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Insecticidal Activity of Pyriproxyfen, A Juvenoid, and Its Suppressive Effect on Growth and Development of The Black Cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae)

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ABSTRACT

The black cutworm *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae), is generally considered to be worldwide in distribution. It is one of the destructive pests attacking nearly all vegetables and different field crops. The objective of the present study was to evaluate the toxicity of pyriproxyfen and its effect on the growth, development, metamorphosis and morphogenesis of this insect. Both the 4th instar and 5th instar larvae were treated with 6 concentrations of this juvenoid (800, 400, 200, 100, 50 & 25 ppm) *via* fresh discs of castor bean leaves. The most important results could be summarized as follows. Pyriproxyfen exhibited strong acute toxic activity against larvae and chronic toxicity against pupae and adults, after treatment of 4th instar or 5th instar larvae. LC₅₀ values were calculated in 65.95 and 99.90ppm, after treatment of 4th instar and 5th instar larvae, respectively, i.e., the 4th instar larvae were found more sensitive to pyriproxyfen than 5th instar larvae. The larval body weight gain was remarkably reduced and the growth was considerably inhibited. The larval and pupal durations were considerably prolonged, in a dose-dependent course. Failure of ecdysis, as a criterion of the disrupted developmental program, was observed only after treatment with certain concentrations, but other features of the disrupted program had not been observed. The pupation was detrimentally suppressed after treatment of 4th or 5th instar larvae with pyriproxyfen. No deformed pupae were observed. Therefore, pyriproxyfen could be recommended as an eco-friendly alternative to synthetic insecticides for the IPM program of *A. ipsilon*.

INTRODUCTION

The black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae), is widely distributed all over the world, particularly in moderate and subtropical countries of the northern and southern hemispheres (Kononenko, 2003; Harrison, and Lynn, 2008; Binning *et al.*, 2015; Mishra, 2020; Rodingpuia and Lalthanzara, 2021). It is one of the most serious species of underground pests and can destroy more than 100 species of host plants (e.g., corn, wheat, cotton, soybean, vegetables and a variety of weeds) (Abd El-Aziz *et al.*, 2007; Binning *et al.*, 2015; Liu *et al.*, 2015).

This pest is a nocturnal insect. During the day, larvae remain buried in the ground, which hinders its viewing field and consequently its control is difficult (Bento *et al.*, 2007).

Therefore, *A. ipsilon* is one of the most challenging agricultural pests when it comes to control and management (Andersch and Schwarz, 2003). Although integrated pest management (IPM) strategies are increasingly being developed (Veres *et al.*, 2020), the majority of treatments for pest insects still rely exclusively on the use of neurotoxic insecticides (Jeschke *et al.*, 2011; Meslin *et al.*, 2021). Some studies have focused on the effectiveness of various insecticides to control *A. ipsilon* (Shakur *et al.*, 2007). In Egypt, the control measure of this insect pest depends mainly on the application of conventional insecticides, particularly organophosphates (Vattikonda and Sangam, 2017).

Over the years, the intensive and improper uses of conventional insecticides usually cause serious toxicological problems to the ecosystems (Haqet *et al.*, 2004; Tiryaki and Temur, 2010; Chowański *et al.*, 2014). Many insecticides are not soluble in water, so large quantities of organic solvents are required and most of these solvents increase the environmental pollution, as well as contamination of ground waters, plants and soil (Arias-Estevez *et al.*, 2008; Holoubek *et al.*, 2009; Sanni and Mutta, 2014; Adrees *et al.*, 2015). In addition, the widespread use of neurotoxic insecticides exhibits negative effects on the physiology and behavior of non-target beneficial insects, such as honeybees (Davies *et al.*, 2007; Blacquièrre *et al.*, 2012; Vattikonda and Sangam, 2017). Unfortunately, the majority of current agrochemicals have adverse effects on human health (Shahzad *et al.*, 2020). Therefore, the agrochemical research institutions have focused on the discovery of alternative selective compounds which interfere with the pest insect growth and development and are less toxic to non-target organisms and with negligible effects on the ecosystem (Dhadialla *et al.*, 2005; Dubey *et al.*, 2010; Chandler *et al.*, 2011; Korrat *et al.*, 2012; Derbalah *et al.*, 2014). Among the alternative control agents are the insect growth regulators (IGRs) which have been classified as 'biorationals' to distinguish these compounds from the conventional insecticides. This term implies that IGRs are selective and specific to the target pests (Ishaaya *et al.*, 2005; Horowitz *et al.*, 2009; Sarwar, 2015). IGRs are now used to control various insect pests and can assist in the development of sustainable agriculture (Raslan, 2002; Zhou *et al.*, 2003; Wang and Wang, 2007; Sabry and Abdu, 2016). Such compounds have much greater metabolic and environmentally more stable than earlier analogs and are much better suited to insect pest control (for some detail, see reviews of Dhadialla *et al.*, 1998; Dhadialla and Jansson, 2000; Dhadialla *et al.*, 2005). Depending on the specific mode of action, IGRs had been classified into three categories: (i) juvenile hormone analogues (JHAs, also called Juvenoids), (ii) Ecdysteroid agonists and (iii) Chitin synthesis inhibitors (CSIs) or moult inhibitors (Dhadialla *et al.*, 1998; Oberlander and Silhacek, 2000). They had been, also, grouped in CSIs and substances that interfere with the action of insect hormones (i.e. JHAs, and ecdysteroids) (Tunaz and Uygun, 2004). Since juvenile hormone does not occur in vertebrates, the juvenoid IGRs are considered safe to humans (Jindra and Bittova, 2020). JHAs are excellent tools for studying endocrinological mechanisms in insects (Ramaseshadri *et al.*, 2012), since disrupting metamorphosis is the main insecticidal activity of juvenoid IGRs (Jindra and Bittova, 2020).

Pyriproxyfen is cited as 2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine or 4-Phenoxyphenyl (*R/S*)-2-(2-pyridyloxy) propyl ether or 2-[1-(4-Phenoxyphenoxy) propan-2-yloxy] pyridine. Its molecular formula is $C_{20}H_{19}NO_3$. It was first registered as KNACK[®], SUMILARV[®], and ADMIRAL[®]; Sumitomo Chemical Co., Japan in 1991 for controlling public health pests (Yokoyama and Miller, 1991). It is a relatively stable compound (Mohandass *et al.*, 2006). Due to the widespread use of pyriproxyfen worldwide, it is important to know how this IGR behaves in the terrestrial environment and the effect on non-target organisms. For the fate of pyriproxyfen in soil and plants, see the review of Devillers (2020). Pyriproxyfen has been reported to be safe for a variety of predatory

arthropods (Naranjo et al., 2003) and compatible with natural enemy conservation (Liu and Chen, 2000; Liu and Stansly, 2004) as well as much less toxic to the ecosystem (Korrat *et al.*, 2012). Also, it has a relatively low mammalian toxicity (Mohandasset *et al.*, 2006) and mild toxicity to some aquatic organisms but is non-toxic to bees (Dhadialla *et al.*, 2005).

Pyriproxyfen is a potent JHA available today affecting the hormonal regulation of different vital processes in insects (Devillers, 2009; Hatakoshi, 2012; Chłopecka *et al.*, 2018) resulting thereby in a strong suppression of embryogenesis (Maharajan et al., 2018), metamorphosis (Barbosa *et al.*, 2018) and adult metamorphosis in several insect orders (Aribiet *et al.*, 2006). Also, it was reported to suppress oviposition, reduce the viability of eggs (Ghasemi *et al.*, 2010; Ohba *et al.*, 2013) and reduce the fecundity of insects (Singh and Kumar, 2015; Meng *et al.*, 2018). Therefore, Pyriproxyfen, as a broad-spectrum IGR, is usually applied against many public health insect pests (Korrat *et al.*, 2012), and has been successfully used to control important pests of many agricultural crops all over the world (Sazo *et al.*, 2008; Moadeli *et al.*, 2014). The objective of the present study was to evaluate the toxicity of pyriproxyfen and its effect on the growth, development, metamorphosis and morphogenesis of *A. ipsilon*.

