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DECREASE IN PUPATION AND ADULT EMERGENCE OF *Plutella xylostella* (L.) TREATED WITH HEXAFLUMURON

Mohammad Mahmoudvand[1, 2*], Habib Abbasipour[1], Aziz Sheikhi Garjan[3], and Ali Reza Bandani[4]

The oligophagous pest *Plutella xylostella* (L.) is a major crucifer pest in Tehran Province, Iran. Hexaflumuron is an insect growth regulator insecticide with good effects on immature insect stages. The objective of this study was to investigate the effects of two sublethal concentrations (LC_{10} and LC_{25}) of hexaflumuron on some biological parameters of *P. xylostella* larvae, such as birth rate (b), death rate (d), finite rate of increase (λ), generation time (T), sex ratio, pupation rate, and adult emergence. Results showed that hexaflumuron decreased the total number of eggs and oviposition and post-oviposition periods, pupation, and adult emergence in the treated generation, b, and λ. Hexaflumuron also increased T, d, and the pre-oviposition period. However, sex ratio, percentage of pupation, and adult emergence in the offspring generation were not affected by hexaflumuron. Overall, these results indicated that sublethal concentrations of hexaflumuron can affect the biological parameters of *P. xylostella*.

Key words: *Plutella xylostella*, hexaflumuron, sublethal, birth rate, death rate, oviposition period.

The diamondback moth *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae) is one of the most destructive pests of cruciferous crops in the world, including Iran. The pest is widely distributed all over the world and can attack a wide range of wild or cultivated Brassicaceae plants, including mustard (Sarfraz *et al*., 2006; Mahmoudvand *et al*., 2009). This insect is much feared because it easily develops insecticide resistance, although the selected pesticides used to control it belong to different chemical classes with unique modes of action; it has been ranked in the top 20 resistant insects worldwide. This pest is the first reported case of field resistance to *Bacillus thuringiensis* (Perez *et al*., 1995; Mota-Sanchez *et al*., 2002).

Sublethal concentrations of insecticides can influence the physiological and behavioral characteristics of insects (Haynes, 1988; Curkovic and Brunner, 2005; Curkovic *et al*., 2009), including larval and pupal weight (Jun *et al*., 1999), adult emergence (Yin *et al*., 2008), developmental time (Michaud and Grant, 2003; Golmohammadi *et al*., 2009), survival (Yin *et al*., 2008), and fecundity (Galvan *et al*., 2005; Kellouche and Soltani, 2006; Wang *et al*., 2009). They also affect reproductive parameters, such as net reproductive rate (R_0), intrinsic rate of increase (r_m), finite rate of increase (λ), generation time (T) (Hui-Dong *et al*., 2004; Zanuncio *et al*., 2005; Mahmoudvand *et al*., 2011b), pre-oviposition, oviposition and post-oviposition periods (Josan and Singh, 2000; Hamedi *et al*., 2010), hatchability (Sammour *et al*., 2008), adult longevity (Cutler *et al*., 2005; Suh *et al*., 2009), egg size (Fujiwara *et al*., 2002), and sex ratio (Delpuech and Meyet, 2003).

In contrast with neurotoxic pesticides, which account for most of the active ingredients registered in all countries, insect growth regulators (IGRs) are targeting molt and metamorphosis and are more selective (Dhadialla *et al*., 1998; Hoffmann and Lorenz, 1998). A group of IGRs are benzoylphenylurea (BPU) compounds that interfere with insect growth, disturb molting, and deform the cuticle (Mian and Mulla, 1982; Reynolds, 1987). Because of their selectivity properties and efficacy on immature stages, BPUs can be used in Integrated Pest Management (IPM) programs (Wright and Retnakaran, 1987; Moser *et al*., 1992). Hexaflumuron [1-[3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy)phenyl]-3-(2,6-difluorobenzoyl) urea] is a BPU insecticide that inhibits chitin synthesis and interrupts the molting process in target insects. It has ingestion, contact, and ovicidal toxicity (Sbragia *et al*., 1983; El-Barkey *et al*., 2009; Mahmoudvand *et al*., 2011a). The impacts of hexaflumuron's sublethal effects on insects have been previously examined (Vasuki and Rajavel, 1992; Coppen and Jepson, 1996; Marco and Castañera, 1996; Abo-Elghar *et al*., 2003; Kellouche and Soltani, 2006; Bakr *et al*., 2009).

