

IMPAIRED SURVIVAL AND DEVELOPMENT OF THE FALSE STABLE FLY, *MUSCINA STABULANS* (FALLEN) (DIPTERA: MUSCIDAE) BY PYRIPROXYFEN (A JUVENILE HORMONE ANALOGUE)

By

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Abstract

The false stable fly *Muscina stabulans* (Diptera: Muscidae) is worldwide distributed with medical, veterinary and forensic importance. This study investigated the disruptive effects of Pyriproxyfen on survival, development, metamorphosis and morphogenesis of this fly. A series of Pyriproxyfen (5.0, 1.0, 0.5, 0.1 & 0.01 µg/larva) was topically applied onto the early last (3rd) instar larvae and prepupae. Regardless the time of treatment, different larval, pupal and adult mortalities were recorded. LD₅₀ values of pyriproxyfen were calculated as 0.242 & 0.444 µg/stage, respectively. Pyriproxyfen exhibited a shortening action on larval and pupal durations, with an exception of prolongation of pupal duration after treatment of the early last instar larvae. Adult longevity was pronouncedly shortened; and pupation was suppressed regardless the time of treatment. Adult emergence was partially blocked parallel to the dose level or in no certain trend. Various percentages of larval-pupal intermediates were recorded after treatment of early last instar larvae and some pupal-adult intermediates were produced after treatment of prepupae with the higher three doses. After prepupae treatment with lower three doses of pyriproxyfen, some failed to metamorphose into pupae and remained as permanent ones. Some deformed pupae were observed only after treatment of the last instar larvae. Topical application of pyriproxyfen onto the last instar larvae or prepupae caused emergence of some deformed adults. All deformed flies died within few days without mating.

Keywords: Adult, emergence, larva, longevity, metamorphosis, morphogenesis, mortality, pupa, toxicity.

Introduction

The intensive and discriminate uses of many conventional insecticides led to several dangerous problems, such as the environmental hazards, destruction of the natural enemies, serious toxicological problems to humans, as well as the development of insect resistance toward different insecticides (Rose, 2001; Davies *et al*, 2007; Costa *et al*, 2008; Mosallanejad and Smaghe, 2009). The alternative control agents were searched to minimize the insecticide hazards. In the last few decades, new class safe compounds were developed (IGRs) insect growth regulators (Dhadialla *et al*, 1998; Khan and Qamar, 2012). IGRs were not directly toxic, but act selectively on the development, metamorphosis and/or reproduction of the target pests (Nicholas *et al*, 1999; Martins and Silva, 2004) owing to their disruptive effects on the activity of endocrine system (Wang and Liu, 2016). On the basis of the mode of

action, IGRs were grouped in three categories: i- Juvenile hormone analogues (JHAs) (=Juvenoids), ii- Ecdysteroids or ecdysone agonists and iii- Chitin synthesis inhibitors (CSIs) or moult inhibitors (Dhadialla *et al*, 1998; Oberlander and Silhacek, 2000).

Pyriproxyfen was registered as Knack[®], Sumilarv[®] and Admiral[®]; (Sumitomo Chemical Co., Japan for controlling public health pests (Yokoyama and Miller, 1991). It is a stable compound (Mohandass *et al*, 2006) and safe for a variety of predatory arthropods, compatible with natural enemy conservation, less toxic to ecosystem and mammals (Naranjo *et al*, 2003; Korrat *et al*, 2012) with mild toxicity to some aquatic organisms, but nontoxic to bees (Dhadialla *et al*, 2005). Pyriproxyfen is a potent JHA (Hatakoshi, 2012) affecting the endocrine regulation in insects resulting thereby in a strong suppression of embryogenesis, metamorphosis and adult formation in several insects

(Ishaaya and Horowitz, 1995; Aribi *et al.*, 2006). It suppressed oviposition, reduced eggs viability (Ghasemi *et al.*, 2010; Ohba *et al.*, 2013), reduced fecundity (Singh and Kumar, 2015), and affected some biochemical processes (Nasr *et al.*, 2010). Pyriproxyfen was successfully used to control pests of many agricultural, horticultural crops (Korrat *et al.*, 2012) and medical insect-vectors (Sazo *et al.*, 2008; Moadeli *et al.*, 2014).

