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Research Article
Evaluation of the Toxic Effects of Novaluron on *Muscina stabulans* (Fallen) (Diptera: Muscidae)

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Abstract

Background and Objective: The worldwide fly species, *Muscina stabulans* (Diptera: Muscidae) is known as 'false stable fly'. It has veterinary, forensic and medical importance. The present study aimed to examine the toxicity of novaluron (chitin synthesis inhibitor) via its effect on the growth and reproductive potential of *M. stabulans*. **Materials and Methods:** The early last (3rd) instar larvae and prepupae have been treated with novaluron using five doses: 5.0, 1.0, 0.5, 0.1 and 0.01 µg/larva. Student's t-test analysis has been used for data processing as well as refined by Bessel correction for significant differences among means. **Results:** Current study revealed that, after the treatment different mortalities of larva, pupa and adult have been estimated. LD_{50} values of novaluron were 0.018 and 0.057 µg/insect, respectively. Furthermore, the larval period was insignificantly shortened while the pupal duration has been significantly extended and the developmental rate has been slightly enhanced. On the other hand, the adult longevity of females was considerably reduced and the adult emergence was considerably blocked, regardless the dose. However, only after the treatment, some deformed pupae were observed and some adult deformities were observed. Both fecundity and fertility were drastically reduced and sterilizing activity on novaluron increased in a dose-dependent course, regardless the time of treatment. **Conclusion:** Novaluron can be used as an effective IGR in the integrated control program for this medically and veterinary serious fly.

Key words: Fertility, fecundity, metamorphosis, mortality, pupation, sterility

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In flies, family Muscidae includes a small genus *Muscina* which currently comprises 14 valid species and 30 additional species[1]. *Muscina stabulans* (Diptera: Muscidae) is commonly known as 'false stable fly'. It is worldwide fly species[2] that mostly found at urban and suburban areas[3]. Also, maggots are frequently found in animal excrement and decaying vegetable materials as well as in poultry houses [4]. Also, it is reported to inhabit latrines and household wastes and other filthy habitats[5]. The fly is reported to cause myiasis in humans and animals[6]. Mian *et al*.[7] isolated *Salmonella enteritidis* from the fly adults in California and Yoshida *et al*.[8] isolated the trypanosomatid flagellate *Herpetomonas mariadeanei* (Protozoa) from this fly species. In addition, *M. stabulans* larvae are reported among flies of the forensic importance[2,9-11]. Despite the potential of adults as a vector of pathogens, larvae prey on the immature stages of other dipterous species and subsequently limit their abundance[12]. Thus, these larvae can play a role in the control of some Dipter[13].

The intensive and discriminate uses of many broad-spectrum conventional insecticides cause several serious problems, such as toxicological problems to humans, destruction of the biological enemies and the development of insect resistance toward different insecticides[14-17]. Therefore, alternative control agents have been searched recently to minimize insecticide hazards. Few years ago, a recent group of safe compounds had been synthesized. These compounds are known as Insect Growth Regulators (IGRs)[18]. IGRs are not directly toxic but act selectively on the growth, metamorphosis and/or reproduction of serious insect pests[19,20] owing to their disruptive effects on the normal activity of endocrine system of insects[21].

According to their modes of action, these Insect Growth Regulators (IGRs) were categorized in three groups: (i) Juvenile Hormone Analogues (JHAs), (ii) Ecdysteroids or ecdysone agonists and (iii) Chitin Synthesis Inhibitors (CSIs)[22,23]. CSIs were known as safe or low toxicity to the beneficial biota and non-target organisms with expected have no residual impact[24] because they interfere with chitin biosynthesis and thus disrupt molting[25].

In the late decades, some new benzoylphenylurea (BPU) compounds were synthesized, such as diflubenzuron, hexaflumuron, noviflumuron, lufenuron and novaluron[26-28]. Novaluron was reported to exhibit different effects on several dipterous species[29-32]. Also, its powerful suppression was recorded for lepidopterous larvae[33-35] and whiteflies[36,37]. In addition, novaluron exhibited various effects on some coleopteran insects[38-40]. This compound has no or moderate impacts on parasitoids and other natural enemies[33,36]. Recently, novaluron was found to exhibit various effects on the pink bollworm *Pectinophora gossypiella*, such as reduced survival, retarded development, impaired metamorphosis[41], disrupted adult performance, disturbed reproductive ability[42] and declined main metabolites[43]. Therefore, the current study was conducted to assess the toxic effects of novaluron and its impacts on the development and the reproduction of *M. stabulans*.

