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Effect of the Non-steroidal Ecdysone Agonist, RH-5849, as a Control Agent Against the False Stable Fly, *Muscina stabulans* (Falle'n)

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Abstract: The non-steroidal ecdysone agonist, RH-5849, was assessed as IGR against the false stable fly *Muscina stabulans* (Falle'n). A dose range of 0.025, 0.100, 0.400, 1.000 and 4.000 μg/insect was topically applied on 1 day old 3rd larval instar and the newly moulted pupae. The larval mortality was increased by increasing RH-5849 doses. While the pupal and adult mortalities which resulted from treated larvae were increased by decreasing doses. On the other hands, the higher of pupal deformation (40.3%) was obtained due to effect the dose of 1.000 μg/larva. Although some various deformities of the adult stage, there is no certain trend could be encountered for the adult deformities. No perfect adults could be emerging as affected by RH-5849 at the dose levels of 4.000 and 1.000 μg/larva. In case of treated pupae with RH-549 the percentage of pupal mortalities were increased by increasing the dose levels. Such relation could not determine completely for the adult mortalities or even the adult deformities. It is noteworthy to mention that, sex ratio of adults was encountered as two fold males than females after treating the newly moulted pupae with 1.000 μg /pupa. Some other data of sex ratio indicated the male predominance after treating the larvae with 0.025 or 0.100 μg/larva. RH-5849 was severely suppressed the reproductive performance of *M. stabulans*, particularly at the two higher doses of 1.000 and 4.000 g/larva or /pupa. Also the resulted deformed females were not lay eggs.

Key words: IGR, RH-5849, *Muscina stabulans*, percent mortalities, morphogenic, reproduction potential

INTRODUCTION

The use of the classical insecticides to control the harmful insects has given rise not only to many adverse effects on man and his environment but also to development of resistant strains or treated insects. There exist, therefore, a bad need to find more effective and safer compounds for chemical control of insects (El-Ibrashy, 1984). The non-steroidal ecdysone, RH-5849, represents a new class of insect growth regulators (Aller and Ramsay, 1988). It was tested against insects of various orders showing different effects on development and reproduction in purpose to use it as safe control agent. Wing (1988) on *Drosophila*, Wing *et al.* (1988) on *Manduca sexta*; Gadenne *et al.* (1990) on *Ostrinia nubilalis*, Nassar and Abdullah (2005) on the Indian meal moth; Ghoneim (1991) and Ghoneim *et al.* (2001) on *Musca domestica*.

The present investigation was conducted to asses the potency of the non-steroidal ecdyson mimic, RH-5849, on the different developmental criteria and also on the productivity potentially of one of the medically important pest, *Muscina stabulans* (Falle'n).

MATERIALS AND METHODS

Insect culture: A susceptible strain of *Muscina stabulans* (Falle'n) was used for this study from the permanent culture at the Entomology Department, Faculty of Science, Cairo University in October 2003. The culture was maintained at 25±°C and 50-60% RH. Adult flies have been confined in wooden cages of 45×45×45 cm and given sugar, water and meat. Water was supplied by dipping a piece of cotton, as a wick in a bottle filled with water. The meat was introduced in a petri-dish and changed daily. Larvae were supplied with meat and saw dust in the breeding pans covered with muslin fitted with rubber bands. All stages were checked daily. Culture was kept and continues according to the method of Nassar (1989) with some modification.

The non-steroidal ecdysone agonist, RH-5849: The RH-5849 is a product synthesized by the Research laboratories, Rohm and Hass Company, in Pennsylvania, USA. Its chemical formula is 1,2-dibenzoyl-1-tert-butylhydrazine.

Treatments of the larvae and pupae: The 24% EC of RH-5849 was diluted to obtain the dosages of: 0.025, 0.100. 0.400, 1.000 and 4.000 μg/insect. Two different stages were undergone to the treatment of RH-5849: 1 day old last larval instar and newly moulted pupae. All these stages received the compound topically on the thoracic dorsum by means of Hamilton micropipette in one mL water. Four replicates (50 insect/replicate) were used per each dosage.

Similar replicates were used for the control insects without any treatment.

Morphogenic and developmental parameters: Just after the day of treatment, the insects were checked daily to estimate all morphological effects could be occurred by the action of RH-5849. Mortalities, as larvae and pupae were counted and presented as percentages relatively to the total number of treated larvae. As well as, mortalities as adults were counted and presented as percentages relatively to the number of treated pupae or the number of perfect pupae (those produced from the treated larvae). The morphogenic efficiency of the compound used according to Vargas and Sehnal (1973). For calculating the developmental durations, (Dempster, 1957), was used and for calculating the developmental rates (Richard's, 1957) was used.

Reproductive potentially: The normally appeared treated pupae were transferred from the breeding pans into small cages (20×20×20 cm) in order to study the reproduction. After adult emergence, females only were used to estimate the fecundity and sterility index.

