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Sublethal Effects of Fenoxycarb on the *Plutella xylostella* (Lepidoptera: Plutellidae)

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ABSTRACT. The effects of fenoxycarb, a Juvenile hormone analogue, at sublethal concentrations were tested on some biological parameters of *Plutella xylostella* (L.) in two consecutive generations. The calculated LC_{10}, LC_{25}, and LC_{50} values of the insecticide were 21.58, 43.25, and 93.62 mg/liter on third-instar larvae, respectively. Fenoxycarb significantly reduced pupal weight and oviposition period in parent generation. In addition, the fecundity of treated groups (LC_{10} = 71.06, LC_{25} = 40.60 eggs per female) in parents was significantly lower than control (169.40 eggs per female). Although fenoxycarb could not affect gross reproductive rate and death rate, it decreased net reproductive rate, intrinsic rate of increase, finite rate of increase, and birth rate in offspring generation. Also, mean generation time and doubling time of treated insects was significantly longer than control at LC_{10} level. Therefore, the data from this study suggested that fenoxycarb could adversely cause population decline in the subsequent generation.

Key Words: *Plutella xylostella*, sublethal, fenoxycarb, biological and oviposition parameter

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is the most destructive insect pest of Brassicaceae worldwide (Talekar and Shelton 1993). In the last two decades, its damage has increased in Tehran and other areas of Iran. Verkerk and Wright (1996) reported that the DBM produces 20 or more generations in tropical regions and cause up to 90% of yield loss. Although, there are many novel methods for insect control, chemical control using insecticides remained as a dependable method (Lee 2000). Up to now, DBM shows the significant resistance to several groups of insecticides (Talekar and Shelton 1993). Following the rules of resistance management, it is necessary to make a follow-up treatment with a pesticide of different mode of action. IGRs are new group of pesticides that is effective even at sublethal concentrations. They are effective on physiological or behavioral parameters of exposed treatments (Haynes 1988, Desneux et al. 2006, Alizadeh et al. 2012). Some of these changes are developmental time (Kumar and Chapman 1984, Coppen and Jepson 1996, Mahmoudvand et al. 2011b), larval and pupal weight (Abro et al. 1993, Jun et al. 1999, Yin et al. 2008), pupal rate and adult emergence (Sial and Brunner 2010, Han et al. 2012), fecundity (Elzen 2001, Cho et al. 2002), egg size and hatching (Yin et al. 2008, Han et al. 2012), adult longevity (Ergin et al. 2007, Hamedi et al. 2010, Mahmoudvand et al. 2012) as well as other biological parameters such as net reproductive rate and intrinsic rate of increase (Mahmoudvand et al. 2011a; Ahmad et al. 2012). Juvenile Hormone Analogues (JHAs) can alter the endocrine balance and therefore cause to abnormal development in some insects especially after metamorphosis (Retnakaran et al. 1985, Dhadialla et al. 1998). Fenoxycarb, [2(phenoxy–phenoxy)-ethyl carbamate], is one of these insecticides that was discovered by the Roche-Socar and Maag societies. It was the first compound from JHAs that was introduced to agricultural pest control (Masner et al. 1980, Miyamoto et al. 1993). It is recognized to have ovicidal and larvicidal activity and have impact on population control of Lepidoptera (Masner et al. 1987). In addition, fenoxycarb has low soil mobility, it does not accumulate, and it breaks down relatively quickly in the environment (Bicchi et al. 1990, Sullivan 2000, Michel et al. 2001). Therefore, this insecticide could be a rational candidate for integrated pest management of DBM. Fenoxycarb as a source of juvenilizing agent can have remarkable impact by disrupting growth, development, and behavior of insect pests. It may affect population size even in the subsequent generation. However, the adverse effects of this pesticide at sublethal concentrations on parents and subsequent generation of DBM have not been studied. In this study, we first, tried to determine lethal and sublethal concentrations of fenoxycarb against DBM. Thereafter, the experiments were established to investigate the sublethal effects on biological and population growth parameters of the insect in two consecutive generations.