MATERIALS AND METHODS

1. Experimental Insect:

A culture of the black cutworm *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) was raised under constant conditions ($27\pm 2^\circ\text{C}$ and $65\pm 5\%$ R.H.) at Department of Zoology and Entomology, Faculty of Science, Al-Azhar University, Cairo, Egypt. It was originated by a sample of eggs from the susceptible strain culture maintained for several generations in Plant Protection Research Institute, Doqqi, Giza, Egypt. The rearing technique was carried out according to Abdin (1979) with the improvement of El-Shershaby (2010). The eggs were kept in wide-mouth plastic jars (1000 ml) fitted with filter paper until hatching. Newly hatched larvae were kept into new jars and provided with clean castor bean leaves *Ricinus communis* as food every day. After the first molt, a sawdust layer was put on the floor to avoid moisture, and renewed daily. At reaching the 4th instar, larvae were reared in few numbers in separate jars to avoid crowding and cannibalism. These jars were covered with pieces of cloth for preventing larval escape. Sawdust and fresh castor bean leaves were renewed daily until pupation. The pupae were then placed in plastic jars (10 x 25 cm) covered with muslin and fitted with filter paper, as an oviposition site for future moths. After the adult emergence, a piece of cotton wool soaked in 10% sugar solution was suspended from the top of each jar and renewed every 48 hrs for feeding moths. The plastic jars were examined daily for collecting papers containing eggs.

2. Pyriproxyfen Concentrations and Larval Treatment:

Pyriproxyfen (S-3113) is cited as 2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine or 4-Phenoxyphenyl (*R/S*)-2-(2-pyridyloxy) propyl ether or 2-[1-(4-Phenoxyphenoxy) propan-2-yloxy] pyridine. Its molecular formula is $\text{C}_{20}\text{H}_{19}\text{NO}_3$. It was first registered as KNACK[®], SUMILARV[®], and ADMIRAL[®]; Sumitomo Chemical Co., Japan. It was purchased from Milipore Sigma, Burlington, MA 01803, USA Merk Ltd., Egypt. A series of 6 concentrations of pyriproxyfen was prepared by diluting with distilled water in volumetric flasks: 800.0, 400.0, 200.0, 100.0, 50.0 and 25.0 ppm.

Bioassay test was carried out against 4th and 5th instar larvae of *A. ipsilon* using the dipping technique. Discs of fresh castor bean leaves were dipped in each concentration for 5 minutes and air dried before the introduction to larvae as food for 24 hr under the aforementioned laboratory conditions. Control larvae were provided with water-treated castor leaves. Thirty larvae in three replicates (10 larvae/replicate) of treated and control

larvae were kept separately in plastic vials. After 24 h feeding on treated leaves, larvae were provided with fresh untreated castor bean leaves and all results were recorded daily.

3. Criteria of Study:

3.1. Toxicity and Insecticidal Effect:

All mortalities of treated and control (larvae, pupae and adults) of *A. ipsilon* were recorded every day and corrected according to Abbott's formula (Abbott, 1925) as follows:

$$\text{Corrected Mortality \%} = \frac{\text{Observed Mortality \%} - \text{Control Mortality \%}}{100 - \text{Control Mortality \%}} \times 100$$

The LC₅₀ values were calculated for general mortality by Microsoft® office Excel (2007), according to Finny (1971).

3.2. Growth, Development and Metamorphosis:

Larval Body Weight Gain: Each individual larva (treated and control) was carefully weighted every day using a digital balance for calculating the body weight gain as follows: Initial weight (before the beginning of the experiment) - final weight (at the end of the experiment).

Larval Growth Rate: Growth rate (GR) can be calculated according to (Waldbauer, 1968) as follows:

GR = fresh weight gain during feeding period of larvae / feeding period x mean fresh body weight of larvae during the feeding period.

Developmental Duration and Rate: Dempster's equation (1957) was applied for calculating the developmental duration, and Richard's equation (1957) was used for calculating the developmental rate.

Pupation Rate: Pupation rate was calculated according to Jimenez-Peydro et al. (1995) as follows:

$$\text{P.R.} = [\text{No. pupated larvae} / \text{No. treated larvae}] \times 100$$

Deranged Metamorphosis: Deranged metamorphosis program of *A. Ipsilon* was observed and calculated in larval-pupal or pupal-adult intermediates (%). Also, pupal deformation was calculated in %. Features of impaired development were recorded in photos.

4. Statistical Analysis of Data:

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction (Moroney, 1956) for the test significance of the difference between means using GraphPad InStat® v. 3.01 (1998).

RESULTS

In the present study, toxicity and bio-efficacy of pyriproxyfen (a juvenoid) were evaluated against *A. ipsilon* after treatment of 4th and 5th instar larvae. For achieving this aim, six concentrations of pyriproxyfen were prepared: 800, 400, 200, 100, 50 & 25 ppm. Using the dipping technique discs of fresh clean castor bean leaves were treated with each concentration and then air-dried. Groups of newly molted larvae of both instars were allowed to no-choice feed on these discs for 24 hr. Control larvae were allowed to feed on untreated leaf discs. The insecticidal activity and effect on growth, development, metamorphosis, morphogenesis, adult performance and reproductive potential were recorded as follows.

1. Insecticidal Activity of Pyriproxyfen Against *A. ipsilon*:

After treatment of the newly molted 4th instar larvae of *A. ipsilon* with 6 concentrations of pyriproxyfen, data of the insecticidal activity were assorted in Table (1). Depending on these data, the treated larvae with the highest concentration completely died. At other concentrations, the larval mortalities were recorded in a dose-dependent course (56.7, 50.0, 50.0, 20.0 & 6.7% larval mortality at 400, 200, 100, 50 & 25 ppm, respectively,

vs. 0.0% mortality of control larvae). According to the data listed in the same table, pyriproxyfen exhibited chronic toxicity against the successfully developed pupae, in a dose-dependent trend (46.7, 37.2, 17.8, 12.5 & 14.1% of pupal mortalities, at 400, 200, 100, 50 & 25 ppm, respectively, vs., 3.3% mortality of control pupae). Also, the successfully emerged adults suffered the toxic action of pyriproxyfen, almost in a dose-dependent manner (38.9, 19.4, 25.0, 14.3 & 8.3% mortality of treated adults, at 400, 200, 100, 50 & 25 ppm, respectively, vs. 0.0% mortality of control adults). However, the corrected mortality was determined in a dose-dependent course. LC₅₀ value was calculated at 65.95 ppm.

After treatment of the newly molted 5th instar larvae with pyriproxyfen concentrations, data of the insecticidal activity were arranged in Table (2). Depending on these data, pyriproxyfen exhibited a strong acute toxicity against larvae, in a dose-dependent course (100, 50.0, 43.3, 30.0, 6.7 & 3.3% larval mortality, at 800, 400, 200, 100, 50 & 25 ppm, respectively, vs. 0.0% mortality of control larvae). As obviously shown in the same table, pyriproxyfen displayed chronic toxicity against the successfully developed pupae of which mortality increased with the increasing concentration (46.7, 23.3, 19.3, 14.4 & 3.3% pupal mortality, at 400, 200, 100, 50 & 25 ppm, respectively, compared to 0.0% mortality of control pupae). Also, the tested IGR displayed chronic toxicity against the successfully emerged adults of which mortality was found almost in a dose-dependent course (33.3, 38.3, 22.9, 7.9 & 7.0% mortality of treated adults, at 400, 200, 100, 50 & 25 ppm, respectively, compared to 0.0% mortality of control adults). As clearly seen in the aforementioned table, the corrected mortality was found in a dose-dependent course. LC₅₀ was calculated at 99.90ppm.