MATERIALS AND METHODS

Experimental insect

Study area: A culture of susceptible strain of false stable fly *Muscina stabulans* (Fallen) (Diptera: Muscidae) was established in the laboratory of Entomology, Faculty of Science, Al-Azhar University, Cairo, Egypt. Controlled laboratory conditions (25±2°C, 55±5% R.H., photoperiod 12 L:12 D) were justified for a number of generations during the period 2017-2019. Larvae were fed on an artificial diet (200 g wheat bran, 100 g powdered milk, 3 g yeast and 200 mL tap water) based on the rearing technique of the house fly *Musca domestica* described by Busvine[44]. Feeding larvae were kept in breeding pans covered with muslin and fitted with rubber bands. After pupation (pupariation), pupae were gently collected and confined in Petri dishes. These Petri dishes were transferred into wooden cages (45×45×45 cm). Each cage was structurally provided with wire-gauze sides except the bottom and the front which was fitted with a small circular window attached with a cloth sleeve for routine daily work, such as feeding, handling and cleaning. After emergence, the adult flies were supplied with a milk diet and a piece of cotton soaked in 10-15% sugar solution.

Novaluron administration: Novaluron ($C_{17}H_9ClF_8N_2O_4$) was purchased from Sigma-Aldrich Chemicals. A sequence of five dose levels has been formulated using acetone for novaluron dilutions (5.0, 1.0, 0.5, 0.1 and 0.01 µg/larva). Fifty larvae in 10 replicates (5 larvae/replicate) of the early last (3rd) instar and a similar number of prepupae were topically treated, individually, with each dose using a Hamilton micro applicator (NHN 737). A similar number of replicates of early last instar larvae and prepupae were topically treated with 1 µL acetone only as controls. All treated and control replicates were confined in small tubes and kept under the previously mentioned laboratory conditions. Treated and control insects were observed every day for feeding of the larvae and recording all criteria of the study.

Toxicity of novaluron: Toxicity of novaluron was determined by observed mortality. Mortality of larvae, pupae and adults (treated and control) have been noted every day and corrected as stated by Abbott's formula[45] as follows:

$$\text{Corrected mortality (\%)} = \frac{\text{Test mortality (\%)-Control mortality (\%)}}{\text{100-Control mortality (\%)}} \times 100$$

The values of LD_{50} for the tested compound, after treatment of larvae and prepupae, were calculated according to Finney[46].

Developmental durations and rates: Developmental durations of larvae and pupae had been calculated (mean days±SD) using Dempster's equation[47]. The developmental rate was calculated according to Lotka[48]. Total longevity of adult female flies was also calculated in mean days±SD.

Metamorphosis and morphogenesis: Pupation rate has been calculated as % of the developed pupae and the adult emergence has been calculated in percentage as mentioned by Jimenez-Peydro *et al.*[49] as follows:

$$\text{Adult emergence} = \frac{\text{Number of completely emerged adults}}{\text{Number of pupae}} \times 100$$

All of the possible aberrations of metamorphosis and morphogenesis were calculated in percentage.

Reproductive potential: For determination of the most important reproductive parameters, treated and control pupae were transferred into small cages (20×20×20 cm). Each treated female has been coupled with two normal males of the same age. Fecundity was calculated by the number of eggs per female. Fertility has been estimated by hatching the percentage of laid eggs. Sterility index has been evaluated as mentioned by Toppozada *et al.*[50] as follows:

$$\text{Sterility index} = 100 - \frac{ab}{AB} \times 100$$

Where:

- a = Indicate the number of eggs laid per female in the treatment
- b = Ratio of hatching in the treatment
- A = Indicate the number of eggs laid per female in the controls
- B = Percentage of hatching in the controls

Data analysis: Data have been processed by the student's t-test and refined by Bessel correction[51] for the test significant differences between means.