Materials and Methods

Insect Rearing. The initial colonies of DBM were established by collection of larvae from infested leaves of cauliflower, *Brassica oleracea* L. (Brassicaceae) in a pesticide-free plantation located in the fields of Tarbiat Modares University, Tehran, Iran, in July 2013. Adults were introduced to cabbage leaves for egg laying in a plastic cage (58 by 20 by 28 cm) given access to 10% sugar solution. Insect stock was maintained at 25 ± 1°C and 65 ± 5% relative humidity under a photoperiod of 16:8 (L:D) h. The experiments were started after three generations.

Bioassay and Insecticide. Fenoxycarb (Insegar 25 WG) was supplied by Syngenta, Basel, Swaziland. Circular leaf disks (4.5 cm diameter) dipped in aqueous solutions of insecticide (0.02% Tween-20) for 10 s. Water containing 0.02% Tween-20 was used as control (Larew et al. 1985). Leaf disks dried at room temperature put in a plastic cup (3 by 5.5 cm) containing 10 third-instar larvae. The experiment was replicated four times and the mortality was recorded 96 h after treatment. The mortality data were subjected to probit analysis using SAS Ver. 9.1 (SAS Institute 2002) to calculate lethal (LC_{50}) and sublethal concentrations (LC_{10} and LC_{25}).

Effects of Sublethal Doses of Fenoxycarb.

Sublethal Effects on Parent of P. xylostella. Leaf disks dipped in solution equivalent to LC_{10} and LC_{25} for 10 s as described above. Then 25 third-instar larvae put on air-dried leaves and allowed to feed for 96 h. Then survived larvae let to feed and pupate on untreated leaves. Then pupae were maintained individually until adult emergence. The weight of each pupa was recorded on second day of pupation. Fifteen pairs of adults from each treatment were introduced into a mating box (8.5 by 6.5 by 4 cm) containing a piece of cabbage leaf for oviposition. The leaves were collected daily and replaced by a new one. The fecundity and egg hatch was recorded every day until death.

The pre-oviposition, oviposition, and post-oviposition periods were recorded in parents. The experiment was conducted with eight replications in a growth chamber similar to stock culture condition.

Sublethal Effects on Offspring Generation. The study of sublethal effects was continued on the subsequent generation by rearing the insect from egg to adult in untreated leaves. In this experiment, numbers of 100 eggs were transferred to a plastic cage by a fine brush separately and the pre-embryonic development time and the hatching rate were recorded. The post-embryonic development was studied until adult fecundity and ultimately death as described in the previous section.

Data Analysis. Biological parameters were calculated based on Carey (1993). Jackknife technique (Maia et al. 2000) which is similar to bootstrapping was used to estimate the sample mean and standard error of biological parameters. Intrinsic rate of increase (r_m) that is the immediate rate of increase of a population under marked condition in discrete time (Birch 1948) was calculated by the following equation: $\sum_{x=0}^{\omega} e^{-r(x)} l_x m_x = 1$, where "$x$" is the age, "$m_x$" the age-specific fecundity, and "l_x" is the survival rate. Gross reproductive rate (GRR $= \sum_{x=\alpha}^{\beta} m_x$) and net reproductive rate ($R_0 = \sum_{x=\alpha}^{\beta} l_x m_x$) are the age-specific fecundity and the average number of female offspring that produced by a females through their lifetime (Pressat 1985). Finite rate of increase ($\lambda = e^{r_m}$) is the multiplication of increase per unit time. Doubling time ($\mathrm{Dt} = \frac{\ln(2)}{r}$) and mean generation time ($T = \frac{\ln(R_0)}{r}$) are defined as the period that a population needs to increase to two and R_0 fold of initial size, respectively (Carey 1993). Birth rate ($b = \frac{1}{\sum_{x=0}^{\omega} e^{-r(x)} l_x}$) and death rate ($b = b - r$) were calculated in this study. The terms of pre-oviposition period (TPOP) is the time between birth to first oviposition and the adult pre-oviposition period (APOP) is referred to the time from adult emergence to first oviposition. Post-oviposition period is the time from last egg laying to death (Yin et al. 2008).