Depending on the data of both tables (1 & 2), pyriproxyfen exhibited stronger insecticidal potency against the 4th instar larvae than the 5th instar larvae, i.e., the 4th instar larvae were found more sensitive to pyriproxyfen than 5th instar larvae.

Table 1: Toxicity (%) of pyriproxyfen on *A. ipsilon* after treatment of the 4th instar larvae.

| Conc. (ppm) | Larval mortality | Pupal mortality | Adult mortality | Total mortality | Corrected mortality | LC ₅₀ (ppm) |
|-------------|------------------|-----------------|-----------------|-----------------|---------------------|------------------------|
| 800 | 100 | --- | --- | 100 | 100 | 65.95 |
| 400 | 56.7 | 46.7 | 38.9 | 86.7 | 86.2 | |
| 200 | 50.0 | 37.2 | 19.4 | 80.0 | 79.3 | |
| 100 | 50.0 | 17.8 | 25.0 | 73.3 | 72.4 | |
| 50 | 20.0 | 12.5 | 14.3 | 40.0 | 38.0 | |
| 25 | 6.7 | 14.1 | 8.3 | 26.7 | 24.2 | |
| Control | 0.0 | 3.3 | 0.0 | 3.3 | 00.00 | |

Conc.: concentration. ---: no developed pupae or emerged adults.

Table 2: Toxicity (%) of pyriproxyfen on *A. ipsilon* after treatment of the 5th instar larvae.

| Conc. (ppm) | Larval mortality | Pupal mortality | Adult mortality | Total mortality | Corrected mortality | LC ₅₀ (ppm) |
|-------------|------------------|-----------------|-----------------|-----------------|---------------------|------------------------|
| 800 | 100 | --- | --- | 100 | 100 | 99.90 |
| 400 | 50.0 | 46.7 | 33.3 | 83.3 | 83.3 | |
| 200 | 43.3 | 23.3 | 38.3 | 73.3 | 73.3 | |
| 100 | 30.0 | 19.3 | 22.9 | 56.7 | 56.7 | |
| 50 | 6.7 | 14.4 | 7.9 | 26.7 | 26.7 | |
| 25 | 3.3 | 3.3 | 7.0 | 13.3 | 13.3 | |
| Control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |

Conc., ---: see footnote of Table (1).

2. Effect of Pyriproxyfen on Growth, Development, Metamorphosis and Morphogenesis of *A. ipsilon*:

After treatment of the newly molted 4th instar larvae with 5 concentrations of pyriproxyfen, data of the somatic weight gain, growth rate, larval and pupal duration, developmental rate, corrupted larval-pupal transformation, pupation rate and pupal morphogenesis were assorted in Table (3). After treatment of the newly molted 5th instar larvae with 5 concentrations of pyriproxyfen, data of the previously mentioned criteria were listed in Table (4).

2.1. Effect of Pyriproxyfen on Weight Gain and Growth:

Data of Table (3) clearly revealed that pyriproxyfen considerably reduced the larval somatic weight gain (wtg), in a dose-dependent course (264.81±10.54, 238.52±9.64, 208.24±1.73, 202.42±3.61 & 181.02±3.61mg of treated larvae, at 25, 50, 100, 200 & 400ppm, respectively, vs. 286.67±4.51mg of control larvae) after-treatment of the newly molted 4th instar larvae. Also, the growth was greatly inhibited, since the growth rate (GR) of treated larvae was drastically regressed, in a dose-dependent course (15.53±0.62, 12.53±0.51, 10.95±0.09, 9.18±0.16 & 8.23±0.16, at 25, 50, 100, 200 & 400ppm, respectively, vs. 23.89±0.38 of control larvae).

After treatment of the newly molted 5th instar larvae with pyriproxyfen, data of Table (4) obviously showed the dramatically reduced wtg, in a dose-dependent course (121.00±8.19, 105.32±2.08, 101.27±4.93, 89.62±12.50 & 71.33±13.32, at 25, 50, 100, 200 & 400ppm, respectively, vs. 126.05±8.19 mg of control larvae). Also, GR was severely regressed, in a dose-dependent course (9.31±0.63, 7.55±0.15, 6.35±0.31, 5.27±0.74 & 3.96±0.74, at 25, 50, 100, 200 & 400ppm, respectively, vs. 10.50±0.68 of control larvae).

2.2. Effect of Pyriproxyfen on Developmental Durations and Rate:

After treatment of the newly molted 4th instar larvae with pyriproxyfen, data of Table (3) clearly demonstrated remarkable prolongation of the larval duration, in a dose-dependent course (18.1±0.45, 20.4±0.50, 23.9±1.01, 24.0±0.05 & 27.9±1.27 days of treated larvae, at 25, 50, 100, 200 & 400ppm, respectively, vs. 15.2±0.23 days of control larvae). Also, the developmental rate (DR) was considerably regressed (5.52, 4.90, 4.18, 4.17 & 3.58, at 25, 50, 100, 200 & 400ppm, respectively, vs. 6.57 of control larvae).

After treatment of the newly molted 5th instar larvae with pyriproxyfen, data of Table (4) obviously showed significantly prolonged larval duration, in a dose-dependent course (14.4±0.97, 16.9±0.66, 18.3±1.02, 22.1±1.71 & 25.7±0.30 days of treated larvae, at 25, 50, 100, 200 & 400ppm, respectively, vs. 11.2±0.76 days of control larvae). Also, DR was greatly regressed (6.94, 5.92, 5.46, 4.52 & 3.89, at 25, 50, 100, 200 & 400ppm, respectively, vs. 8.93 of control larvae).

With regard to the pupal duration, treatment of the newly molted 4th instar larvae with pyriproxyfen, resulted in a significant prolongation, in a dose-dependent course, with no exception (12.0±0.17, 12.1±0.06, 13.1±0.36, 13.7±0.58 & 14.9±0.55 days, at 25, 50, 100, 200 & 400ppm, respectively, vs. 09.6±0.26 days of control pupae, Table 3). A similar result was recorded for pupae after-treatment of the newly molted 5th instar larvae with pyriproxyfen, since the pupal duration was remarkably prolonged, in a dose-dependent course (12.1±0.15, 14.2±0.21, 14.7±0.64, 14.0±0.44 & 15.5±0.41days, at 25, 50, 100, 200 & 400ppm, respectively, vs. 09.9±0.40 days of control pupae, Table, 4).

2.3. Effect of Pyriproxyfen on The Developmental Program:

Data of Table (3) displayed a criterion of the disrupted developmental program, Failure of ecdysis, after treatment of 4th instar larvae only with the higher two concentrations of pyriproxyfen (20.00 & 26.66% failed larvae to molt, at 200 & 400ppm, respectively, compared to 0.0% failure of control larvae to molt). Plate (1) demonstrated photos of incompletely ecdysed 5th instar larvae with attached 4th instar cuticles. A similar

result was obtained after treatment of 5th instar larvae only with the highest concentration of Pyriproxyfen (13.3% failure of ecdysis, Table 4). Plate (2) contains photos of failure of 5th instar larvae to moult into 6th instar, since old 5th instar cuticle remained with the 6th instar larvae, in addition to some abdominal constrictions. However, no larval-pupal intermediates were produced, as another feature of the disrupted developmental program, irrespective of the treated larval instar with pyriproxyfen.