Statistical analysis: Data obtained were analysed using the t-test distribution (Moroney, 1956) for the test significance of difference between means.

RESULTS AND DISCUSSION

Effect on survival potential and morphogenesis: Results of Table 1 reaveled that the insecticidal properties of the compound increased initially by the increasing dosages (67.0 and 18.4% larval mortalities at the dose levels of 4.000, highest and 0.025, lowest μg/larva vs. 4.0% larval mortality of control corresponding. These died larvae have been morphologically found to suffer from premature lethal moult. The premature moult could be induce and initiate by RH-5849 in *M. Sexta* (Wing, 1988: Wing *et al*., 1988). Also, Gadenne *et al*. (1990) reported the premature moult by the RH-5849 in the last instar larvae of *O. nubilalis*; Ghoneim *et al*. (2001) recorded some degrees of premature lethal moult for *M. domestica* by the action of the same compound, RH-5849, after treatment of 1 day old and 4 day old larvae.

In the present study, the cumulative (pupal and adult) mortalities reversibly increased. Pupal mortalities were clearly observed in a reverse course of the dose levels (03.3 till 23.0% pupal mortalities at the dose levels of 4.000 and 0.025 μg/larva). The same tendency could be easily observed during the adult stage with an exceptional case of the mortality % caused by the lowest dosage.

Regarding to the morphological defects appeared during the pupal and adult stages (with an exceptional of the pupal deformities caused by the highest dosage), pupae have been affected in a dose-dependent trend. The meet tremendous malformations of pupae have been resulted as a response of the RH-5849 action at the dosage of 1.000 μg/larva. No certain trend could be appreciated for the same score of adult, albeit some various degrees of deformities were determined. While the Morphogenetic Efficiency (ME) of the compound was deduced as 86.36 for pupae, previously treated as larvae with the highest dosage 4.000 μg/larva, the morphogentic effect of the compound was counted as 34.92 for adults produced from larvae treated with 0.100 μg/larva. These results agreed with the finding of many authors against *M. domestica*. Abou El-Ela *et al*. (1990) by using altosid ZR-515; Bakr *et al*. (1991) by using Dimilin, Bay Sir-8514 and altosid ZR-515 and Ghoneim (1991) using RH-5849.

The percentage of pupal mortality rates was clearly observed in a consecutive relationship to the dose levels (71.4 and 15% pupal mortality at the dose levels of 4.000 and 0.025 μg/pupa) (Table 2). Similar relationship could not maintain completely for the adult mortalities or even for the adult deformities. The compound, RH-5849, at the highest dosage, deranged completely the emergence of perfect adults. The other dose levels caused different prohibition degrees of perfect adult eclosion. These results of the adult eclosion as affected by the action of the ecdysone mimic agree to some extent with the results obtained by Ghoneim (1991) recorded different degrees of the blockage of adult emergence and they had shown that the highest concentration rate of RH-5849 caused the highest degree of adult eclosion blockage. The author obtained the highest degree of adult blockage by the concentration rate of 200 ppm (the conc. Just before the highest one) if the RH-5849 was applied to 4 day old larvae. They concluded the 1 day old larvae were more sensitive to RH-5849 than the 4 day old larvae. The obtained results assorted in Table 1 and 2 show an agreement with the reverse findings of Ghoneim (1991) in respect to this criterion.

Referring to sex ratio as affected by the action of RH-5849, Table 1 shows no remarkable deviations far from the sex ratio of the control adults. It is noteworthy to mention that; sex ratio of adults was encounted as two fold of females than males after treatment of the newly molted pupae with 1.000 μg/pupa of RH-5849. Some other data of sex ratio indicated the female predominance after treating larvae with 0.025 or 0.100 μg/larva or pupa. On the contrary, many authors measured a suppressing action of several chitin inhibitors on the development of immature stages (Bakr *et al*., 1991; Osman, 1984; Soltani *et al*., 1989; Ghoneim *et al*., 2001; Vasuki and Rajavel 1992; Van Laeck, 1993a, b; Ghoneim and Ismail, 1995; Mohapatra *et al*., 1996; Smagghe and Degheele, 1994). However, the presence of variation in developmental effects of chitin

Table 1: Percent mortalities and morphogenic effects of RH-5849 on the different developmental stages of *Muscina stabulans* treated as 1-day old last instar larvae