[1]Shahed University, Faculty of Agricultural Sciences, Department of Plant Protection, P.O. Box 18151/159, Tehran, Iran.
[2]Islamic Azad University, Department of Plant Protection, Khorramabad Branch, 68135 Khorramabad, Iran.
[*]Corresponding author (msco_1381@yahoo.com).
[3]Iranian Research Institute of Plant Protection, P.O. Box 1454-19395, Tehran, Iran.
[4]University of Tehran, University College of Agriculture and Natural Resources, Department of Plant Protection, P.O. Box 4111, Karaj, Iran.
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Since the last decade, *P. xylostella* has become a serious pest for members of the Brassicaceae crop family in Tehran Province. Iran. Based on our knowledge, no insecticide has been registered yet in Iran to control it. The present study is focused on the effects of sublethal doses of the IGR hexaflumuron on some biological parameters of the diamondback moth. Some data from this project were previously reported (Mahmoudvand *et al*., 2011c; 2012). Total number of eggs laid by females, sex ratio, pupation, and adult emergence (in parents and the next generation), oviposition period, and four reproductive parameters, including generation time, finite rate of increase, birth rate, and death rate of *P. xylostella* were reported here.

MATERIALS AND METHODS

Insect rearing

The primary population of *P. xylostella* was collected from cauliflower (*Brassica oleracea* L. var. *botrytis*) (Brassicaceae) crops in Shahre-Rey in southern Tehran, Iran. Approximately 500 *P. xylostella* adults were released in a plastic cage (50 cm × 30 cm × 30 cm); the eggs were then transferred to cauliflower leaves to continue their development. Insect stock was maintained at 25 ± 1 °C and 65 ± 5% RH under a 16:8 h photoperiod in a growth chamber. The colony was reared two generations before testing.

Bioassay of third-instar larvae

The bioassay used a leaf dip method (Tabashnik and Cushing, 1987). Cabbage leaf disks (3 cm diameter) were dipped in seven concentrations of hexaflumuron (Consult 10% EC, Dow Agro Sciences, Indianapolis, Indiana, USA) solutions containing 0.02% Tween-80 for 30 s. In the control group, leaf disks were dipped in water with 0.02% Tween-80. The treated leaf disks were allowed to dry at room temperature and were then placed in a plastic cup (3 cm depth and 5.5 cm diameter). Ten third-instar larvae that had been starved for 2 h were placed on the leaf disks. These tests were replicated four times and at least 60 third-instar larvae made up each concentration. Mortality was recorded 96 h after treatment. Data were analyzed with SAS software for probit analysis (SAS Institute, 1997). Values of LC_{10} and LC_{25} for leaf dip tests on third-instar *P. xylostella* were 0.59 and 0.91 (mg L^{-1}) after 96 h ($\chi^2 = 2.57$, $P = 0.76$).

Treatment with sublethal hexaflumuron concentrations

Cabbage leaf disks treated with LC_{10} and LC_{25} hexaflumuron concentrations and the control were prepared in 0.02% Tween-80. After drying, 25 third-instar larvae that had been starved for 2 h were placed on treated leaves in a plastic cup (as described above). Eight replicates were measured for each treatment. Live larvae were transferred to fresh cabbage leaves after 96 h and allowed to continue their development until the pupal stage. Pupae were placed individually in a Petri dish (8 cm diameter) until adults emerged. Afterwards, 20 pairs of adults of each of the sublethal concentrations or control were selected and each pair (male and female) was put in a plastic cage (14 cm × 11 cm × 5 cm). A sugar solution (10%) was used to feed moth adults. Adults were allowed to lay eggs on cabbage leaves placed in each cage. Leaves were replaced with fresh ones and the number of eggs laid was recorded daily. This process continued until the adult's death. Furthermore, pre-oviposition, oviposition, and post-oviposition periods were evaluated.

Data analysis

Data obtained were subjected to one-way ANOVA ($P < 0.05$) after checking for normality. Means were compared with Tukey's studentized range test with significant differences at $P < 0.05$. Differences in biological parameters were tested by the Jackknife method (Maia *et al*., 2000). All analyses were with SAS software (SAS Institute, 1997).