2.4. Effect of Pyriproxyfen on The Metamorphosis:

As shown in Table (3), treatment of 4th instar larvae with pyriproxyfen resulted in considerably suppressed pupation rate, since pupation % remarkably decreased, in no certain trend (93.3, 80.0, 40.0, 40.0 & 43.3% pupation, at 25, 50, 100, 200 & 400ppm, respectively, vs. 100% pupation of control larvae. Data of Table (4) revealed that the pupation was adversely hindered proportional to the Pyriproxyfen concentration (96.7, 93.3, 70.0, 56.7 & 50.0% pupation, at 25, 50, 100, 200 & 400ppm, respectively, vs. 100% pupation of control larvae).

2.5. Effect of Pyriproxyfen on The Morphogenesis Program:

As clearly seen in Table (3) and Table (4), pyriproxyfen failed to affect the pupal morphogenesis, since no malformed pupae were observed, regardless the treated larval instar.

Table 3: Influenced growth and development of *A. ipsilon* after treatment of 4th instar larvae with pyriproxyfen.

| Conc. (ppm) | Larval stage | | | | | | Pupal stage | | |
|-------------|------------------------------|------------------------|----------------------------|-------------------|------------------------|-------------------------|--------------|--------------------|----------------------------|
| | Weight gain (mg) (mean ± SD) | Growth rate (mean± SD) | Duration (days) (mean ±SD) | Develop. rate (%) | Failure of ecdysis (%) | Larval-pupal Inter. (%) | Pupation (%) | Deformed pupae (%) | Duration (days) (mean ±SD) |
| 400 | 181.02±3.61 d | 8.23±0.16 d | 27.9±1.27 d | 3.58 | 26.66 | 0.0 | 43.3 | 0.0 | 14.9±0.55 d |
| 200 | 202.42±3.61 d | 9.18±0.16 d | 24.0±0.05 d | 4.17 | 20.00 | 0.0 | 40.0 | 0.0 | 13.7±0.58 d |
| 100 | 208.24±1.73 d | 10.95±0.09 d | 23.9±1.01 d | 4.18 | 00.00 | 0.0 | 40.0 | 0.0 | 13.1±0.36 d |
| 50.0 | 238.52±9.64 c | 12.53±0.51 d | 20.4±0.50 d | 4.90 | 00.00 | 0.0 | 80.0 | 0.0 | 12.1±0.06 d |
| 25.0 | 264.81±10.54 b | 15.53±0.62 d | 18.1±0.45 d | 5.52 | 00.00 | 0.0 | 93.3 | 0.0 | 12.0±0.17 d |
| Control | 286.67±4.51 | 23.89±0.38 | 15.2±0.23 | 6.57 | 00.00 | 0.0 | 100.0 | 0.0 | 09.6±0.26 |

Conc.: See footnote of Table (1). Develop.: Developmental. Mean±SD followed with letter a: insignificant (P >0.05), b: significant (P<0.05), c: highly significant (P<0.01), d: very highly significant (P<0.001).

Table 4: Influenced growth and development of *A. ipsilon* after treatment of 5th instar larvae with pyriproxyfen.

| Conc. (ppm) | Larval stage | | | | | | Pupal stage | | |
|-------------|------------------------------|------------------------|-----------------------------|-------------------|------------------------|-------------------------|--------------|--------------------|-----------------------------|
| | Weight gain (mg) (mean ± SD) | Growth rate (mean± SD) | Duration (days) (mean ± SD) | Develop. rate (%) | Failure of ecdysis (%) | Larval-pupal Inter. (%) | Pupation (%) | Deformed pupae (%) | Duration (days) (mean ± SD) |
| 400 | 71.33±13.32 c | 3.96±0.74 d | 25.7±0.30 d | 3.89 | 13.3 | 0.0 | 50.0 | 0.0 | 15.5±0.41 d |
| 200 | 89.62±12.50 b | 5.27±0.74 c | 22.1±1.71 d | 4.52 | 00.0 | 0.0 | 56.7 | 0.0 | 14.0±0.44 d |
| 100 | 101.27±4.93 b | 6.35±0.31 b | 18.3±1.02 d | 5.46 | 00.0 | 0.0 | 70.0 | 0.0 | 14.7±0.64 d |
| 50 | 105.32±2.08 b | 7.55±0.15 b | 16.9±0.66 d | 5.92 | 00.0 | 0.0 | 93.3 | 0.0 | 14.2±0.21 d |
| 25 | 121.00±8.19 a | 9.31±0.63 a | 14.4±0.97 b | 6.94 | 00.0 | 0.0 | 96.7 | 0.0 | 12.1±0.15 d |
| Control | 126.05±8.19 | 10.50±0.68 | 11.2±0.76 | 8.93 | 00.0 | 0.0 | 100.0 | 0.0 | 09.9±0.40 |

Conc.: See footnote of Table (1). Develop., a, b, c, d,: see footnote of Table (3).

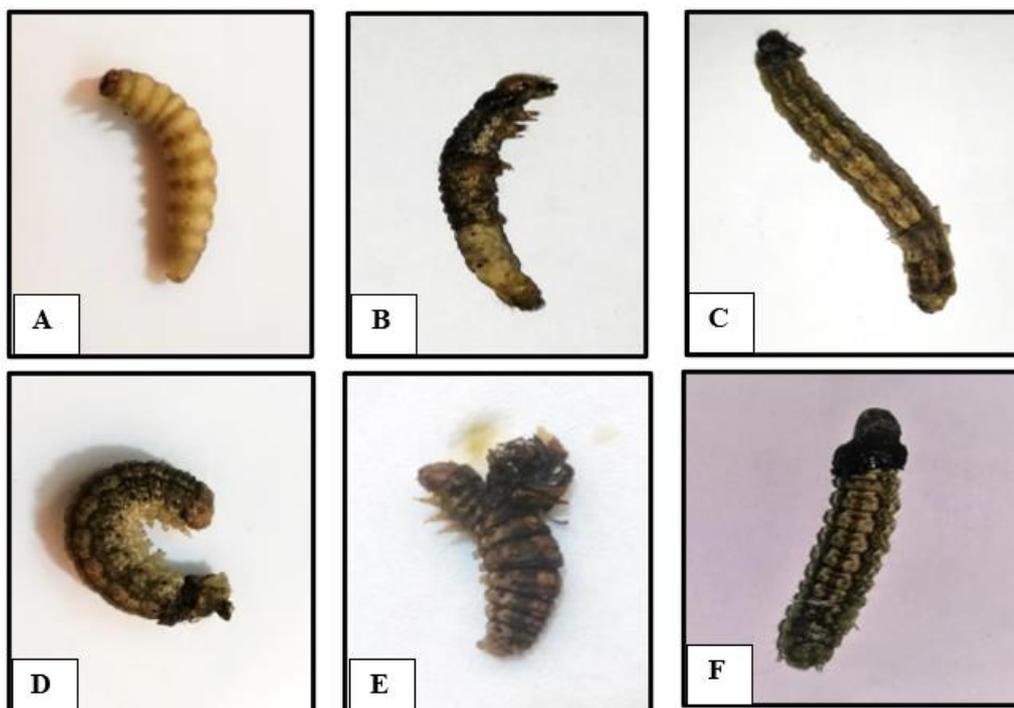


Plate 1: Failure of ecdysis of *A. ipsilon* after treatment of the newly moulted 4th instar larvae with pyriproxyfen. A: Normal 5th instar larva. B, C, D, E & F: photos of incompletely ecdysed 5th instar larva with attached 4th instar cuticle.