RESULTS

Toxic impact of novaluron against *M. stabulans*: Novaluron showed high toxicity that was expressed as mortality of larvae (maggots), pupae (puparia) and adult flies when early last (3rd) instar larvae have been treated as assorted in Table 1. Following topical treatment of prepupae, data of mortalities were arranged in Table 2. Last instar larvae treated with Novaluron showed high larval mortality in a dose-dependent course (10, 18, 28, 38 and 62% mortalities, at 0.01, 0.1. 0.5, 1.0 and 5.0 µg/larva, respectively, vs. 4% mortality among control larvae) as presented in Table 1. It was also observed that, pupae have completely died after the application of the highest two doses and the mortality of pupae was detected in a dose-dependent course, at other applied doses (22.2, 43.9 and 69.4% pupal mortality, at 0.01, 0.1 and 0.5 µg/larva, respectively, vs. control pupae mortality 2.1%).

Furthermore, pupae have completely died after the highest dose of Novaluron was applied for prepupae treatment as presented in Table 2. At other dose levels, the pupal mortality has been noted in a dose-dependent way (37.5, 47.6, 50.0 and 81.3% pupal mortality, at 0.01, 0.1, 0.5 and 1.0 µg/prepupa, respectively, vs. control pupae mortality, 4.1%). Depending on the data of the same table, no adult mortality could be recorded at the higher two doses of Novaluron because all pupae died. Following application of other three doses on last instar larvae, the adulticidal effect of Novaluron was exhibited parallel to the dose level, i.e., the mortality of adult has been noted in a dose-dependent manner (20.0, 47.8 and 63.6% adult mortality, at 0.01, 0.1 and 0.5 µg/larva, respectively, in comparison with 6.0% mortality of control adults). As clearly seen in Table 2, also, no adult mortality could be recorded after treatment of prepupae with the highest dose of novaluron because all pupae died. In addition, the adult mortality was recorded in a dose-dependent style (37.5, 47.6, 50.0 and 81.3% adult mortality, at 0.01, 0.1, 0.5 and 1.0 µg/prepupa, respectively, vs. control congeners mortality, 4.1%).

Depending on the corrected mortality percentages, Novaluron exerted a lethal potency against *M. stabulans* parallel to the dose level, regardless the time of treatment (Table 1 and 2). The calculated LD_{50} values of Novaluron against *M. stabulans* were 0.018 and 0.057 µg/insect for last

Table 1: Toxic effect (%) of Novaluron on *M. stabulans* after topical treatment of the early last instar larvae

Dose (µg/larva)	Larval mortality	Pupal mortality	Adult mortality	Total mortality	Corrected mortality	LD_{50} (µg/larva)
5.0	62	100.0	---	100	100.0	
1.0	38	100.0	---	100	100.0	
0.5	28	69.4	63.6	92	90.9	0.018
0.1	18	43.9	47.8	76	72.7	
0.01	10	22.2	20.0	44	36.4	
Control	4	2.1	6.0	12	---	

-: No adult mortality could be calculated because no adult flies emerged

Table 2: Toxic effect (%) of Novaluron on *M. stabulans* after topical treatment of the prepupae

Dose (µg/prepupa)	Pupal mortality	Adult mortality	Total mortality	Corrected mortality	LD_{50} (µg/prepupa)
5.0	100	---	100	100.0	0.057
1.0	36	81.3	88	87.2	
0.5	24	50.0	62	59.6	
0.1	16	47.6	56	53.2	
0.01	4	37.5	40	36.2	
Control	2	4.1	06	---	

-: No adult mortality could be calculated because no adult flies emerged

Table 3: Affected developmental durations (mean days±SD) of *M. stabulans* after topical application of Novaluron onto the early last instar larvae

		Pupal stage		
Dose(µg/larva)	Larval duration	Duration	Developmental rate	Adult longevity
5.0	3.8±0.4[a]	---	---	---
1.0	3.7±0.5[a]	---	---	---
0.5	3.7±0.4[a]	8.1±3.11[b]	12.32	05.3±0.48[d]
0.1	3.8±0.2[a]	8.0±1.84[b]	12.50	08.3±0.94[d]
0.01	3.8±0.2[a]	6.6±1.24[a]	15.52	10.7±1.70[d]
Control	3.9±0.4	6.6±1.38	15.50	19.2±0.20

-: Some pupae died as morphologically normal pupae and other pupae died as deformed pupae, thus pupal duration could not be measured. Mean±SD followed with the same letter a: Insignificantly different (p>0.05), b: Significantly different (p<0.05), d: Very highly significantly different (p<0.001)

Table 4: Affected developmental durations (mean days±SD) of *M. stabulans* after topical application of Novaluron onto the prepupae