Doses	% larval	% Pupation				% Adult emergence				Sex ratio		
(µg/larva)	Mort.	Mort.	Deform. (a)	Pupa (b)	ME (c)	Mort.	Deform. (a)	Adult (b)	ME (c)	Male		Female
control	04.0	02.0	00.0	93.8	---	06.0	---	87.8	---	50.6	:	49.4
0.025	18.4	23.0	16.8	41.8	20.24	08.2	05.3	28.3	19.04	61.4	:	38.6
0.100	24.6	16.8	27.2	31.4	31.91	13.6	11.2	06.6	34.92	57.1	:	42.9
0.400	35.2	12.0	38.6	14.2	59.23	06.4	04.4	03.4	31.30	50.0	:	50.0
1.000	47.2	09.6	40.6	0.26	77.14	01.6	01.0	---	32.00	---	:	---
4.000	67.0	03.3	28.7	1.0	86.36	01.0	---	---	---	---	:	---

(a) Deformities: mean all shapes of failure of larvae to pupate or failure of pupae to adult stages, (b) Means: normal pupae or normal adults, (c) ME: Means morphogenic efficiency is a percentage calculated for the compound used by the equation of Vargas and Sehnal (11) as follows: ME = Malformed insects All insects succeeded to metamorphose

Table 2: Percent mortalities and morphogenic effects of RH-5849 on the different developmental stages of *Muscina stabulans* (Falle'n) topically applied as newly moulted pupae

Doses	% pupal	% Adult emergence				Sex ratio		
(µg/larva)	Mortalities	Mort.	Deform. (a)	Adult (b)	ME (c)	Male		Female
Control	02.4	04.8	---	92.8	---	50.6	:	49.4
0.025	15.0	11.8	20.4	52.8	24.12	60.4	:	39.8
0.100	23.4	29.8	15.2	31.6	20.26	63.5	:	36.5
0.400	30.0	38.2	18.6	13.2	26.42	50.0	:	50.0
1.000	42.8	40.4	12.6	---	21.73	66.7	:	33.3
4.000	71.4	---	28.6	---	100.00	---	:	---

(a), (b) and (c): see the footnote of the Table 1

Table 3: Reproductive potentiality of adult female of *Muscina stabulans* (Fallen) as influenced by the action of RH-5849

Doses	Flies treated as pupa	
(µg/larva)	Fertility	Fecundity
control	189.3±1.64	167.4±2.5
0.025	145.2±2.31	113.7±1.81
0.100	116.3±1.56	68.7±2.11
0.400	86.7±1.32	33.4±1.77
1.000	00.0±00	00.0±00
4.000	-	-

(a), (b) and (c): see the footnote of the Table 2

inhibitors may be largely due to the large species-variation in respect to relative potency of this compound. This variation may, also be resulted from the different mechanism of ecdysteroid metabolism existing in different insects.

Effect on the reproductive potentiality: The higher dose levels (1.000 and 4.000 g/larva) when found as deformed. These deformed females could not lay eggs (Table 3). The same case of precluded oviposition was obtained only by the highest d osage when applied on the pupa using RH-5849 of adult Coleoptera: *Epilachna varivestis, Leptinotarsa decemlineata* and *Anthronomus granadis*, affects prevention or cessation of oviposition (Aller and Ramsy, 1988). They also found that feeding of neonate adult *M. domestica vicina* on diet treated with RH-5849 inhibited the oviposotion. In addition, Wing (1988) and Wing *et al.* (1988) reported the RH-5849 inhibited of the ovarian development, affecting prevention or cessation of oviposition in the lepidopterous larvae of *M. sexta*. All other dosages of RH-5849, in the present study, severely suppressed the female fertility and fecundity. Where female fertilities were, 145.2, 116.3 and 86.7 after pupal treatment with the doses of 0.025, 0.100 and 0.400 µg/insect, respectively comparison to 189.3 of control fertility. The same previous doses were suppressed the female fecundity by 113.7, 68.7 and 33.4 hatched eggs/female, respectively comparison to 167.4 of control fecundity (Table 3). To some extent, Ghoneim (1991) reported similar results, owing to the action of RH-5849 in the house fly, *M. domestica vicina*. Current data show the sterility indices were pronouncedly dose-dependent. It should be overlooked that such trend of effectuation was reversed by the hatching percentages (egg viability). The egg viability increased by the decreasing dose levels of RH-5849 and vice versa. This was also that finding when the topical application was carried out for pupae. Such our results disagree with that results obtained by Aller and Ramsy (1988) for some coleopterans. Reviewing our results, a case had turning the attention, that was the hatchability of the very few eggs oviposited by females after treatment of the pupae with 1.000 µg/pupa. These very few eggs were completely non-viable.

In conclusion, the non-steroidal ecdysone mimic, RH-5849, was found affecting not only the development and metamorphosis but also the reproductive performance of the false stable fly, *Muscina stabulans*. Some authors tested the same ecdysonoid against Coleopterans, lepidopterans and also dipterans. They concentrated their attention on the ecdysis aiming to explore the role of this ecdysonoid. Using our experimental insect, *M. stabulans*, we should conduct some other studies in future for this purpose, partially or exactly, depending on the facilities and equipment available.

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