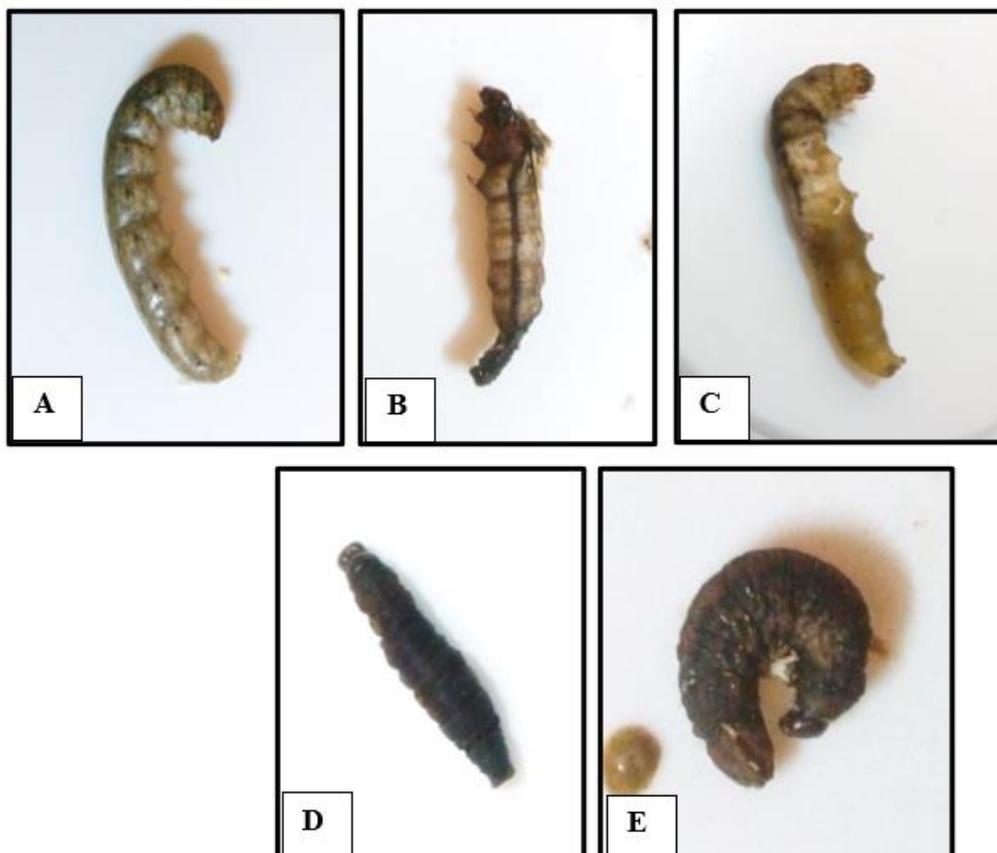


Plate 2: Failure of ecdysis of *A. ipsilon* after treatment of the newly moulted 5th instar larvae with pyriproxyfen. A: Normal 6th instar larva. B, C, D & E: photos of incompletely moulted 6th instar larvae with old 5th instar cuticles and abdominal constrictions.

DISCUSSION

Insecticidal Activity of Pyriproxyfen against *A. ipsilon*:

Toxic effects of various insect growth regulators (IGRs) on the black cutworm *Agrotis ipsilon* had been reported, such as flufenoxuron and methoprene (Khatter, 2014), chlorfluazuron and triflumuron (Fahmy, 2014), methoprene (Abdou and Abdel-Hakim, 2017), chlorfluazuron and flufenoxuron (Shaurub *et al.*, 2018) and chlorantraniliprole (He *et al.*, 2019). Results of the present study were in agreement with these reported results, since pyriproxyfen exhibited strong acute insecticidal activity against larvae of *A. ipsilon*, in a dose-dependent course, after treatment of 4th instar or 5th instar larvae. Also, the same IGR exhibited considerable chronic toxicity against the pupae and adults, in a dose-dependent trend, regardless of the treated larval instar.

Also, the present results were in corroboration with various reported results of toxicity of different IGRs against several insects, other than *A. ipsilon*, such as methoprene against the common house mosquito *Culex pipiens* (Gelbic *et al.*, 2002), the Asian tiger mosquito *Aedes albopictus* (Khan *et al.*, 2016), the rice meal moth *Corcyra cephalonica* (Tripathi and Tiwari, 2006), the yellow fever mosquito *Aedes aegypti* (Braga *et al.*, 2005) and the flesh fly *Sarcophagaruficornis* (Singh *et al.*, 2017); Flufenoxuron (El-Naggar, 2013), Lufenuron (Bakr *et al.*, 2013), Buprofezin (Nasr *et al.*, 2010), Cyromazine (Tananiet *et al.*, 2015), Teflubenzuron (Mead and Khedr, 2018) and chlorfluazuron (Shaurub *et al.*, 2020) against the Egyptian cotton leafworm *Spodoptera littoralis*; pyriproxyfen against the Sunn pest *Eurygaster integriceps* (Mojaver and Bandani, 2010) and the lawn armyworm *Spodoptera mauritia* (Resmitha and Meethal, 2016); kinoprene against *C. pipiens* (Hamaidia and Soltani, 2014); Lufenuron against the red flour beetle *Tribolium castaneum* (Gadoet *et al.*, 2015); Tebufenozide (RH-5992) against the Mediterranean flour moth *Ephestia kuehniella* (Taziret *et al.*, 2016); Lufenuron against the lesser mulberry snout moth *Glyphodes pyloalis* (Aliabadi *et al.*, 2016) and the corn earworm *Helicoverpa armigera* (Vivan *et al.*, 2017); Fenoxycarb against *C. Cephalonica* (Begum and Qamar, 2016); Cyromazine against the flies *Musca domestica*, *Stomoxys calcitrans* and *Fannia canicularis* (Donahue *et al.*, 2017); Novaluron against the pink bollworm *Pectinophora gossypiella* (Ghoneimet *et al.*, 2017a) and the mosquito *Aedes aegypti* larvae (Gunathilaka *et al.*, 2020); Novaluron (Ghoneim *et al.*, 2017b) and Methoxyfenozide (Hamadah and Abo Elsoud, 2018) against olive leaf moth *Palpita unionalis*; Pyriproxyfen against the false stable fly *Muscina stabulans* (Hamadah, 2018); *etc.*

In the current investigation, 4th instar larvae of *A. ipsilon* were found more sensitive to the pyriproxyfen toxicity than 5th instar larvae. This result was consistent with the results of larval sensitivity of the same insect to some IGRs, such as some chitin synthesis inhibitors (El-Kady *et al.*, 1990; Abou El-Ghar *et al.*, 1994) and methoprene (a juvenoid) (Abdou and Abdel-Hakim, 2017).

It is important to discuss the variation of LC₅₀ values in the present study, compared to various values, as reported for other insects. In the current study on *A. ipsilon*, LC₅₀ values of pyriproxyfen were calculated at 65.95 and 99.90 ppm, after treatment of 4th instar and 5th instar larvae, respectively. Various LC₅₀ values against *A. ipsilon* were reported for different IGRs, such as 3.5 and 4.0 ppm for triflumuron and chlorfluazuron, respectively (Fahmy, 2014); 1.00 and 4.68 mg/L for chlorfluazuron and flufenoxuron, respectively (Shaurub *et al.*, 2018) and 0.187 µg.g⁻¹ for chlorantraniliprole (He *et al.*, 2019).

