feeds on a wide range of important crops and is one of the most destructive pests in the tropical and subtropical areas of the world [1]. It is the main pest of cotton in some cotton growing countries, especially Egypt, Israel and India. It is damaging vegetables in North Africa, cotton in Egypt and the glasshouses plant and flower production in Southern Europe [2, 3]. In East, West and North Africa, it often causes extensive damage to soybeans, vegetables and cowpeas. It could, therefore, threaten their cultivation in the future, if adequate control measures are not taken. When large numbers of the pest are present complete crop loss is possible [4].

As reported by many authors [5-11], although some farmers in Egypt laboriously and pick the egg batches to control *S. littoralis* population, most farmers prefer using chemical pesticides, which are detrimental to natural enemies, pollinators and all other non-target insects. In addition, *S. littoralis* developed resistance to organophosphorus, synthetic pyrethroid and many registered pesticides making their control even more difficult.

Owing to the socioeconomic importance of *S. littoralis*, the insect is subject to extensive research, much of which is envisioned to finding new ways to control it as a pest and to improve the effects of known pest control methods [12]. At present, using insect growth regulators (IGRs) is considered as the possible alternative way of synthetic insecticides for controlling this pest [13]. IGRs differ widely from the commonly used insecticides, as they exert their insecticidal effects through their influence on development, metamorphosis and reproduction of the target insects by disrupting the normal activity of the endocrine system [14, 15]. Their comprehensive effects and high selectivity as well as lower toxicity to non-target animals and the environment provide new tools for integrated pest management [16, 17].

Chitin synthesis inhibitors (CSIs) are usually classified in IGRs interfering with chitin biosynthesis in insects [18-20] and thus prevents moulting, or produces an imperfect cuticle [21, 22]. These compounds are effective suppressors of development for the entire life cycle of insect pests [23, 24]. Novaluron is a relatively new benzoylphenyl urea IGR with low mammalian toxicity [25, 26]. The compound has no appreciable effect on parasitoids and phytoseiids and has probably a mild effect on other natural enemies [27,28]. Its residues tend to dissipate with half-life of 2.08 days and the safe use of it on tomatoes, and possibly on other crops in Egypt was established [29]. Novaluron is a powerful suppressor of the whiteflies *Bemisia tabaci* and *Trialeurodes vaporariorum* [30]. It acts by ingestion and contact against lepidopterans (*S. littoralis, S. exigua, S. frugiperda, Tuta absoluta* and *Helicoverpa armigera*), whitefly, *B. tabaci*, eggs and larvae, and different stages of the leafminer, *Liriomyza huidobrensis* [31]. It exhibited, also, a good activity against the Colorado potato beetle [32-35]. The present study was conducted to investigate the effects of Novaluron on the adult performance of *S. littoralis*.

2. MATERIALS AND METHOD

2.1. Experimental Insect

A sample of Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) pupae was kindly obtained from the culture of susceptible strain maintained for several generations in Plant Protection Research Institute, Agricultural Research Center, Doqqi, Giza, Egypt. In laboratory of Entomology, Faculty of Science, Al-Azhar University, Cairo, a culture was reared under laboratory controlled conditions (27±2°C, 65±5% R.H., photoperiod 14 h L and 10 h D). Rearing procedure was carried out according to [36] and improved by [37]. Larvae were provided daily with fresh castor bean leaves *Ricinus communis*. The emerged adults were provided with 10% honey solution on a cotton wick as a food source. Moths were allowed to lay eggs on *Oleander* branches, then the egg patches were collected daily, and transferred into Petri dishes for another generation.

2.2. Larval treatments with Novaluron

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). Its molecular formula is $C_{17}H_9ClF_8N_2O_4$. A series of concentration levels using distilled water (1.0, 0.1, 0.01, 0.001& 0.0001ppm) was prepared. Newly moulted larvae of 5th (penultimate) instar were treated with all concentration levels and the newly moulted larvae of 6th (last) instar were treated with 0.1, 0.01, 0.001& 0.0001ppm. Fresh castor bean leaf discs were dipped in each concentration for 5 minutes and air dried before introduction to larvae for feeding. Control congeners were provided with water-treated leaf discs. Ten replicates of treated and control larvae (one larva/replicate) were kept separately in glass vials. The larvae were left to feed on treated leaf discs for 24 hrs and then were provided with untreated fresh leaf discs every day. The larvae (control and treated) were carefully handled until the adult emergence just after which all parameters of adult performance were recorded.