The false stable fly *Muscina stabulans* (Diptera: Muscidae) is a blood-sucker fly (Grzywacz *et al.*, 2015). Its maggots were frequently found in household wastes and animal excreta, decaying vegetable matter and poultry houses (Queiroz and Carvalho, 1987). Laos *et al.* (2004) reported that *M. stabulans* often inhabits latrines and habitats created by agriculture and other human activities. The maggots caused myiasis in animals and humans (Zumpt, 1965), with forensic importance as maggots attacked exposed and diseased human body (Schroeder *et al.*, 2003; Gaudry, 2010) as well as dead ones (Grzywacz *et al.*, 2017). Gunn and Bird (2011) reported that larvae were accidentally seen moving of buried corpses in tombs. *M. stabulans* is a mechanical vector of diseases, its larvae can prey on immature stages of other dipterous species as an effective control agent (Duarte *et al.*, 2013).

The present study aimed to investigate the disruptive effects of pyriproxyfen on survival, development, metamorphosis and morphogenesis of *M. stabulans* (Fallen).

Materials and Methods

A culture of susceptible strain of the false stable fly *Muscina stabulans* was established at the Department of Zoology and Entomology, Faculty of Science, Al-Azhar University, under controlled laboratory conditions (25±2°C, 55±5% R.H., photoperiod 12h L & 12h D) for several generations. The culture

was originated by a sample of maggots collected from cow's manure. Maggots (larvae) were fed on an artificial diet (200g wheat bran, 100g powdery milk, 3g yeast & 200ml water) after Busvine (1962). Feeding larvae were kept in breeding pans covered with muslin and fitted with rubber bands. After pupation, pupae were gently collected and confined in Petri-dishes into wooden cages (45x45x45cm). Each cage was provided with wire-gauze sides except the bottom and front side which was fitted with a small circular window attached with a cloth sleeve for feeding, handling and cleaning. Emerged adults were supplied with a food as milk diet and a piece of cotton soaked in 10-15% sugar solution.

Pyriproxyfen, a juvenile hormone analogue with a commercial name: Admiral 10% S C, Sumitomo® and systematic one: 2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine with the molecular formula: C₂₀H₁₉NO₃, was kindly obtained from Plant Protection Research Institute, Giza. Pyriproxyfen was diluted with acetone to five dose levels: 5.0, 1.0, 0.5, 0.1 & 0.01µg/larva. Fifty larvae in ten replicates (5larvae/replicate) of the early last (3rd) instar and same number of prepupae were topically treated, individually, with each dose using Hamilton microapplicator (NHN 737). Similar number of replicates of early last instar larvae and prepupae were only topically treated with 1µ acetone as controls. All treated and control replicates were confined in small tubes and kept under the previously mentioned laboratory conditions. They were checked daily for feeding of larvae and recording all criteria of study.

Toxicity of pyriproxyfen was determined by mortality. All mortalities of treated and control (larvae, pupae and adults) were recorded every day and corrected according to Abbott's formula (Abbott, 1925) as follows:

$$\text{Corrected mortality\%} = \frac{\% \text{ of test mortality} - \% \text{ of control mortality}}{100 - \% \text{ of control mortality}} \times 100$$

LD₅₀ values were calculated by Microsoft office Excel, 2007 (Finny, 1971).

Developmental durations of larvae and pupae had been calculated (mean days±SD).

Richard's equation (1957) was used to calculate developmental rate. Total longevity of adult females was calculated $M \pm SD$ days.

Metamorphosis and morphogenesis: Pupation rate was expressed in % of the developed pupae. Adult emergence was determined in % (Jimenez-Peydro *et al.* (1995) as No. of completely emerged adults / No. of pupae $\times 100$. All of the possible aberrations of metamorphosis and morphogenesis were calculated in %.