Apart from *A. ipsilon*, the available literature contains many reported results of LC₅₀ values of different IGRs against several insects, such as LC₅₀ values of novaluron and lufenuron against the tobacco cutworm *Spodoptera litura* larvae were 350.45 and 453.78

ppm, respectively (Sharma and Pathania, 2014); LC₅₀ of Pyriproxyfen was found to be 0.025% against *S. litura* larvae (Kaur and Chandi, 2015); LC₅₀ of hexaflumuron against *H. armigera* was 8.47 mg /L (Taleh *et al.*, 2015); LC₅₀ values of lufenuron and chlorfluazuron against 4th instar larvae of *S. littoralis* were 1.7 and 2.2 ppm, respectively (Aboutaleb *et al.*, 2015). LD₅₀ values of the ecdysone agonists RH-5849 and RH-5992 (tebufenozide) against *E. kuehniella* were 0.05 and 0.005 µg/insect, respectively (Tazir *et al.*, 2016); LC₅₀ of the ecdysone agonist methoxyfenozide against *Cx. pipiens* was 24.54 µg/L (Hamaidia and Soltani, 2016); LC₅₀ of Lufenuron against *G. pyloalis* was 19 ppm (Aliabadi *et al.*, 2016); LC₅₀ values of chlorfluazuron, cyromazine, lufenuron and precocene I against the cat flea *Ctenocephalides felis* were 0.19, 2.66, 0.20, and 10.97 ppm, respectively (Rust and Hemsarth, 2016); LD₅₀ values of RH-5849 and tebufenozide against *E. kuehniella* were 0.05 and 0.005 µg/insect, respectively (Tazir *et al.*, 2016); LC₅₀ of teflubenzuron against *P. gossypiella* was 78.59 ppm (Said *et al.*, 2017); LC₅₀ values of Novaluron against *P. gossypiella* were 0.187 ppm and 0.765 ppm, after treatment of newly hatched and full grown larvae, respectively (Ghoneim *et al.*, 2017a); LC₅₀ values of flufenoxuron, chlorfluazuron and triflumuron against 4th instar larvae of *S. littoralis* were 0.14 ppm, 0.42 and 1661.58 ppm, respectively (Abdel-Mageed *et al.*, 2018); LC₅₀ of pyriproxyfen against the mosquitoes *Aedes aegypti* and *Aedes albopictus* were 1.63ppm and 1,56ppm, respectively (Sucipto *et al.*, 2018); LC₅₀ values of diofenolan against *P. gossypiella* were 0.028 ppm and 0.036 ppm, after treatment of newly hatched and full grown larvae, respectively (Tanani and Bakr, 2018); LC₅₀ value of methoxyfenozide against last instar (6th) larvae of *P. unionalis* was 0.176 ppm (Hamadah and Abo Elsoud, 2018); LD₅₀ values of pyriproxyfen against the false stable fly *Muscina stabulans* were 0.242 & 0.444µg/stage, after treatment of early last (3rd) instar larvae and prepupae, respectively (Hamadah, 2018); LD₅₀ of novaluron against 9th instar larvae of the red palm weevil *Rhynchophorus ferrugineus* was 14.77 ppm (Hussain *et al.*, 2019); LC₅₀ values of hexaflumuron, lufenuron and chlorfluazuron against *H. armigera* larvae were 6.16, 61.31 and 31.75 mg ai/l, respectively (Khorshidi *et al.*, 2019); LC₅₀ of fenoxycarb against 4th instar larvae of *S. littoralis* was 25.943 ppm (Gad *et al.*, 2019); LC₅₀ values of novaluron and methoxyfenozide against 3rd instar *S. litura* larvae were 29.56 mg/l and 21.06 mg/l, respectively (Khan *et al.*, 2021); *etc.* However, LC₅₀ value depends on several factors, such as susceptibility of the insect and its treated stage or instar, lethal potency of the tested compound and its concentration levels, method and time of treatment, source of stock insect referring to potential genetical/geographical variations, as well as the experimental conditions.

To explicate the recorded toxic effect of pyriproxyfen on larvae, pupae and adults of *A. ipsilon*, in the current study, IGRs exhibit their toxic effects on insects with a mode of action other than that of conventional insecticides. It was suggested that the tested juvenoid IGR interfered with the transport system of UDP-N-acetyl amine across the membrane (Eto, 1990). For some detail, the larval deaths of *A. ipsilon* by pyriproxyfen, in the current study, might be due to the failure of larvae to moult owing to the inhibition of chitin formation (Abdel Rahman *et al.*, 2007; Adel, 2012) or the prevention of molting larvae to swallow volumes of air for splitting the old cuticle and expand the new one during ecdysis (Linton *et al.*, 1997) or to the inability of the molting larvae to shed their exocuticle (Zorzetti *et al.*, 2015). Also, these larval deaths might be due to the prevented feeding and continuous starvation of the present insect (Ghoneim *et al.*, 2000).

Although the disturbance of hormonal regulation or the disruption of the normal activity of the endocrine system in insects by IGRs was reported (Oberlander *et al.*, 1997; Mulla *et al.*, 2003; Djeghader *et al.*, 2014), the pupal deaths in *A. ipsilon*, in the present study, could be due to some causes, such as suffocation, bleeding and desiccation due to

imperfect exuviation, failure of vital homeostatic mechanisms, *etc.* (Smagghe and Degheele, 1994).

In addition, the adult mortality of *A. ipsilon* after treatment of newly moulted 4th and 5th instar larvae with pyriproxyfen, in the current study, could be explained by the retention and distribution of this juvenoid IGR in the insect body as a result of rapid transport from the gut of treated larvae into other tissues, by the direct and rapid transport *via* the haemolymph to other tissues, and/or by lower detoxification capacity of adults against the tested compound (Osman *et al.*, 1984).

Disrupted Growth, Development, Metamorphosis and Morphogenesis of *A. ipsilon* by Pyriproxyfen:

1. Reduced Weight Gain and Inhibited Growth:

In the present study, treatment of 4th instar or 5th instar larvae of *A. ipsilon* with pyriproxyfen resulted in a remarkable reduction of the larval somatic weight gain, in a dose-dependent course. Also, the growth was considerably inhibited, since the growth rate of treated larvae was drastically regressed, in a dose-dependent course. These results were in accordance with the reported results of reduced body weight and inhibited the growth of the same insect after treatment of chlorfluazuron or triflumuron (Fahmy, 2014) and chlorantraniliprole (He *et al.*, 2019).