2.3. Adult Performance Parameters

For investigation the adulticidal activity of Novaluron on *S. littoralis*, mortality of adult females was observed throughout the adult longevity and calculated in percentage. For investigating the morphogenic efficiency, the adult deformities were observed and calculated in percentage as follows:

[No. of deformed adults / No. of emerged adults] × 100

For investigation the effects of Novaluron on the adult longevity, total adult longevity of females was measured in mean days±SD. The major compartments of adult longevity are pre-oviposition (ovarian maturation) period, oviposition period (reproductive life-time) and post-oviposition period. With regard to *S. littoralis*, the post-oviposition period usually elapses only few hours, therefore, no post-oviposition period was recorded. All durations were measured in mean days±SD.

2.4. Statistical Analysis of Data

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction [38] for the test significance of difference between means.

3. RESULTS

3.1. Adult Survival Potential of *S. Littoralis* as Affected by Novaluron

Assessment of this Novaluron on the adult performance was carried out using concentration range of 1.0-0.0001 ppm against the newly moulted penultimate (5th) instar larvae. According to data assorted in Table (1), the adults were subjected to a latent lethal action of Novaluron at only 0.10 and 0.01 ppm

(33.33% vs. 00.00% of control adults). At other concentration levels (higher or lower), Novaluron failed to affect the adult survival.

Assessment of this CSI on the adult performance was also carried out using concentration range of 0.1-0.0001 ppm against the newly moulted last instar larvae. Data of Table (2) obviously reveal the failure of Novaluron to affect the adult survival except at the higher two concentration levels (14.34 and 12.50%, respectively, vs. 00.00% mortality of control adults).

3.2. Effects of Novaluron on the Adult Morphogenesis of *S. Littoralis*

In connection with the morphogenesis program of adults after treatment of penultimate instar larvae with Novaluron, no effect was observed, regardless the concentration level (Table 1).

After treatment of last instar larvae, data of Table (2) exiguously show the impairment of adult morphogenesis only at the higher two concentration levels because adult deformities had been recorded in 14.33 and 12.50% (compared to 00.00% deformity among control adults). As clearly seen in Plate (1), some moths failed to completely emerge from the pupal exuvia.

3.3. Effects of Novaluron on the Adult Longevity of *S. Littoralis*

As usually observed in the life cycle of *S. littoralis*, under laboratory controlled conditions, adult females remain alive for only few hours after egg laying. Therefore, two compartments of longevity could be observed in the present study: ovarian maturation (pre-oviposition) period and reproductive life-time (oviposition period).

With regard to the ovarian maturation period of adult females after treatment of penultimate instar larvae with different concentration levels of Novaluron, data arranged in Table (1) exiguously reveal a predominant accelerating effect on the maturation rate at all concentration levels except the highest one that delayed it.

It is important to recall the data of Table (2) which unexceptionally show a diverse effect of Novaluron on the ovarian maturation period, and subsequently on the maturation rate, after treatment of last instar larvae with different concentration levels of Novaluron. At the higher two concentration levels, Novaluron prohibited the ovarian maturation rate expressed in significantly prolonged period (2.50±0.71 and 2.33±0.58 days, respectively, vs. 2.00±0.00 days of control adult females) while it remarkably enhanced such vital process during pronouncedly shortened period at the lower two concentration levels (1.75±0.50 and 1.67±0.52 days, respectively, vs. 2.00±0.00 days of control adult females).

Just a look at the data assorted in Table (1) and Table (2) indicate a prevalent stimulatory effect of Novaluron on the reproductive life-time of adult females to quickly lay eggs during a very short interval, regardless the time of larval treatment. Although such effect did not run in a dose-dependent course, the strongest stimulation was exhibited after application of the highest concentration level on penultimate instar larvae (3.00±0.00 in comparison with 8.80±0.45 days of control adult females, Table 1) and last instar larvae (7.00±1.41 in comparison with 8.80±0.45 days of control adult females, Table 2).