One-way analysis of variance (ANOVA) was performed to check significant differences among treatments after checking for normality. Post-test ANOVA was used to separate means by Tukey's Studentized Range test at $P < 0.05$. SAS software was used for all analyses (SAS Institute 2002).

Results

Toxicity of Fenoxycarb. The data indicated that the fenoxycarb had high toxicity against third-instar larvae. The estimated LC_{50} was 93.92 mg/liter after 96 h. Also, the LC_{10} and LC_{25} values were 21.58 and 43.25 mg/liter, respectively (Table 1).

Development Time. The development time of various stages in offspring generation are shown in Table 2. Sublethal concentrations at LC_{10} and LC_{25} level extended embryonic development for 2.88 and 3.21 days in the offspring, respectively ($F = 120.37$, df = 2, 291, $P < 0.0001$). Also fenoxycarb prolonged larval development time significantly (1st: $F = 4.46$, df = 2, 204, $P = 0.0127$; 2nd: $F = 9.95$, df = 2, 188, $P < 0.0001$; 3rd: $F = 1.29$, df = 2, 178, $P = 0.2962$; 4th: $F = 33.14$, df = 2, 162, $P < 0.0001$). Although the pupal developmental time was increased ($F = 24.87$, df = 2, 141, $P < 0.0001$), but prepupal period was not statistically different ($F = 0.91$, df = 2, 159, $P = 0.4055$). The results also indicated that the developmental time of pre-adult stage increased when fenoxycarb treated ($F = 88.88$, df = 2, 140, $P < 0.0001$). The total life span of males and females did not affect by fenoxycarb at LC_{10} and LC_{25} concentrations (Male: $F = 1.70$, df = 2, 72, $P = 0.1906$; Female: $F = 1.82$, df = 2, 67, $P = 0.1697$).

Oviposition Period in Parent and Offspring. Fenoxycarb at LC_{10} and LC_{25} extended the APOP significantly ($F = 5.40$, df = 2, 42, $P = 0.0082$) but decreased the oviposition period in a dose-dependent manner in parents ($F = 14.63$, df = 2, 42, $P < 0.0001$); however, it had no effect on the post-oviposition period ($F = 1.01$, df = 2, 42, $P = 0.3719$) as well as the adult longevity (both male and female) of parents (Male: $F = 0.61$, df = 2, 42, $P = 0.5477$; Female: $F = 1.51$, df = 2, 42, $P = 0.2326$). In offspring generation, APOP was not significantly affected ($F = 3.06$, df = 2, 67, $P = 0.0534$). In addition, total pre-oviposition period (TPOP) was extended significantly at sublethal concentrations ($F = 27.37$, df = 2, 67, $P < 0.0001$), but oviposition and post-oviposition periods did not affect in the offspring (oviposition period: $F = 0.27$, df = 2, 67, $P = 0.7608$; post-oviposition period: $F = 1.02$, df = 2, 67, $P = 0.3675$). At LC_{25}, the male and female longevity were diminished (Male: $F = 4.34$, df = 2, 70, $P = 0.0168$, and Female: $F = 3.48$, df = 2, 67, $P = 0.0366$) (Table 3).

Table 1. Toxicity of fenoxycarb on third-instar larvae of *P. xylostella*

Treatment	n^a	df	LC_{10} (mg/liter)[b]	LC_{25} (mg/liter)[b]	LC_{50} (mg/liter)[b]	Slope ± SE	χ^2	*P*-value
Fenoxycarb	280	5	21.58 (8.11–33.92)	43.25 (24.52–57.48)	93.62 (74.85–115.65)	2.01 ± 0.39	2.04	0.84

[a]Number of larvae.
[b]95% confidence limits in parenthesis.