Also, the current results were concomitant with many reported results of reduced body weight and/or inhibited growth of other insects by various IGRs, such as *S. littoralis* by flufenoxuron (Bakr *et al.*, 2010), lufenuron (Adel, 2012), and novaluron (Ghoneim *et al.*, 2015); the common lime butterfly *Papiliodemoleus* by diofenolan (Singh and Kumar, 2011); *S. litura* by chlorfluazuron (Perveen, 2012); *Cx. pipiens* by novaluron (Djehaderet *et al.*, 2014) and kinoprene (Hamaidia and Soltani, 2014); the bean aphid *Aphis craccivora* (Vadja and Kalasariya, 2015), the brown planthopper *Nilaparvata lugens* (Alam and Das, 2017) and *S. litura* (Khatun *et al.*, 2017) by buprofezin; the mosquitoes *A. aegypti* and *Aedes albopictus* by pyriproxyfen (Sucipto *et al.*, 2018); *P. unionalis* by Methoxyfenozide (Hamadah and Abo Elsoud, 2018); *H. armigera* by hexaflumuron, lufenuron and chlorfluazuron (Khorshidi *et al.*, 2019); the okra jassid *Amrasca biguttula* by pyriproxyfen, lufenuron and buprofezin (Joarder *et al.*, 2020); *etc.*

On the other hand, the present results disagreed with some reported results of increased body weight and/or enhanced growth of some insects by certain IGRs, such as *A. ipsilon* by fenoxycarb (Abdel-Hakim and El-Mandarawy, 2017) and methoprene (Abdou and Abdel-Hakim, 2017) and *S. littoralis* by cycloheximide (Basiouny and Ghoneim, 2018). In addition, some IGRs failed to affect the growth of different insects, such as the house fly *Musca domestica* (Ghoneim *et al.*, 1991), the American cockroach *Periplaneta americana* and the large milkweed bug *Oncopeltus fasciatus* (Darvas *et al.*, 1992), the African armyworm *Spodoptera exempta*, the beet armyworm *Spodoptera exigua* and the Colorado potato beetle *Leptinotarsa decemlineata* (Smagghe and Degheele, 1994). For the interpretation of body weight gain reduction and growth inhibition of *A. ipsilon* after treatment with pyriproxyfen, in the present study, it may be important to mention that Lepidoptera belongs to the most sensitive groups of insects regarding the growth-regulating effects of IGRs. The reduction of the body weight and inhibition of larval growth of *A. ipsilon* might be a result of the blocked release of morphogenic peptides, causing alteration in the ecdysteroid and juvenoid titers (Barnby and Klocke, 1990), disrupted tissues and cells undergoing mitosis (Nasiruddin and Mordue, 1994), disruption of chitin synthesis and loss of body fluid during the molting stage (Vojudi *et al.*, 2017) or might be due to the reduced food intake during the larval period or high metabolic costs required for the detoxification of ingested xenobiotic compounds (Khorshidi *et al.*, 2019).

2. Prolonged Developmental Duration and Regressed Developmental Rate of *A. ipsilon* by Pyriproxyfen:

In the present study, treatment of 4th instar or 5th instar larvae of *A. ipsilon* with pyriproxyfen resulted in remarkable prolongation of the larval and pupal durations, in a dose-dependent course. Also, the developmental rate was considerably regressed, in a dose-dependent course. These results were in agreement with some reported results of prolonged larval and pupal durations of the same insect after treatment of larvae with different IGRs, such as chlorfluazuron or triflumuron (Fahmy, 2014), methoprene and flufenoxuron (Khatter, 2014), methoprene (Abdou and Abdel-Hakim, 2017), fenoxycarb (Abdel-Hakim and El-Mandarawy, 2017), chlorfluazuron or flufenoxuron (Shaurub *et al.*, 2018) and chlorantraniliprole (He *et al.*, 2019).

Also, the present results were in accordance with many reported results of prolonged larval and pupal durations indicating regressed developmental rate of other insects after treatment with IGRs of various categories, such as *Cx. pipiens* after treatment with kinoprene (Hamaidia and Soltani, 2014) and methoxyfenozide (Hamaidia and Soltani, 2016); the diamondback moth *Plutella xylostella* after treatment with Pyriproxyfen (Mahmoudvand *et al.*, 2015); *S. littoralis* after treatment with diflubenzuron (Aref *et al.*, 2010), Lufenuron (Gaaboub *et al.*, 2012), novaluron (Ghoneim *et al.*, 2015), methoxyfenozide (Khaled and Farag, 2015) and cyromazine (Tanani *et al.*, 2015); *G. pyloalis* after treatment with lufenuron (Aliabadi *et al.*, 2016); *C. cephalonica* after treatment with methoprene (Tripathi and Tiwari, 2006) and fenoxycarb (Begum and Qamar, 2016); *P. gossypiella* after treatment with buprofezin (Al-Kazafy, 2013), teflubenzuron (El-Khayat *et al.*, 2015; Said *et al.*, 2017), chromafenozide (Salem, 2015), pyriproxyfen (Sabry and Abdou, 2016), novaluron (Hamadah and Ghoneim, 2017; Ghoneim *et al.*, 2017a) and diofenolan (Tanani and Bakr, 2018); the false stable fly *Muscina stabulans* after topical application of pyriproxyfen onto the early last (3rd) instar larvae (Hamadah, 2018); *H. armigera* after feeding on diet mixed with hexaflumuron, lufenuron or chlorfluazuron (Khorshidi *et al.*, 2019); *etc.*

On the contrary, the present results disagreed with the reported results of shortened larval and pupal duration of some insects after treatment with different IGRs, such as *A. ipsilon* after treatment with flufenoxuron (El-Sheikh, 2002); *Rh. ferrugineus* after treatment with lufenuron and diofenolan (Tanani, 2001); the desert locust *Schistocerca gregaria* after treatment with lufenuron (Bakr *et al.*, 2008); *P. gossypiella* after treatment with methoxyfenozide (Sabry and Abdou, 2016); *P. unionalis* after treatment with novaluron (Ghoneim *et al.*, 2017b); *Cx. pipiens* after treatment with halofenozide (Bouaziz *et al.*, 2017); *M. stabulans* after topical application of Pyriproxyfen onto the prepupae (Hamadah, 2018) and *S. littoralis* after treatment with chlorfluazuron (Shaurub *et al.*, 2020). In addition, the larval duration of *S. littoralis* was not affected after treatment with flufenoxuron or pyriproxyfen (Shaurub *et al.*, 2020).

To explicate the remarkable prolongation of larval and pupal duration and considerably regressed developmental rate after treatment 4th instar or 5th instar larvae of *A. ipsilon* with pyriproxyfen, in the present study, pyriproxyfen might affect the tissues and cells undergoing mitosis (Nasiruddin and Mordue, 1994) or might exhibit a delaying effect on the ecdysis and transformation (Linton *et al.*, 1997). Also, pyriproxyfen might exhibit a delaying effect on the pupation of *A. ipsilon*. On the other hand, the final step of the chitin biosynthesis pathway was inhibited by this juvenoid IGR and the precursor was not converted into chitin leading to a prolongation of the developmental duration (Djeghader *et al.*, 2014). In general, the prolongation of pupal duration might be due to the persistence of juvenile hormone (JH) and its elevated level in the haemolymph where it is only in the absence of JH that ecdysone could be activated and lead to the formation of the next stage

(Kuwano *et al.*, 2008). In addition, because the pyriproxyfen-treated *A. ipsilon* larvae must spend more resources on detoxification rather than development, larval development takes significantly longer duration (Hannig *et al.*, 2009). In other words, the pyriproxyfen-treated larvae should divert the energy stream from development and reproduction to detoxification or rehabilitation of tissues following possible damages. Moreover, hormonal imbalances after IGR treatment may cause diverse physiological discrepancies to be taken care of by the survived larvae which require energy (Khorshidi *et al.*, 2019).