Table1. *Adult performance of S. littoralis as affected by Novaluron treatments on the newly moulted penultimate instar larvae.*

Conc. (ppm)	Adult mortality (%)	Adult deformities (%)	Ovarian maturation period (mean days±SD)	Reproductive life-time (mean days±SD)	Total longevity (mean days±SD)
1.00	00.00	00.00	5.00±0.01 d	3.00±0.001 d	08.00±0.001 d
0.10	33.33	00.00	1.50±0.71 d	5.50±0.70 d	05.67±2.31 d
0.01	33.33	00.00	3.00±1.41 d	5.00±0.20 d	07.00±2.00 d
0.001	00.00	00.00	1.86±0.38 d	4.09±0.60 d	06.00±0.00 d
0.0001	00.00	00.00	2.00±0.00 a	6.57±0.53 d	08.43±0.53 d
Control	00.00	00.00	2.00±0.00	8.80±0.45	10.80±0.45

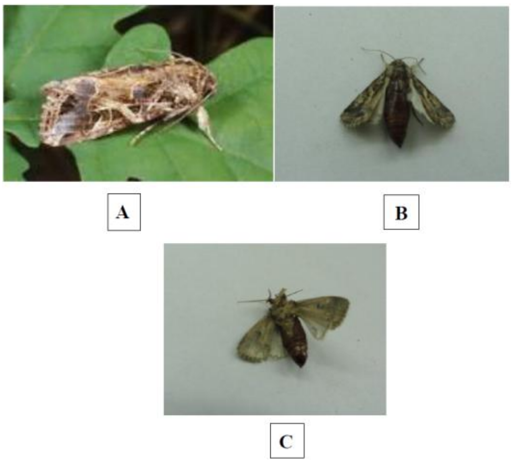
Conc.: concentration level. Mean ± SD followed with the letter (a): insignificantly different (P >0.05), (d): very highly significantly different (P<0.001).

In respect of the total longevity, Novaluron exerted an accelerating action on the adult females to quickly pass aging ending in death during considerably shortened interval. In other words, data assorted in Table (1) and Table (2) clearly shows significantly shortened adult longevity, regardless the time of larval treatment with Novaluron. As for example, the shortest total longevity was measured in 5.67±2.31 days (vs. 10.80±0.45 days of control adult females) after treatment of penultimate instar larvae with 0.1 ppm of Novaluron and the shortest total longevity was determined in 9.00±0.89 days (vs. 10.80±0.45 days of control adult females) after treatment of last instar larvae with the lowest concentration level.

Table2. *Adult performance of S. littoralis as affected by Novaluron treatments on the newly moulted last instar larvae*

Conc. (ppm)	Adult mortality (%)	Adult deformities (%)	Ovarian maturation period (mean days±SD)	Reproductive life-time (mean days±SD)	Total longevity (mean days±SD)
0.10	14.34	14.34	2.50±0.71 d	7.00±1.41 d	09.50±2.12 d
0.01	12.50	12.50	2.33±0.58 d	8.00±1.00 d	10.33±0.58 d
0.001	00.00	00.00	1.75±0.50 d	7.75±0.96 d	09.50±0.58 d
0.0001	00.00	00.00	1.67±0.52 d	7.33±0.82 d	09.00±0.89 d
Control	00.00	00.00	2.00±0.00	8.80±0.45	10.80±0.45

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



Plate(1): *Deformed adults of S. littoralis after treatment of larvae with Novaluron. (A) Normal adult moth. (B & C) Adult moth failed to completely emerge from the pupal exuvium (Dorsal view and ventral view).*

4. DISCUSSION

Chitin synthesis inhibitors (CSIs) are usually classified in IGRs interfering with chitin biosynthesis in insects [19, 20] and thus prevents moulting, or produces an imperfect cuticle [21,22]. These compounds are effective suppressors of development for the entire life cycle of insect pests [23]. They also affect the hormonal balance in insects, thereby resulting in physiological disturbances, such as, inhibition of DNA synthesis [39,40]; alteration of carbohydrates [41]; increase in phenyloxidase activity [42]; cuticular lipids [43] and microsomal oxidase [44].

4.1. Influenced Adult Survival of *S. Littoralis* by Novaluron

The available literature contains many reported toxicities of IGRs and CSIs on the immature stages of *S. littoralis* [37, 45-56] and other insects [57-69] while their lethal effects on adults are scarcely reported [70]. In the present study, adult females of *S. littoralis* were subjected to a latent lethal action of Novaluron after treatment of penultimate instar larvae only with 0.10 and 0.01 ppm. After treatment of last instar larvae, Novaluron failed to affect the adult survival except at the higher two concentration levels.