Table 2. Effect of fenoxycarb on development time of *P. xylostella* offspring when treated in parents third-instar larvae

Entries	Development time (Mean ± SE) (day)[a]			*F*	*P*	$df_{t,e}$
	Control	LC_{10}	LC_{25}			
Egg	2.18 ± 0.03 c	2.88 ± 0.05 b	3.21 ± 0.05 a	120.37	<0.0001	2, 291
First-instar larvae	2.98 ± 0.05 b	3.34 ± 0.09 a	3.07 ± 0.10 ab	4.46	0.0127	2, 204
Second-instar larvae	1.61 ± 0.08 b	1.82 ± 0.08 b	2.19 ± 0.10 a	9.95	<0.0001	2, 188
Third-instar larvae	1.25 ± 0.07 a	1.34 ± 0.06 a	1.21 ± 0.06 a	1.22	0.2962	2, 178
Fourth-instar larvae	1.51 ± 0.06 b	2.40 ± 0.08 a	2.28 ± 0.12 a	33.14	<0.0001	2, 162
All larvae	7.44 ± 0.23 c	8.89 ± 0.16 a	8.25 ± 0.13 b	17.54	<0.0001	2, 159
Prepupa	0.40 ± 0.06 a	0.35 ± 0.06 a	0.48 ± 0.08 a	0.91	0.4055	2, 159
Pupa	3.70 ± 0.10 b	4.98 ± 0.16 a	4.71 ± 0.16 a	24.87	<0.0001	2, 141
Pre adult stages	13.61 ± 0.19 b	17.22 ± 0.21 a	16.62 ± 0.24 a	88.88	<0.0001	2, 140
Total life span (male)	30.78 ± 1.26 a	32.38 ± 1.39 a	28.76 ± 1.33 a	1.70	0.1906	2, 72
Total life span (female)	31.30 ± 1.18 a	32.20 ± 1.57 a	28.12 ± 1.47 a	1.82	0.1697	2, 67

[a]Means followed by the same letters within a row are not significantly different (Tukey's test; $P < 0.05$).

Pupal Weight in Parent and Offspring. The pupal weight was significantly decreased compared with control in parents. Also LC_{25} was more effective than LC_{10} ($F = 8.09$, df = 2, 72, $P = 0.0007$) (Fig. 1). Pupal weight of offspring was significantly decreased only at LC_{25} ($F = 7.03$, df = 2, 72, $P = 0.0016$).

Sublethal Effects on Fecundity and Population Growth Parameters. The fecundity and population growth parameters of DBM were significantly affected by fenoxycarb (Tables 4 and 5). In parent generation, fenoxycarb reduced the fecundity in a dose-dependent manner significantly ($F = 24.63$, df = 2, 42, $P < 0.0001$), however, it had no significant impact on fecundity of offspring (LC_{25}: F = 1.51, df = 2, 67, $P = 0.2287$). In addition, the daily fecundity and fertility did not affect by the LC_{10} and LC_{25} (fecundity: $F = 1.70$, df = 2, 67, $P = 0.1911$; fertility: $F = 1.99$, df = 2, 67, $P = 0.1452$) (Table 4).

Although the net reproductive rate (R_0) was significantly decreased by LC_{10} and LC_{25} ($F = 6.75$, df = 2, 67, $P = 0.0021$) (Fig. 2), however, no significant differences was observed in GRR ($F = 0.42$, df = 2, 67, $P = 0.6167$). In contrast to LC_{25}, the LC_{10} had a significant effect on the intrinsic rate of increase (r_m) ($F = 8.59$, df = 2, 67, $P = 0.0005$). Increasing the fecundity at LC_{25} dose is the main reason of this difference. The finite rate of increase (λ) at two sublethal concentrations was significantly decreased ($F = 7.79$, df = 2, 67, $P = 0.0009$). The doubling time (Dt) and mean generation time (T) were significantly extended only by the LC_{10}. Dt and T are related to r_m. Hence, similar to