	Pupal stage		
Dose (µg/prepupa)	Duration	Developmental rate	Adult longevity
5.0	---	---	---
1.0	6.4±1.62[a]	16.47	06.8±0.12[d]
0.5	6. 5±1.74[a]	16. 45	08.3±2.88[d]
0.1	4.6±0.80[b]	19.67	10.0±0.80[d]
0.01	6.1±1.70[a]	16.48	13.5±1.28[d]
Control	6.6±4.60	16.40	19.7±1.50

-: Some pupae died as morphologically normal pupae and other pupae died as deformed pupae, thus pupal duration could not be measured. Mean±SD followed with the same letter a: Insignificantly different (p>0.05), b: Significantly different (p<0.05), d: Very highly significantly different (p<0.001)

instar larvae and prepupae, respectively. Therefore, the last instar larvae were more sensitive to the toxicity of Novaluron than prepupae.

Effect of novaluron on the developmental durations and rates of *M. stabulans*: Data about the durations of different developmental stages of the last instar larvae treated with Novaluron were arranged in Table 3. Based on these data, the larval duration was insignificantly shortened, in no certain trend. In contrast, the pupal duration was remarkably prolonged at 0.5 and 0.1 µg/larva (8.1±3.11 and 8.0±1.84 days, respectively, vs. 6.6±1.38 days of control pupae). Also, the developmental rate of treated pupae had been seriously suppressed at these two doses. The adult female longevity was considerably shortened (5.3±0.48, 8.3±0.94 and 10.7±1.7 days at 0.5, 0.1 and 0.01 µg/larva respectively, in comparison to 19.2±0.2 days of control adult females.

Information about the effect of application of Novaluron onto the prepupae development was shown in Table 4. According to these data, the pupal duration has been significantly or insignificantly shortened, based on the dose concentration (6.4±1.62, 6.5±1.74, 4.6±0.8 and 6.1±1.70 days, at 1.0, 0.5, 0.1 and 0.01 µg/prepupa, respectively, vs. 6.6±4.6 days of control pupae). The developmental rate of

Table 5: Metamorphic and morphogenic effects of Novaluron on *M. stabulans* after topical treatment of early last instar larvae

	Pupal stage		Adult stage	
Dose (µg/larva)	Pupation (%)	Pupal deformities (%)*	Emergence (%)	Adult deformities (%)**
5.0	39.0	32.0	00.0	---
1.0	61.3	19.8	00.0	---
0.5	71.6	30.6	87.0	06.4
0.1	81.8	17.6	69.2	10.6
0.01	89.9	11.4	93.3	05.0
Control	100	00.0	93.3	00.0

*: Deformed pupae perished without metamorphosis into adult flies, **: Deformed adults with curly wings, atrophied mouth parts and ill-developed legs. These adult females perished within few days without mating

Table 6: Metamorphic and morphogenic effects of Novaluron on *M. stabulans* after topical treatment of prepupae

	Pupal stage		Adult stage	
Dose (µg/prepupa)	Pupation (%)	Pupal deformities (%)*	Emergence (%)	Adult deformities (%)**
5.0	90.0	0	00.0	---
1.0	100	0	51.6	30.2
0.5	100	0	76.7	22.3
0.1	100	0	83.3	16.1
0.01	100	0	86.7	26.0
Control	100	0	93.3	00.0

*: Deformed pupae perished without metamorphosis into adult flies, **: Deformed adults with curly wings, atrophied mouth parts and ill-developed legs. These adult females perished within few days without mating

treated pupae was slightly elevated, especially at 0.1 µg/prepupa. Data of Table 4 obviously revealed that the adult longevity was conspicuously shortened, in a dose-dependent style, following prepupae topical treatment (13.5±1.28, 10.0±0.80, 8.3±2.88 and 6.8±0.12 days, at 0.01, 0.1, 0.5 and 1.0 µg/prepupa, respectively, vs. days of adult females control, 19.7±1.5).

Effects of novaluron on metamorphosis of *M. stabulans*: Following Novaluron application on the last instar larvae with, data of metamorphosis and morphogenesis were arranged in Table 5. Following current application of Novaluron on the prepupae, data of metamorphosis and morphogenesis were assorted in Table 6. As clearly seen in Table 5, the pupation rate (%) was regressed in a dose-dependent manner (89.9, 81.8, 71.6, 61.3 and 39% pupation, at 0.01, 0.1, 0.5, 1.0 and 5.0 µg/larva, respectively, vs. 93.3% pupation of control congeners). Additionally, data of Table 6 showed no effect of Novaluron on the pupation rate after the treatment of prepupae.