These adult mortalities at the higher concentrations of Novaluron can be explained by the retention and distribution of it in the insect body as a result of rapid transport from the gut of treated larvae into other tissues, the direct and rapid transport of haemolymph to other tissues, and/or to lower detoxification capacity against the tested CSI [71, 72]. Also, an extended or chronic lethal effect of Novaluron may be due to feeding that leading to continuous starvation and subsequently death [73], on the homeostasis leading to increasing loss of body water as a direct cause of death [74] or disturbed adult enzymatic pattern and hormonal hierarchy [75].

4.2. Deranged Adult Morphogenesis of *S. Littoralis* by Novaluron

Deranged adult morphogenesis, as expressed in the production of deformed adults, was widely reported in the literature for *S. littoralis* after treatment of larvae with different CSIs or IGRs, such as chlorfluazuron and triflumuron [46], lufenuron [76], flufenoxuron [37], ecdysteroid agonists tebufenozide and methoxyfenozide [49,77], etc. Also, impaired morphogenesis of adults had been reported for other insect species by larval treatment with various IGRs, such as *Simulium vittatum* [78], *Blattella germanica* [79], *Mamestra brassicae* [80] and *Muscina stabulans* [81] by Diflubenzuron; *Cydia pomonella* [82] and *Rhynchophora ferrugineus* [83] by Diofenolan; *Schistocerca gregaria* by chlorfluazuron [84]; *Spodoptera exigua* [85] and *Choristoneura fumiferana* [57] by tebufenozide and methoxyfenozide; *Tribolium castaneum* and *Tribolium confusum* by Cyromazine [86]; *Eurygaster integriceps* by pyriproxyfen [62]; *Dysdercus koenigii* by flucycloxuron [63]; *Anagasta kuehniella* by diflubenzuron and hexaflumuron [87]; etc. In accordance with these reported results, treatment of newly moulted last instar larvae of *S. littoralis* with Novaluron resulted in some features of impaired adult morphogenesis only at the higher two concentrations, in the present study, and some moths failed to completely get rid the pupal exuvia. On the contrary, no adult deformities had been produced by this CSI after treatment of penultimate instar larvae, regardless the concentration. The latter result agrees with the reported result of unaffected adult morphogenesis of *Musca domestica* by lufenuron or Diofenolan [70]. Thus, the morphogenic action of CSIs depends on the time of treatment and concentration level beside other factors like the application method [88].

The adult deformities of *S. littoralis*, observed in the current study, may be explained by an action of Novaluron on the hormonal imbalance during the adult differentiation, in particular the modification of ecdysteroid titer which led to changes in lysosomal enzyme activity causing overt morphological abnormalities [89]. In addition, other suggestions can be appreciated, such as the interference of the tested CSI with juvenile hormone or ecdysteroid metabolism causing a disruption in the chitin metabolic system [90], inhibition of chitin synthase by metabolites of the CSI [91], inhibition of DNA synthesis [92] and/or inhibition of facilitated diffusion and active transport across cell membranes of nucleosides and amino acids [93].

4.3. Disturbed Adult Longevity of *S. Littoralis* by Novaluron

4.3.1. Total Adult Longevity

After the attainment of sexual maturity, insects often show degenerative changes in some tissues and organs which can be called 'senility' or 'aging'. In insects, the affected adult longevity can be considered an informative indicator for the adult aging, i.e., prolongation of longevity may denote a delay of aging and *vice versa*. In the present study, the total adult longevity of *S. littoralis* was significantly shortened, regardless the time of treatment with Novaluron. Thus, an accelerating action of Novaluron can be detected on the adult females to quickly pass aging ending in death. The present result is in agreement with those reported results for the same lepidopteran by other CSIs or IGRs, such as chlorfluazuron and flufenoxuron [94], Lufenuron [95] and methoxyfenozide [96]. No effect on the adult longevity was reported after treatment of 4^{th} instar larvae of the same insect with buprofezin [97]. In addition, shortened adult longevity of other insect species was recorded after treatment with some IGRs, such as *Tenebrio molitor* by diflubenzuron [98], *Agrotis ipsilon* by flufenoxuron [99], *Grapholita molesta* [100] and *S. exigua* [101] by methoxyfenozide. In contrast to our results, a prolongation of adult longevity was determined in some insects as an effect of some IGRs, such as *Lipaphis erysimi* by pyriproxyfen [102]. Moreover, no effect was exhibited on adult longevity of *M. domestica* by Diofenolan [103], *Cydia pomonella* by tebufenozide or methoxyfenozide [104] and *Lygus lineolaris* by Novaluron [105].