Table 3. Effect of sublethal concentrations of fenoxycarb on pre-oviposition, oviposition, post-oviposition periods and adult longevity of *P. xylostella* in parents and offspring

Generations	Stages	Mean ± SE (day)[a]			*F*	*P*	$df_{t,e}$
		Control	LC_{10}	LC_{25}			
Parent	APOP[b]	2.13 ± 0.66 b	7.53 ± 1.80 a	8.33 ± 2.14 a	5.40	0.0082	2, 42
	Oviposition	11.26 ± 0.95 a	7.46 ± 1.04 b	3.26 ± 1.13 c	14.63	<0.0001	2, 42
	Post-Oviposition	0.86 ± 0.30 a	1.46 ± 0.40 a	0.80 ± 0.38 a	1.01	0.3719	2, 42
	Male longevity	20.07 ± 1.98 a	18.27 ± 1.79 a	17.00 ± 2.13 a	0.61	0.5477	2, 42
	Female longevity	14.26 ± 1.16 a	16.46 ± 1.39 a	12.40 ± 2.22 a	1.51	0.2326	2, 42
Offspring	APOP[b]	4.13 ± 0.78 a	2.70 ± 0.46 a	2.75 ± 1.61 a	3.06	0.0534	2, 67
	TPOP[c]	13.17 ± 0.26 b	16.79 ± 0.32 a	17.31 ± 1.06 a	27.37	<0.0001	2, 67
	Oviposition	10.65 ± 0.89 a	10.37 ± 1.35 a	9.43 ± 0.93 a	0.27	0.7608	2, 67
	Post-oviposition	2.06 ± 0.57 a	1.29 ± 0.43 a	1.25 ± 0.44 a	1.02	0.3675	2, 67
	Male longevity	16.60 ± 1.27 a	14.65 ± 1.32 ab	11.76 ± 1.33 b	4.34	0.0168	2, 70
	Female longevity	16.62 ± 1.20 a	14.33 ± 1.52 ab	11.17 ± 1.50 b	3.48	0.0366	2, 67

[a]Means followed by the same letters within a row are not significantly different (Tukey's test; $P < 0.05$).
[b]Adult pre-oviposition period, time between adult emergence and first oviposition.
[c]Total pre-oviposition period, time from birth to first reproduction in female.

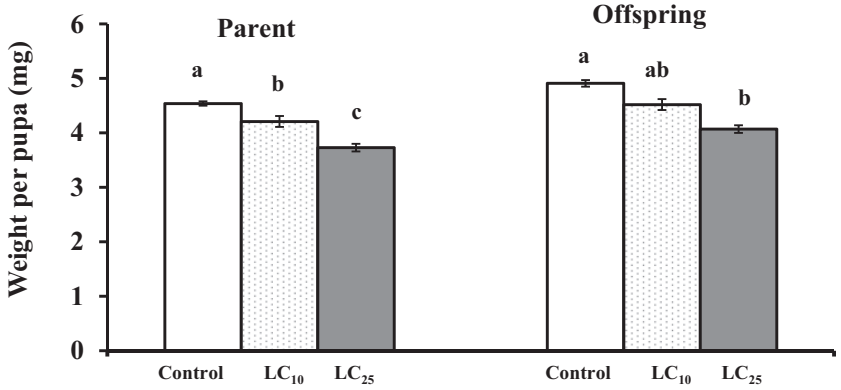

Fig. 1. Effects of fenoxycarb on pupal weight of *P. xylostella* in the parent and offspring when third-instar larvae were fed on fenoxycarb treated leaves for 96 h in the first generation.