RESULTS

Pupation rate and adult emergence percentage

Pupation rate of the treated diamondback moth generation was significantly affected by hexaflumuron (Figure 1); however, no significant differences were found between the exposed and control groups in the offspring generation in the pupation trend (Parent: $F = 63.55$, $P < 0.0001$, df = 2, 21; Offspring: $F = 1.56$, $P = 0.2625$, df = 2, 9). The percentage of adult emergence in specimens exposed to hexaflumuron was obviously lower than the control in the treated generation ($F = 48.69$, $P < 0.0001$, df = 2, 21), but this parameter in the LC_{10} and LC_{25} concentrations and control were similar in offspring ($F = 1$, $P = 0.4053$, df = 2) (Figure 2).

Total number of eggs and sex ratio

Total number of eggs and sex ratio of treated and control specimens are shown in Table 1. Both sublethal

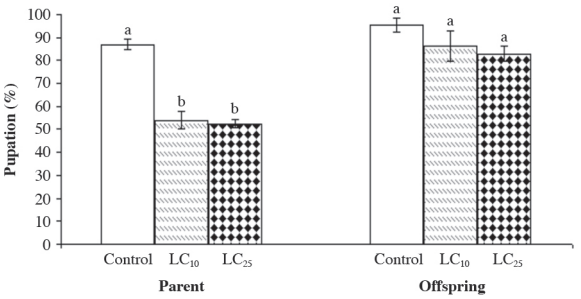

Figure 1. Effect of sublethal doses of hexaflumuron on pupation percentage of *Plutella xylostella* in parent and offspring generations (Parent: $F = 63.55$, $P > 0.0001$, df = 2, 21; Offspring: $F = 1.56$, $P = 0.2625$, df = 2, 9).

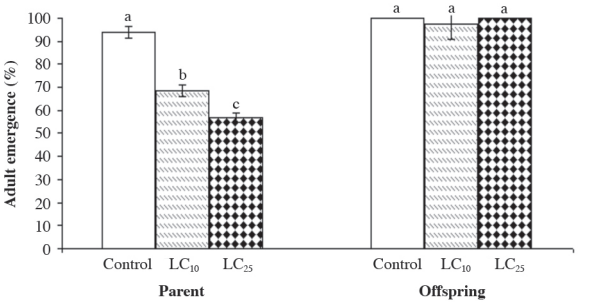

Figure 2. Effect of sublethal doses of hexaflumuron on adult emergence percentage of *Plutella xylostella* in parent and offspring generations (Parent: $F = 48.69$, $P > 0.0001$, df = 2, 21; Offspring: $F = 1$, $P = 0.4053$, df = 2, 9).

concentrations (LC_{10} and LC_{25}) caused a decrease in the total number of eggs laid by females as compared with the control. The total number of eggs differed significantly between LC_{10} and LC_{25} treatments ($F = 814.43$, $P < 0.0001$, df = 2, 57). However, there was no significant difference in sex ratio between control and sublethal hexaflumuron concentrations ($F = 0.5346$, $P = 0.70$, df = 2, 6).

Oviposition period

Table 2 shows the sublethal effects of hexaflumuron on pre-oviposition (time between adult emergence and first oviposition), oviposition, and post-oviposition periods of *P. xylostella*. Hexaflumuron at LC_{25} increased the pre-oviposition period ($F = 6.95$, $P = 0.0020$, df = 2, 57). The oviposition duration of the diamondback moth was lower than the control in the LC_{25} group ($F = 4.12$, $P = 0.0020$, df = 2, 57). In addition, hexaflumuron in both sublethal concentrations significantly declined the post-oviposition period of *P. xylostella* ($F = 35.82$, $P < 0.0001$, df = 2, 57).

Table 1. Comparison of number of eggs laid by all females and sex ratio of *Plutella xylostella* treated with sublethal doses of hexaflumuron and control.

	Total number of eggs of females	Sex ratio (± SE)
Control	3 741.35 ± 32.71a	0.50 ± 0.03a
LC_{10}	3 489.25 ± 17.46b	0.46 ± 0.03a
LC_{25}	2 491.65 ± 15.28c	0.43 ± 0.05a
P	< 0.0001	0.5346
F	814.43	0.70
Df	2, 57	2, 6

Means with different letters in the same column are significantly different according to Tukey's test ($P < 0.05$).
LC_{10}: concentration that killed 10% of population; LC_{25}: concentration that killed 25% of population; P: probability; Df: degree of freedom; SE: standard error.