Statistical analysis: Data were analyzed by Student's *t*-distribution, and refined by Bessel correction (Moroney, 1956) for the test significance of difference between means.

Results

Toxic effect of pyriproxyfen: Mortalities among larvae, pupae (puparia) and adults, after one topical application of pyriproxyfen onto early last (3rd) instar larvae were assorted (Tab. 1). Pyriproxyfen exhibited toxic effect parallel to its dose level because the larval mortality was in a dose-dependent course. Developed pupae completely died at dose (5.0 μ g/larva) and various percentages of mortality were in no certain trend. But, adult mortality was in a reverse relation to pyriproxyfen dose, i.e., increasing mortality % with the decreasing dose level (4.8, 3.7, 6.9 & 11.1% adult mortality, at 1.0, 0.5, 0.1 & 0.01 μ g/larva, respectively, vs. 6.4% mortality of control adults). LD_{50} was calculated as 0.242 μ g/larva.

After topical application of pyriproxyfen onto prepupae, mortalities of different developmental stages were arranged (Tab. 2). Larval mortality% was recorded in no certain trend but pupal mortality% was in a dose-dependent course. Adult mortality% was recorded in a similar trend. LD_{50} was found 0.444 μ g/prepupa. Early last instar larvae were more sensitive to pyriproxyfen than prepupae.

After topical application of pyriproxyfen onto early last instar larvae, data of influenced durations of larvae, pupae and adults were listed (Tab. 3). Depending on these data, larval duration was slightly shortened. In

contrast, the pupal duration was significantly prolonged, especially at the doses 1.0, 0.5 & 0.1 μ g/larva (8.1 \pm 3.11, 8.1 \pm 3.11 & 8.0 \pm 1.84 days, respectively, vs. 6.6 \pm 1.38 days of control pupae). Developmental rate of treated pupae was remarkably regressed at these three dose levels. Adult longevity was pronouncedly shortened, in a dose-dependent course (10.7 \pm 1.70, 8.3 \pm 0.94, 5.3 \pm 0.48 & 5.1 \pm 0.40 days, at 0.01, 0.1, 0.5 & 1.0 μ g/larva, compared to 19.2 \pm 0.20 days of control adult females).

After topical application of pyriproxyfen onto prepupae, the development data were summarized (Tab. 4). Pupal duration was insignificantly shortened at the majority of doses except dose 0.1 μ g/prepupa at which pupal duration was shortened and developmental rate was conspicuously promoted. Adult longevity was significantly shortened, in a dose-dependent fashion (13.5 \pm 1.28, 10.0 \pm 0.80, 8.3 \pm 2.88, 6.8 \pm 0.12 & 6.7 \pm 0.12 days, at 0.01, 0.1, 0.5, 1.0 & 5.0 μ g/prepupa, respectively, vs. 19.7 \pm 1.50 days of controls).

Topical application of pyriproxyfen onto the early last instar larvae resulted in different features of impaired metamorphosis program (Tab. 5). Pupation rate was suppressed in a dose-dependent course. Adult emergence was partially blocked in no certain trend. An interesting feature of development impairment was the production of larval-pupal intermediates in no certain trend.

Topical application of pyriproxyfen onto the prepupae showed various features of deranged development (Tab. 6). Pupation was suppressed in no certain trend. Adult emergence was increasingly blocked parallel to dose (89.6, 78.7, 68.1, 50.0 & 29.8% emergence, at 0.01, 0.1, 0.5, 1.0 & 5.0 μ g/ prepupa, respectively, vs. 98.0% controls).

One of the most attractive features of impaired development was pupal-adult intermediates production after topical application of higher three doses onto prepupae. Some treated prepupae failed to metamorphose into pupae and survived for long period as permanent prepupae, after treatment with the

lower three doses.