In respect of the adult emergence, no adults emerged following application of the highest two doses for the treatment of 3rd instar larvae. The adult emergence was remarkably blocked at the other three doses (93.3, 69.2 and 87.0%, at 0.01, 0.1 and 0.5 µg/larva, respectively, vs. 93.3% emergence of control adults, Table 5). Following treatment of prepupae, the adult eclosion was completely blocked at the highest dose and increasingly blocked parallel to the increasing dose (86.7, 83.3, 76.7 and 51.6% emergence, at 0.01, 0.1, 0.5 and 1.0 µg/prepupa, respectively, vs. 93.3% emergence of control adults, Table 6).

Effect of novaluron on morphogenesis of *M. stabulans*: Following Novaluron application for the treatment of last instar larvae, disruptive effect on the pupal morphogenesis was observed, since different percentages of deformed pupae were recorded, with no certain trend (11.4, 17.6, 30.6, 19.8 and 32.0% pupal deformities, at 0.01, 0.1, 0.5, 1.0 and 5.0 µg/larva, respectively, vs. 0% deformity of control pupae, Table 5). These deformed pupae perished without metamorphosis into adults. On the contrary, Novaluron failed to affect the pupal morphogenesis after topical treatment of prepupae, since no deformed pupae were observed (Table 6).

With regard to the affected adult morphogenesis, treated early last instar larvae with Novaluron showed some deformities of adults, in no certain trend (5.0, 10.6 and 6.4% deformed adults, at 0.01, 0.1 and 0.5 µg/larva, respectively, in comparison to 0% deformation of control adults, Table 5). Also, various percentages of malformed adults were recorded after topical treatment of prepupae with Novaluron, in no certain trend (26, 16.1, 22.3 and 30.2% deformed adults, at 0.01, 0.1, 0.5 and 1.0 µg/prepupa, respectively, in comparison to 0% deformation of control adults, Table 6). Irrespective of the time of treatment with Novaluron, the deformed adults could not sexually be differentiated (males or females). The

Table 7: Reducing effect of Novaluron on the reproductive potential of *M. stabulans*

	After treatment of last instar larvae			After treatment of prepupae		
Dose (µg/larva)	Fecundity (mean No. of egg±SD)	Fertility (hatching %)	Sterility index	Fecundity (mean No. of egg±SD)	Fertility (hatching %)	Sterility index
5.0	---	---	---	---	---	---
1.0	---	---	---	073.5±1.5[d]	13.3	94.99
0.5	140.3±0.9[d]	76.8	27.12	135.8±1.6[d]	77.2	37.09
0.1	151.4±1.2[d]	82.4	13.38	146.8±1.7[d]	81.0	28.76
0.01	153.0±1.3[d]	88.1	07.77	152.7±1.2[d]	85.0	25.58
Control	192.3±1.8	98.5	-	194.8±2.5	100	-

d: Very highly significantly different ($p<0.001$)

major features of deformation had been seen in dwarf bodies with curly wings, atrophied mouthparts and ill-developed legs. All deformed adults perished within few days without mating.

Effect of novaluron on the reproductive capacity of *M. stabulans*: Important parameters of the reproductive capacity of *M. stabulans* have been presented in Table 7 as results of novaluron treatment on the early last instar larvae and prepupae. With regard to the fecundity (mean number of eggs/♀), novaluron exerted increasingly suppressive action on the oviposition females, regardless the time of treatment. In some detail, the female fecundity was drastically reduced after treatment of the early last instar larvae, in a dose-dependent fashion (153.0±1.3, 151.4±1.2 and 140.3±0.9 eggs/treated ♀, at 0.01, 0.1 and 0.5 µg/larva, respectively, compared to 192.3±1.8 eggs/control ♀). In a similar trend, fecundity was dramatically inhibited correspondingly to the dose level, after treatment of prepupae (152.7±1.2, 146.8±1.7, 135.8±1.6 and 073.5±1.5 eggs/treated ♀, at 0.01, 0.1, 0.5 and 1.0 µg/prepupa, respectively, in comparison to 194.8±2.5 eggs/control ♀).