Reproduction parameters

Effects of sublethal hexaflumuron concentrations on finite rate of increase (λ), generation time (T), birth rate (b), and death rate (d) of *P. xylostella* are reported in Table 3. Hexaflumuron at LC_{10} and LC_{25} concentrations significantly diminished λ ($F = 29433.23$, $P < 0.0001$, df = 2, 57) and also decreased b of the diamondback moth ($F = 9462.75$, $P < 0.0001$, df = 2, 57). Generation time (T) was delayed in the parent groups as compared with the control ($F = 19970.60$, $P < 0.0001$, df = 2, 57), and d at these two sublethal concentrations significantly increased ($F = 99999.99$, $P < 0.0001$, df = 2, 57).

Table 3. Comparison of finite rate of increase (λ), generation time (T), birth rate (b), and death rate (d) of *Plutella xylostella* treated with sublethal doses of hexaflumuron and control.

	λ (d^{-1})	T (d)	Birth rate (b)	Death rate (d)
Control	1.19 ± 0.00a	24.80 ± 0.01c	0.21 ± 0.00a	0.036 ± 0.00c
LC_{10}	1.15 ± 0.00b	26.15 ± 0.02b	0.19 ± 0.00b	0.051 ± 0.00b
LC_{25}	1.10 ± 0.00c	30.58 ± 0.02a	0.16 ± 0.00c	0.063 ± 0.00a
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001
F	29 433.23	19 970.60	9 462.75	99 999.99
Df	2, 57	2, 57	2, 57	2, 57

Means with different letters in the same column are significantly different according to Tukey's test ($P < 0.05$).
LC_{10}: concentration that killed 10% of population; LC_{25}: concentration that killed 25% of population; P: probability; Df: degree of freedom.

DISCUSSION

Results of the leaf dip bioassay indicated that hexaflumuron has a good toxicity on the third-instar larvae of *P. xylostella*. Furthermore, hexaflumuron had acceptable effects on pupation, adult emergence, oviposition, and reproductive factors of *P. xylostella* at sublethal concentrations.

In this study, the number of eggs laid by females decreased with hexaflumuron at sublethal concentrations. This suggests that hexaflumuron has a good effect on the physiology of *P. xylostella* and causes reduced egg-laying. Similarly, Abo-Elghar *et al*. (2003) and Kellouche and Soltani (2006) used different insects and reported a decline fecundity of *Callosobruchus maculatus* Fabricius after exposure to hexaflumuron. Sial and Brunner (2010) reported a decrease in fecundity of *Choristoneura rosaceana* (Harris) (Lepidoptera: Totricidae) treated with the IGR pyriproxyfen. Sex ratio of the offspring generation changed in some cases when adults were exposed to pesticides. This can occur due to the effect on the fertilization of the females' ova. This phenomenon

Table 2. Comparison of oviposition period (d ± SE) of *Plutella xylostella* treated with sublethal doses of hexaflumuron.

		Hexaflumuron				
	Control	LC_{10}	LC_{25}	*P*	*F*	df
Pre-oviposition period	0.65 ± 0.10b	1.35 ± 0.23ab	1.75 ± 0.26a	0.0020	6.95	2, 57
Oviposition period	15.7 ± 0.73a	15.55 ± 1.00a	12.65 ± 0.14b	0.0213	4.12	2, 57
Post-oviposition period	4.30 ± 0.27a	2.00 ± 0.24b	1.70 ± 0.19b	< 0.0001	35.82	2, 57

Means with different letters in the same row are significantly different according to Tukey's test ($P < 0.05$).
LC_{10}: concentration that killed 10% of population; LC_{25}: concentration that killed 25% of population; P: probability; Df: degree of freedom; SE: standard error.