As to morphogenesis, pyriproxyfen exhibited a disruptive effect on pupal morphogenesis only after treating early last instar larvae with some deformed pupae, in no certain trend, after larval treatment (2.4, 14.7, 18.2, 12.0 & 7.1% of malformed pupae, at 0.01, 0.1, 0.5, 1.0 & 5.0 µg/larva, respectively, vs. 0% control deformity). All malformed pupae perished without metamorphosis into adults.

In respect of the adult morphogenesis, topical application of pyriproxyfen onto the last instar larvae resulted in the emergence

of some deformed adults, in a reverse relation to the dose level (19.4, 13.8, 11.1 & 00.0% deformed adults, at 0.01, 0.1, 0.5 and 1.0 µg/larva, respectively, vs. 00.0% deformation among control adults). Pyriproxyfen exhibited a similar anti-morphogenic action on the successfully emerged adults after treatment of prepupae. The percentages of deformed adults were recorded in no certain trend. The morphologically deformed adults had dwarf bodies, curly wings, atrophied mouth parts and ill-developed legs. All of them died within few days without mating.

Table 1: Toxic effect (%) of pyriproxyfen 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	72	100	---	100	100	0.242
1.0	50	16	4.8	60	54.6	
0.5	34	18.2	3.7	48	40.9	
0.1	28	19.4	6.9	46	38.6	
0.01	16	14.3	11.1	36	27.3	
Control	4	2.1	6.4	12	---	

No adult mortality as none emerged.

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

Dose (µg/prepupa)	Prepupal mortality	Pupal mortality	Adult mortality	Total mortality	Corrected mortality	LD ₅₀ (µg/prepupa)
5.0	6	70.2	28.6	80	78.7	0.444
1.0	4.0	50.0	20.8	62	59.6	
0.5	6.0	31.9	18.8	48	44.7	
0.1	6.0	21.3	16.2	38	34.0	
0.01	4.0	10.4	4.7	18	12.8	
Control	0.0	2.0	4.1	6	---	

Table 3: Developmental durations of pyriproxyfen *M. stabulans* topical treated as early last instar larvae.

Dose (µg/larva)	Larval duration	Pupal duration	Pupal developmental	Adult longevity
5.0	3.8±0.4 a	---	---	---
1.0	3.7±0.5 a	8.1±3.11 b	12.32	05.1±0.40 d
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

No pupal duration measured as larval-pupal stage died; morphologically normal pupae and deformed pupae. a: insignificant different (P>0.05), b: significant different (P<0.05), d: very highly significant different (P<0.001)

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

Dose (µg/pupa)	Pupal duration	Pupal developmental	Adult longevity
5.0	6.4±1.60 a	16.46	06.7±0.12 d
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

a, b, d: See previous footnote (Tab. 3).

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

Dose (µg/larva)	Larval-pupal intermediates (%)	Pupal stage		Adult stage	
		Pupation (%)	Deformities (%)*	Emergence (%)	Deformities (%)**
5.0	04.0	28	07.1	---	---
1.0	06.0	50	12.0	84.0	00.0
0.5	08.0	66	18.2	81.8	11.1
0.1	12.0	72	14.7	80.1	13.8
0.01	10.0	84	02.4	85.7	19.4
Control	00.00	96	00.0	97.9	00.0

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

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

Dose (µg/prepupa)	Permanent prepupae	Pupal-adult intermediates	Pupal stage		Adult stage	
			Pupation	Deformities*	Emergence	deformities**
5.0	0.0%	2.1%	94%	0.0%	29.8%	28.6%
1.0	0.0%	4.2%	96%	0.0%	50.0%	20.8%
0.5	6.0%	2.9%	94%	0.0%	68.1%	06.3%
0.1	6.0%	0.0%	94%	0.0%	78.7%	24.3%
0.01	4.1%	0.0%	96%	0.0%	89.6%	27.9%
Control	0.0%	0.0%	100%	0.0%	98.0%	00.0%

*&**See previous footnote (Tab. 5).