3. Disrupted Developmental Program of *A. ipsilon*:

Depending on the available literature, the major symptoms and features of the impaired developmental program of an insect after treatment with various IGRs had been described as failure of larval ecdysis, production of larval-pupal and/or pupal-adult intermediates, production of supernumerary larval instars (superlarvae). However, all or some of these features were observed in various insects by the disruptive effects of IGRs. However, all or some of these features were observed in various insects as responses to the disruptive effects of different IGRs, such as *S. littoralis* by flufenoxuron (El-Naggar, 2013), novaluron (Ghoneim *et al.*, 2015) and cyromazine (Tanani *et al.*, 2015). Also, some or all of these symptoms of the impaired developmental program were recorded after treatment of different insects with several IGRs, such as the American serpentine leafminer *Liriomyza trifolii* (Saryazdi *et al.*, 2012) and the cowpea weevil *Callosobruchus maculatus* (Al-Mekhlafi *et al.*, 2012) by cyromazine; *H. armigera* (Murthy and Ram, 2002), *A. aegypti* (Nwankwo *et al.*, 2011) and *M. domestica* (Lohmeyer *et al.*, 2014) by novaluron; the mustard aphid *Lipaphis erysimi* by pyriproxyfen (Liu and Chen, 2001); *Rh. ferrugineus* (Tanani, 2001) and the lime butterfly *Papilio demoleus* (Singh and Kumar, 2011) by diofenolan; the European grapevine moth *Lobesia botrana* by lufenuron (Saenz-de-Cabezón *et al.*, 2005); *Cx. pipiens* by kinoprene (Hamaidia and Soltani, 2014); *P. gossypiella* (Ghoneim *et al.*, 2017a) and *P. unionalis* (Ghoneim *et al.*, 2017b) by novaluron; etc.

In the present study on *A. ipsilon*, ecdysis failure of larvae, as a criterion of the disrupted developmental program, was observed only after treatment of 4th instar larvae with two concentrations and after treatment of 5th instar larvae with the highest concentration of pyriproxyfen. The major symptom of this failure was observed as incompletely ecdysed larvae with the attached old cuticle of the previous instar and some abdominal constrictions.

For the interpretation of this ecdysis failure of treated *A. ipsilon* larvae, it may be important to mention that the molting hormone "ecdysone" plays a major role in the shedding of old cuticles in a phenomenon called "ecdysis" or "molting". Pyriproxyfen might exhibit serious disturbances during larval molting, indicating that it disrupted the function of the larval endocrine system, thereby preventing the completion of molting (Ben Hamouda *et al.*, 2015). For some detail, pyriproxyfen might suppress the activity of ecdysone in larvae leading to the failure of moult and ultimately died (Baskar *et al.*, 2009; Baskar *et al.*, 2011; Jeyasankar *et al.*, 2013; Sivaraman *et al.*, 2014). On the other hand, failure of ecdysis of *A. ipsilon* larvae, in the current work, may be attributed to an inhibitory effect of pyriproxyfen on the chitin formation (Abdel Rahman *et al.*, 2007; Adel, 2012) or to the inability of larvae to shed their exocuticle during ecdysis (Linton *et al.*, 1997).

On the other hand, other features of the disrupted developmental program of *A. ipsilon*, such as larval-pupal intermediates, permanent larvae, giant larvae of supernumerary larvae, had not been observed in the present study after treatment with pyriproxyfen. However, the production of larval-pupal intermediates had been reported for some insects by various IGRs, such as *H. armigera* by hexaflumuron (Taleh *et al.*, 2015), *S. littoralis* by novaluron (Ghoneim *et al.*, 2015) and cyromazine (Tanani *et al.*, 2015), *C.*

cephalonica by fenoxycarb (Begum and Qamar, 2016); *P. gossypiella* (Ghoneim *et al.*, 2017a) *P. unionalis* (Ghoneim *et al.*, 2017b) by novaluron; and *P. unionalis* by methoxyfenozide (Hamadah and Abo Elsoud, 2018).

4. Impaired Metamorphosis of *A. ipsilon*:

Pupation is a crucial process in the life of insects for transformation from one stage to the next one. Depending on the current literature, the pupation rate of *A. ipsilon* was reported to be reduced after treatment with some IGRs, such as chlorfluazuron and triflumuron (Fahmy, 2014), chlorfluazuron and flufenoxuron (Shaurub *et al.*, 2018) and chlorantraniliprole (He *et al.*, 2019). Results of the present study were consistent with those reported results, since treatment of 4th or 5th instar larvae of *A. ipsilon* with a series of pyriproxyfen concentrations led to detrimentally suppressed pupation, proportional to the concentration. The present result was, also, in agreement with many reported results of reduced pupation of different insects, other than *A. ipsilon*, after treatment with various IGRs, such as such as *P. xylostella* after treatment with Hexaflumuron (Mahmoudvand *et al.*, 2012); *S. littoralis* after treatment with novaluron (Ghoneim *et al.*, 2015), cyromazine (Tanani *et al.*, 2015), methoxyfenozide (Khaled and Farag, 2015) and cycloheximide (Basiouny and Ghoneim, 2018); *G. Pyloalis* after treatment with lufenuron (Aliabadiet *et al.*, 2016) and fenoxycarb (Singh and Tiwari, 2016); the whitefly parasitic wasp *Encarsia formosa* after treatment with pyriproxyfen and fenoxycarb (Wang and Liu, 2016); *P. gossypiella* after treatment with novaluron (Ghoneim *et al.*, 2017a), teflubenzuron (Said *et al.*, 2017), noviflumuron (Hamadah and Ghoneim, 2017) and diofenolan (Tanani and Bakr, 2018); *P. unionalis* after treatment with novaluron (Ghoneim *et al.*, 2017b); *M. stabulans* after treatment with pyriproxyfen (Hamadah, 2018); *etc.*

To understand the regressed pupation rate of *A. ipsilon*, in the current investigation, pyriproxyfen might exert a suppressive action on the chitin synthesis and prevented the normal deposition of the new cuticle during apolysis (Retnakaran *et al.*, 1985). For some detail, pyriproxyfen might exert an inhibitory action on the prothoracic gland (ecdysone-producing gland) and hence the ecdysone could not be synthesized and/or released. In other words, pyriproxyfen might block the release of morphogenic peptides, causing a disturbance in titers of both ecdysteroids and juvenoids (Barnby and Klocke, 1990). Also, pyriproxyfen might disrupt the ecdysteroid metabolism or might alternatively act directly to inhibit the release of an ecdysis-triggering hormone (Gaur and Kumar, 2010). In addition, reduction of the pupation rate of *A. ipsilon* might be due to an inhibitory effect of pyriproxyfen on the synthesis of specific storage proteins in the fat body during the last larval instar and their deposition at the time of pupation (Gupta, 1985).

2.5. Impaired Morphogenesis Program of *A. ipsilon*:

According to the currently available literature, there are some reported results of impaired pupal morphogenesis of a number of insects, since deformed pupae were observed after treatment with different IGRs, such as the red flour beetle *Tribolium castaneum* and the confused flour beetle *Tribolium confusum* after treatment with cyromazine (Kamaruzzaman *et al.*, 2006); the fall armyworm *Spodoptera frugiperda* after feeding of 5th instar larvae on a diet treated with methoxyfenozide (Zarate *et al.*, 2011); *C. cephalonica* after topical application of fenoxycarb onto last instar larvae (Begum and Qamar, 2016); *P. gossypiella* after-treatment of the full-grown larvae with novaluron (Ghoneim *et al.*, 2017a) and *P. unionalis* after treatment of newly moulted last instar larvae with novaluron (Ghoneim *et al.*, 2017b). In contrast, the results of the present study disagreed with the previously reported results, since pyriproxyfen failed to affect the pupal morphogenesis and no malformed pupae were observed after treatment of the 4th instar or 5th instar larvae of *A. ipsilon* with a series of its concentrations.

Conclusion:

Depending on the results of the present study, pyriproxyfen exhibited a considerably toxic effect on different development stages of *A. ipsilon*, caused a drastic reduction of the larval weight gain and detrimental inhibition of growth. Also, it remarkably suppressed the pupation and disturbed development program. Therefore, pyriproxyfen could be recommended as an eco-friendly alternative to synthetic insecticides for the management of this dangerous insect.

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