has occurred particularly in haplodiploid insects. Another reason could be the differential survival of treated males and females before the adult stage (Idris and Grafius, 1993). According to these results, hexaflumuron was unable to change the sex ratio of the diamondback moth. Similarly, indoxacarb had no effect on the sex ratio of *P. xylostella* (Mahmoudvand *et al*., 2011b). A major effect of IGRs is in the molting process that disrupts cuticle synthesis. In this study, sublethal hexaflumuron concentrations in the treated generation decreased the pupation rate and adult emergence of *P. xylostella*. This effect was not repeated in the next generation. It is indicated that hexaflumuron had a direct impact on pupation rate and adult emergence, but the next generation exhibited no effects in these parameters. In accordance with the present study, Marco and Viñuela (1999) observed that sublethal hexaflumuron concentrations had a significant decrease on the percentage of pupation and adult emergence rate of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Vasuki and Rajavel (1992) also stated that hexaflumuron significantly decreased the adult emergence rate of *Anopheles stephensi* (Diptera: Culicidae). Analogous to this study's results, Mahmoudvand *et al*. (2011b) reported that sublethal concentrations of indoxacarb decreased the pupation rate and adult emergence of *P. xylostella* in the parent generation, but that these parameters were unchanged in the next generation. The oviposition and post-oviposition period of *P. xylostella* was shorter in the treatment groups than in the control in our study. On the other hand, sublethal concentrations postponed inception of female oviposition. Hexaflumuron delayed the start of oviposition by more than 1 d, whereas this period was 0.65 d for the control. In accordance with the present results, Josan and Singh (2000) showed that the oviposition period of *P. xylostella* significantly decreased when treated with lufenuron which is a CSI insecticide. In addition, Yin *et al*. (2008) reported that the pre-oviposition period of the diamondback moth was increased with spinosad. In this study, ova were affected by hexaflumuron and this caused the oviposition period to decrease. On the other hand, death of parent group females was low after oviposition as compared with the control.

Hexaflumuron sublethal concentrations reduced finite rate of increase (λ) and birth rate (b) of *P. xylostella*, but increased death rate (d) and generation time (T). In addition, Mahmoudvand *et al*. (2011b) recently reported decreases in the intrinsic rate of increase (r_m), net reproductive rate (R_0), and gross reproduction rate (GRR) along with an increase in the doubling time (D_t) of *P. xylostella* after exposure to hexaflumuron. Similarly, Sáenz-de-Cabezón *et al*. (2006) stated that r_m and λ of *Tetranychus urticae* (Koch) (Acari: Tetranychidae) decreased after being treated with the IGR triflumuron. Wang *et al*. (2008) remarked that biological parameters of *Myzus persicae* (Sulzer) (Hom.: Aphididae) were influenced by imidacloprid. In the study by Yin *et al*. (2008), LC_{25} and LC_{50} spinosad concentrations decreased λ and increased T in *P. xylostella*. We showed that the effect of hexaflumuron decreases the number of eggs and λ in *P. xylostella*, and Hui-Dong*et al*. (2004) found that emamectin also decreases these parameters in *P. xylostella*.

CONCLUSION

In conclusion, results suggest that hexaflumuron has a good ingestion effect against the *Plutella xylostella* larval stage. Hexaflumuron also reduced pupation rate, adult emergence, number of total eggs, and biological parameters of *P. xylostella*. Furthermore, hexaflumuron reduced the oviposition and post-oviposition period and increased the pre-oviposition period, whereas it had no impact on the sex ratio of the diamondback moth.

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Disminución en pupación y emergencia de adultos de *Plutella xylostella* (L.) tratados por hexaflumuron. *Plutella xylostella* (L.), una plaga oligofaga, es una importante plaga de crucíferas en la provincia de Teherán, Irán. Hexaflumuron, un insecticida regulador de crecimiento de insectos, tiene buenos efectos sobre estados inmaduros de insectos. El objetivo de este estudio es investigar los efectos de dos concentraciones subletales (LC_{10} y LC_{25}) de hexaflumuron sobre algunos parámetros de larvas de *P. xylostella* tales como tasa de nacimiento (b) y tasa de mortalidad (d), tasa finita de aumento (λ), tiempo de generación (T), proporción de sexos, tasa de pupación, emergencia de adultos, y algunos otros parámetros. Los resultados mostraron que hexaflumuron redujo el número total de huevos y ovipostura y períodos de post-ovipostura, pupación, y emergencia de adultos en la generación tratada, b y λ. Hexaflumuron además aumentó T, d y el período pre-ovipostura. Sin embargo, la proporción de sexos, porcentaje de pupación, y emergencia de adultos en la generación producto no fueron afectados por hexaflumuron. En conclusión, estos resultados indicaron que las concentraciones subletales de hexaflumuron pueden afectar los parámetros biológicos de *P. xylostella*.

Palabras clave: *Plutella xylostella*, hexaflumuron, subletal, tasa de nacimiento, tasa de mortalidad, período de ovipostura.

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