In insects, the adult longevity depends on healthy immature stages. Digestive disorders such as starvation, disturbance in metabolism, degeneration of peritrophic membranes and accumulation of faecal materials at the hind gut may be the cause of untimely adult mortality as a result of CSIs exposure [98]. Generally, the shortened adult longevity of *S. littoralis*, in the current investigation, can be explained by the accumulation of toxic xenobioties in the body which upsets a complicated balance of factors such as absorption, excretion and detoxification [106]. On the other hand, this shortened longevity of *S. littoralis* adult females may be attributed to the effect of tested CSI on a hormonal activity because there is a close relation between certain hormones and adult longevity. This suggestion can be appreciated in the light of reported results in *Drosophila*. In this fly, representatives of peptide hormone, lipophilic hormones and bioactive amines have been shown to modulate longevity by manipulations that directly decrease hormone production [107], through inactivating mutations in hormone receptors or their downstream targets [108,109] or by polymorphic alterations in the genes required for hormone biosynthesis [110]. At least one of the *Drosophila* insulin-linked peptides expressed in the median neurosecretory cells (which produce the prothoracicotropic hormone, PTTH) is likely to contribute to the endocrine regulation of longevity [111]. However, the exact mode of action of the tested CSI on biochemical sites in adults of *S. littoralis* is presently unknown. Therefore, more information on the adult endocrine system is needed before a general interpretation can be formulated on the susceptibility of the life stage toward Novaluron.

4.3.2. Ovarian Maturation Period

In most insects, the pre-oviposition period can be called 'ovarian maturation period' and it may be informative for the ovarian maturation rate, i.e., the shorter period indicates faster rate and *vice versa*. In the present study, treatment of penultimate instar larvae of *S. littoralis* with Novaluron resulted in remarkably speed up the ovarian maturation through significantly prolonged period, at all concentration levels except the highest one that delayed it. On the other hand, a diverse effect was detected after treatment of last instar larvae because Novaluron, at the higher two concentrations, prohibited this vital process through a prolonged duration while CSI remarkably enhanced it through conspicuously shortened period, at the lower two concentration levels. This prolongation of ovarian maturation period agrees with the reported results for the same lepidopteran after treatment with diflubenzuron [51] and for *Ephestia kuehniella* after treatment with tebufenozide [112] but no significant effect of chlorfluazuron or methoxyfenozide was exhibited on this period of *Spodoptera litura* [113].

Many lepidopteran species have a relatively short, non-feeding adult stage, which requires the adult female to emerge with most of her eggs ready to be fertilized and oviposited within hours. This life style constrains these insects to a program of ovarian organogenesis and follicle development that must occur at stages earlier than in other insects. The determinants required for germ cell formation are similar in moths, but there are spatial differences in their localization within the presumptive germ band [114]. In the light of this information, delaying or enhancing effect of Novaluron on the ovarian maturation in the present lepidoptera *S. littoralis* may be understood by influenced germ band or the number of germ cells formed in the embryo [115]. However, the exact mode of action of the retarding or enhancing effect of Novaluron on the pre-oviposition period is unfortunately available right now but its interference with the hormonal regulation of this physiological process needs further investigation.

4.3.3. Reproductive Life-Time

With regard to another compartment of adult longevity, oviposition period (reproductive life-time), very scarcely reported results have been seen in the available literature. No significant effect was detected on such period in *M. domestica* by Diofenolan [103] or *S. litura* by methoxyfenozide and chlorfluazuron [113]. In the current work, Novaluron exhibited a prevalent stimulatory effect on *S. littoralis* adult females to quickly lay their eggs during a very short interval, regardless the time of larval treatment. The exact mechanism of this stimulation is still unknown. However, these females may be enforced to lay their eggs quickly to avoid this toxic xenobiotic factor.

5. CONCLUSION

Since *S. littoralis* has developed resistance to the majority of conventional insecticides, the use of compounds with different mode of action like Novaluron might be considered a part of the integrated pest management since it disruptively affected the adult performance leading to a reduction of the pest population.

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