In respect of the fertility (hatching% = hatchability), it was clearly indicated the inhibitory effect of Novaluron of the egg viability, regardless the time of treatment, since fertility was seriously reduced after treatment of last instar larvae, in a dose-dependent style (88.1, 82.4 and 76.8% reduction of fertility, at 0.01, 0.1 and 0.5 µg/larva, respectively, vs. 98.5% hatchability of control eggs) as noted in Table 7. Also, sterility index increased with the increasing dose level of Novaluron. Similarly, fertility was pronouncedly reduced after treatment of prepupae, in a dose-dependent manner (85.0, 81.0, 77.2 and 13.3% hatching of treated eggs, at 0.01, 0.1, 0.5 and 1.0 µg/prepupa, respectively, vs. 100% hatching of control eggs). In a comparable style, the sterility index increased (Table 7).

DISCUSSION

Findings from the present investigation indicated that the application of novaluron to the early last instar larvae of *M. stabulans* led to larval mortality in a dose-dependent manner.

Following application of the highest two doses against last instar larvae or treatment of prepupae with the highest dose, all developed pupae completely died. At other dose levels, pupal mortality has been noted in a dose-dependent style. Also, the mortality of adult has been noted in a dose-dependent manner after the treatment of larvae or prepupae with novaluron. Therefore, the present outcomes have been, to some extent, in accordance with some stated findings of toxicity of different IGRs against a number of insects, such as fenoxycarb against the parasitoid *Phanerotoma ocularis*[52] and juvenoid pyriproxyfen against the desert locust *Schistocerca gregaria*[53]. Larvicidal and pupicidal impact of methoprene has been reported on the common house mosquito *Culex pipiens*[54], the rice meal moth *Corcyra cephalonica*[55], the yellow fever mosquito *Aedes aegypti*[56] and the flesh fly *Sarcophaga ruficornis*[57]. Also, toxicities of other IGRs were reported against different insects, such as kinoprene against *C. pipiens*[58].

In the current investigation, larval mortalities of *M. stabulans* might be due to the prevention of larvae to split the old cuticle and expand the new one during moulting[59]. Additionally, the mortality of the larva might have resulted from continuous starvation[60]. In addition, pupal mortality might be directly due to failure of vital homeostatic mechanisms and/or some secondary factors, such as bleeding, suffocation and desiccation by imperfect exuvation[61].

On the other hand, in insects, however, LC_{50} of an agent differs according to various factors, such as its lethal potency as well as its concentration, the sensitivity of the insect and its developmental stage or instar, method and time of treatment and the experimental conditions. In this context, the early last

instar larvae of *M. stabulans* were more sensitive to novaluron than prepupae, in the present study. This finding coincides, to a great extent, with many works reporting that the early larval instars of different flies have been more sensitive than the later ones to some IGRs, such as *Ceratitis*[62].

In the present investigation on *M. stabulans*, prolonged pupal period and regressed developmental rate, following the treatment of last instar larvae with novaluron at different doses 0.5 and 0.1 µg/larva of, might be due to the disturbance of the neuroendocrine organs responsible for the synthesis and/or release of their hormones[63]. To explicate the shortened larval period after treatment of last instar larvae with novaluron and shortened pupal period after treatment of prepupae, this might be due to avoiding responses of these stages to novaluron as a xenobiotic agent.

In the current research, novaluron enforced the adult females of *M. stabulans* to survive short longevity. Current findings were, to some extent, in corroboration with many reports resulted in shortened longevity in different insects by several IGRs, such as *S. litura* by ecdysteroid RH-5849[64]. Present findings, the shortened longevity of *M. stabulans* adult females, might be an indication to the interference of novaluron with the hormonal activity in adults, while a close relation between special hormones and adult longevity has been noted in other insects[65,66].

After attainment of the sexual maturity in adult insects, different degenerative changes have been shown in some organs and tissues which could be collectively described as 'senility'. However, the disturbed adult longevity might be considered as a useful sign for the adult aging, i.e., shortened longevity might indicate the accelerated aging and *vice versa*. JH regulates the aging because of its ability for direct affects mechanisms of the somatic survival as reported by Yamamoto *et al*.[67]. Therefore, novaluron could influence the JH level and/or functions resulting in shortening of adult longevity, in the current investigations. Though, the particular mechanism of novaluron on the biochemical processes in adults still needs further investigation.