Discussion

Several insect growth regulators (IGRs) had been reported to exhibit toxic effects on different insect species. However, toxic effects of pyriproxyfen on some insect species had been reported, such as the Sunn pest *Eurygaster integriceps* (Mojaver and Bandani, 2010), the migratory locust *Locusta migratoria* (Hu *et al.*, 2012), the tobacco cutworm *Spodoptera litura* (Kaur and Chandni, 2015) and the lawn armyworm *Spodoptera mauritia* (Resmitha and Meethal, 2016), the southern house mosquito *Cx. quinquefasciatus* (Khan *et al.*, 2016) and the pink boll worm *Pectinophora gossypiella* (Sabry and Abdou, 2016). The present results, to some extent, agreed with the previously reported results, since topical application of pyriproxyfen onto early last (3rd) instar larvae resulted in larval mortality in a dose-de-pendent course. All pupae died at the highest dose and various pupal mortalities were caused by other doses. Adult mortality was observed in a reverse relation to the dose level. After topical application onto prepupae, larval mortality was recorded in no certain trend but pupal and adult mortalities were observed in the dose-dependent course. However, the present larval mortality might be attributed to the moulting pre-

vention to gain air for splitting the old cuticle and expand the new one (Linton *et al.*, 1997). Larval mortality might be due to feed lack and starvation (Ghoneim *et al.*, 2000). Pupal mortality could be related to hormonal activity of pyriproxyfen or other causes, as suffocation, bleeding, desiccation, or failure of vital homeostatic mechanisms (Smagghe and Degheele, 1994). Adult mortality might be due to retention and distribution of pyriproxyfen in insect body by direct and rapid transport via haemolymph to other tissues, and/or by lower detoxification capacity of the adults (Osman *et al.*, 1984). In the current study, pyriproxyfen LD₅₀ was calculated as 0.242 & 0.444µg/insect after larvae & prepupae treatment, respectively. This finding agreed with many studies reporting that the early larval instars of flies were more susceptible than later ones to some IGRs, such as *Musca domestica* (Fouda *et al.*, 1991), *Lucilia cuprina* (Friedel and Mc-Donell, 1985), *Fannia* spp. (Meyer *et al.*, 1987) and *Ceratitis capitata* (Vinuela *et al.*, 1993).

In the present study, pyriproxyfen exhibited a major shortening action on larval and pupal durations, regardless time of treatment. These results were concomitant to short larval duration of some insects after IGRs treatment, as the desert locust *Schis-*

tocerca gregaria treated with lufenuron (Bakr *et al*, 2008), *P. gossypiella* treated with methoxyfenozide (Sabry and Abdou, 2016) and *P. unionalis* treated with novaluron (Ghoneim *et al*, 2017b). The present shortened larval and pupal durations might be due to their avoiding pyriproxyfen adverse action, as a xenobiotic agent. Pyriproxyfen might prevent nuclear receptors formation of the cells, caused disturbance in developmental durations (Riddiford and Truman, 1993).

In the present study, Adult longevity was pronouncedly shortened, in a dose-dependent course, regardless the time of treatment with pyriproxyfen. This result agreed with the shortening action of various IGRs, such as *S. littoralis* by Novaluron (Hamadah *et al*, 2015); the Oriental fruit moth *Grapholita molesta* (Reinke and Barrett, 2007) and the beet armyworm *Spodoptera exigua* (Luna *et al*, 2011) by methoxyfenozide; *G. pyloalis* by lufenuron (Aliabadi *et al*, 2016); *P. gossypiella* by chlorfluazuron (Kandil *et al*, 2005; Salem, 2015). The shortened adult longevity might be attributed to interference of pyriproxyfen with the hormonal regulation of adult longevity because a close relation between certain hormones and adult longevity was reported in other insects, such as the vinegar fly *Drosophila melanogaster melanogaster* (Broughton *et al*, 2005; Carbone *et al*, 2006; Chamseddin *et al*, 2012).

The insect fat body serves many vital functions (Arrese and Soulages, 2010) and thus, the longevity mechanisms occurred within this tissue (Hwangbo *et al*, 2004). Pyriproxyfen might adversely affect the fat bodies resulting in shortened adult longevity.

In the present study, pupation was drastically inhibited, in a dose-dependent course or in no certain trend, depended on pyriproxyfen treatment. This result was more or less consistent with the report of regressed pupation rate of some insects by the suppressive action of various IGRs, such as the grey flesh fly *Parasarcophaga argyrostoma* by pyriproxyfen (Ismail, 1995) and chlorfluazuron (Ghoneim and Ismail, 1995); the

diamondback moth *Plutella xylostella* by hexaflumuron (Mahmoudvand *et al*, 2012); *S. littoralis* by novaluron (Ghoneim *et al*, 2015); *G. pyloalis* by lufenuron (Aliabadi *et al*, 2016) and fenoxycarb (Singh and Tiwari, 2016); the whitefly parasitic wasp *Encarsia formosa* by pyriproxyfen and fenoxycarb (Wang and Liu, 2016); *P. gossypiella* (Ghoneim *et al*, 2017a) and *P. unionalis* (Ghoneim *et al*, 2017c) by novaluron. The inhibited pupation might be due to an inhibitory effect of this compound on the synthesis of specific storage proteins by fat body on last larval instar and their deposition at pupation time (Gupta, 1985).

In the present study, adult eclosion was hindered after topical application of methoprene onto larvae of *Aedes aegypti* (Braga *et al*, 2005), *Cx. cephalonica* (Tripathi and Tiwari, 2006), *Cx. quinquefasciatus* and *Ae. albopictus* (Khan *et al*, 2016; Bibbs *et al*, 2017). Pyriproxyfen treatment resulted in the inhibition of adult emergence of *P. argyrostoma* (Ismail, 1995), *Cx. quinquefasciatus* and *Aedes albopictus* (Khan *et al*, 2016), *D. melanogaster* (Benseba *et al*, 2015) and *E. formosa* (Wang and Liu, 2016). These results more or less agreed with the previously reports, since pyriproxyfen topical application onto last instar larvae or prepupae partially blocked adult emergence. Thus, it was important to emphasize that the adult emergence in insects is a crucial physiological process and regulated by the eclosion hormone. Disturbance of this hormone partially or completely arrested the adults to emerge (Josephraj Kumar *et al*, 1999). On the molecular basis, the JH mimics compounds might cause miss-expression of certain genes, particularly the brood complex (*br-C*) transcription factor gene, leading to symptoms of impaired metamorphosis, like blocking of adult emergence (Wilson, 2004; Nandi and Chakravarty, 2011).

In the current study, pyriproxyfen topical application onto early last instar larvae produced various percentages of larval-pupal intermediates, in no certain trend, but some

pupal-adults were produced after topical application of higher three doses onto the prepupae. These mosaic creatures perished soon after production. This agreed with some of the reported larval-pupal intermediates after IGRs treatment. Some larval-pupal intermediates were formed after treatment of 3rd instar larvae of *P. argyrostoma* with 150µg/larva of chlorfluazuron (Ghoneim and Ismail, 1995). Also, treatment with some juvenoids induced the production of larval-pupal intermediates or larviform pupae in the stable fly *Stomoxys calcitrans* and the flesh fly *Sarcophaga bullata* (Wright, 1970; Weaver and Begley, 1982). In the present study, production of intermediate creatures indicated the metamorphosis disturbance by pyriproxyfen. This juvenoid interfered with the hormonal regulation of pupation program (Al-Sharook *et al.*, 1991). But, pyriproxyfen might inhibit metamorphosis program *via* an ecdysteroid reduction and/or interference with the release of neurosecretion (Josephraj Kumar *et al.*, 1999) that indicated a juvenile property of pyriproxyfen disrupting the perfect larval-pupal transformation or production of these mosaic creatures resulted by pyriproxyfen inhibitory effect on DNA synthesis (Mitlin *et al.*, 1977) or the chitin biosynthesis and chitin synthase (Mayer *et al.*, 1980). The induction had lethal consequences as a rapid moult did not have enough time for the completion transformation. Thus, the insects moulted to nonviable forms between the stages (Tateishi *et al.*, 1993). Molts induced during the early phase of the last instar produce larval-like individuals, while those formed in the late phase generate pupal-like individuals (Eizaguirre *et al.*, 2007). In insects, one symptom of the suspended metamorphosis has attracts a great attention of some entomologists. This feature is usually expressed in 'permanent larvae'. The induction of permanent larvae or nymphs was recorded in some insect species as a response to some IGRs. Permanent larvae of the European corn borer *Ostrinia nubilalis* were induced depending upon the