A dose-dependent regression of the pupation rate of *M. stabulans*, in the present study was recorded after treatment of last instar larvae with novaluron. In contrast, no affect was recorded on this rate after treatment of prepupae. These results were, to some extent, in agreement with some reported results in some insects by various IGRs which regressed the pupation rate[68,21].

Depending on the present results the adult emergence of *M. stabulans* was completely prevented after treatment of early last instar larvae with the higher two doses of novaluron (5.0 and 1.0 µg/larva) and after treatment of prepupae with the highest dose. In addition, the adult emergence was remarkably blocked at the other doses by different percentages. These results were, to a great extent, in corroboration with some reported results since adult emergence in different insect species was partially or completely hindered after larval treatment with various LGRs, such as *S. littoralis* after treatment with novaluron[35].

In this regard, it is critical to point out that the adult eclosion in insects is a vital process and regulated by the Eclosion Hormone (EH). Partial or complete disturbance of EH prohibit the adults to emerge[69]. The blocked adult emergence, in the present study, can be interpreted by a disturbing effect of novaluron on the adult EH release.

In the current study, a disruptive effect was exhibited by Novaluron on the pupal morphogenesis of *M. stabulans*, since different deformed pupae were observed after treatment of the last instar larvae. For interpretation of the anti-morphogenic activity of novaluron against pupae of *M. stabulans*, in the present study, it might exhibit suppressive effect on the chitin synthesis leading to prevent the normal deposition of new cuticle[70].

In the current investigation, some adult deformations were observed after treatment of last instar larvae and prepupae of *M. stabulans* with novaluron. The production of anomalous *M. stabulans* adult flies might be interpreted by an adverse action of novaluron on the hormonal balance during the adult metamorphosis, since the disturbance of ecdysteroid titer leads to changed activity of the lysosomal enzyme.

In the present study the female fecundity of *M. stabulans* was drastically inhibited after novaluron application on last instar larvae or prepupae. In the current investigation, fecundity of *M. stabulans* was drastically prohibited which might resulted from the interference of novaluron with some reproductive developments, including the ovarian follicle growth and the egg maturation. In some detail, this can be explained by some reasons as follows:

- Some disorders in ovaries might be caused by novaluron[71]
- Novaluron might disrupt the synthesis of proteinaceous contents during the oogenesis[72]
- Novaluron might disturb the production and/or function of the gonadotropic hormone responsible for vitellogenesis (yolk precursors)[73]

- Eggs might develop normally in ovaries but they could not be lay, owing to the severely deformed ovipositor or to the reduced mechanical strength[74] or their reabsorption before oviposition[75]

In the present study, the fertility of *M. stabulans* adult females was seriously reduced after treatment of last instar larvae or prepupae with novaluron. This result was in accordance with some noted outcomes of reduced fertility in different insects by some IGRs, such as *L. dispar*[76] by methoxyfenozide.

In the current research, several recommendations might be given for explicating the fertility reduction in *M. stabulans* by novaluron, (1): Vitellogenins are necessarily required for the embryogenesis in the insect egg[77]. These vitellogenic metabolites are synthesized mainly by fat body during the developmental stages or inside the ovary itself[78]. Novaluron might disrupt the production and/or accumulation of these metabolites in adult females of *M. stabulans*, (2): Novaluron might disrupt the vitellogenin deposition into oocytes through the inhibition of gonadotropic hormone responsible for the egg maturation or/and exhibit a disruptive effect on the intracellular spaces in the follicular epithelium[79], (3): From the treated *M. stabulans* immature stages, some residual amounts of novaluron penetrated into mothers and then into eggs. The residual material affected the cuticle synthesis resulting in weakened mouth parts of mature embryos to perforate the vitellin membrane for hatching[42], (4): In the current study, reduction of *M. stabulans* fertility might be due to the negative influence at certain stages of novaluron on the developing embryos.

CONCLUSION

Depending on the present results, novaluron exhibited toxicity against larvae, pupae and adults of *M. stabulans*. Also, the tested compound exerted disruptive action on development, metamorphosis and morphogenesis of pupae and adults in addition to its reducing action on fecundity and sterilizing activity. Therefore, novaluron can be used as an effective IGR in the integrated control program for this medically and veterinary serious fly.

SIGNIFICANCE STATEMENT

This study discovered the tested compound, novaluron that can be beneficial for impairing the developmental and reproduction of *M. stabulans*. This study will help the researchers to uncover some critical areas of the control of the present medically dangerous fly that many researchers were not able to explore.

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