dose of fenoxycarb and the timing of application onto the 5th instar larvae (Gadenne *et al.*, 1990). Permanent larvae of the grey flesh fly *Parasarcophaga argyrostoma* were induced after chlorfluazuron treatment with 100µg/ larva (Ghoneim and Ismail, 1995). Feeding of the greater wax moth *Galleria mellonella* larvae, for a long time, on a diet treated with the JH analogue (0.1 mg/g of diet) induced permanent larvae (Slama and Lukas, 2013). Results of the present investigation on *M. stabulans* were in agreement with the previously reported results, since treatment of prepupae with the lower three doses of pyriproxyfen suppressed some prepupae to metamorphose into pupae but survived for long period as permanent prepupae. To understand appearance of the permanent prepupae in the present study, pyriproxyfen might disrupt the ecdysteroid metabolism or might alternatively act directly to inhibit the release of ecdysis-triggering hormone (Gaur and Kumar, 2010; Gibbens *et al.*, 2011).

In the present study, pyriproxyfen exerted an impaired pupal morphogenesis only after early last instar larvae treatment, some deformed pupae were observed. These agreed with the report of impaired pupal morphogenesis in *S. frugiperda* after feeding of 5th instar larvae on methoxyfenozide treated diet (Zarate *et al.*, 2011), *C. cephalonica* after fenoxycarb topical application on last instar larvae (Begum and Qamar, 2016), *P. gossypiella* after novaluron treatment of the full grown larvae (Ghoneim *et al.*, 2017a) and *P. unionalis* after novaluron treatment of newly moulted last instar larvae (Ghoneim *et al.*, 2017c). Anti-morphogenic activity of pyriproxyfen against pupae might exert suppressive action on the chitin synthesis and prevented normal deposition of new cuticle during apolysis led to pupal deformities (Retnakaran *et al.*, 1985). Pyriproxyfen might block the release of morphogenic peptides and alternated titers of ecdysteroids and juvenoids (Barnby and Klocke, 1990).

Disrupted adult morphogenesis occurred

after IGRs treatment as *S. littoralis* treated with novaluron (Hamadah *et al.*, 2015); *E. integriceps* treated with pyriproxyfen (Mojaver and Bandani, 2010); *S. frugiperda* treated with methoxyfenozide (Zarate *et al.*, 2011); *E. kuehniella* treated with hexaflumuron (Ashouri *et al.*, 2014); *H. armigera* treated with hexaflumuron (Taleh *et al.*, 2015); *C. cephalonica* treated with fenoxycarb (Begum and Qamar, 2016). Pyriproxyfen exerted ecdysteroid titer disturbance in lysosomal enzyme activity causing abnormal adult (Josephraj Kumar *et al.*, 1999), or chitin synthase was inhibited by the compound metabolites (Cohen and Casida, 1980), inhibited DNA synthesis or facilitated diffusion and active transport across nucleosides and amino acids cell membranes (Mayer *et al.*, 1988).

Conclusion

Pyriproxyfen exhibited toxic effects on larvae, pupae and adults as some disruptive effects on development and metamorphosis as well as anti-morphogenic action on pupae and adults. Pyriproxyfen proved an effective agent in controlling this fly.

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