Table 4. Comparison of fecundity and fertility of *P. xylostella* treated with sublethal doses of fenoxycarb and control in parents and offsprings

Entries	Parent (mean ± SE) (day)[a]	Offsprings (mean ± SE) (day)[a]		
	Fecundity (eggs/female)	Fecundity (eggs/female)	Fecundity (egg/female/day)	Fertility (fertile egg/female/day)
Control	169.40 ± 12.84 a	148.00 ± 12.22 a	4.33 ± 0.42 a	4.33 ± 0.42 a
LC_{10}	71.06 ± 14.34 b	120.25 ± 15.25 a	3.28 ± 0.43 a	3.18 ± 0.41 a
LC_{25}	40.60 ± 13.46 b	153.06 ± 15.11 a	4.20 ± 0.35 a	4.07 ± 0.34 a
F	24.63	1.51	1.70	1.99
P	<0.0001	0.2287	0.1911	0.1452
$df_{t,e}$	2, 42	2, 67	2, 67	2, 67

[a]Means followed by the same letters within a column are not significantly different (Tukey's test; $P < 0.05$).

r_m, these parameters were affected only by LC_{10} (Dt: $F = 6.72$, df = 2, 67, $P = 0.0022$; T: $F = 4.05$, df = 2, 67, $P = 0.0218$). The birth rate (b) at LC_{10} was obviously lower than those of control ($F = 7.45$, df = 2, 67, $P = 0.0026$), but no significant differences was observed in death rate of treatments (d) ($F = 0.14$, df = 2, 67, $P = 0.8708$) (Table 5).

Discussion

There are several studies showing the toxicity of fenoxycarb on a number of insects (Chandler et al. 1992, Singh and Tiwari 2015), however, this is the first study reporting the sublethal effects of fenoxycarb on DBM. In this study, fenoxycarb was detrimental to DBM in parent and offspring generation. However, it should be caution that fenoxycarb may enhance the fecundity of some insects such as *Cacopsylla pyricola* (Forster) (Homoptera: Psyllidae) (Solomon and Fitzgerald 1987, Horton and Lewis 1996). Other insecticides such as pyriproxyfen, a JH analogue (Oouchi 2005) and methomyl and fanvalerate (Sota et al. 1998, Fujiwara et al. 2002) may also promote reproduction of DBM. Similar to neemarin (Ahmad et al. 2012) and metaflumizone (Zhang et al. 2012), fenoxycarb could reduce the reproductive and biological parameters of DBM. The disruption in reproduction has been reported when insects were treated by JHAs such as fenoxycarb at larval stage (Retnakaran et al. 1985, Rumpf et al. 1998). Impaired reproduction may occur as a result of production of underweight pupae by failure of food uptake or disturbing somatic physiology (Grosch and Hoffman 1973). In this study, fenoxycarb caused underweight pupae in parent and offspring. In contrast, Mauchamp et al. (1989) have reported an increase in body weight of tobacco budworm, *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) fenoxycarb-treated insects. However, this study is accordant with studies of Biddinger and Hull (1999), who reported the production of small pupae

Table 5. Effect of fenoxycarb on biological parameters of *P. xylostella* offspring when treated in parents third-instar larvae

Entries	Biological parameters (mean ± SE)[a]							
	GRR	R_0	r_m (day^{-1})	λ (day^{-1})	T (day)	Dt (day)	b (birth rate)	d (death rate)
Control	98.68 ± 12.02 a	50.76 ± 4.00 a	0.190 ± 0.008 a	1.21 ± 0.01 a	20.73 ± 0.71 b	3.61 ± 0.18 b	0.26 ± 0.01 a	0.07 ± 0.003 a
LC_{10}	91.39 ± 12.84 a	31.31 ± 3.95 b	0.148 ± 0.008 b	1.16 ± 0.01 b	23.27 ± 0.78 a	4.68 ± 0.26 a	0.21 ± 0.01 b	0.06 ± 0.002 a
LC_{25}	112.09 ± 14.15 a	32.20 ± 2.13 b	0.164 ± 0.006 ab	1.18 ± 0.08 b	21.26 ± 0.54 ab	4.17 ± 0.20 ab	0.22 ± 0.01 ab	0.06 ± 0.001 a
F	0.42	6.75	8.59	7.79	4.05	6.72	7.45	0.14
P	0.6761	0.0021	0.0005	0.0009	0.0218	0.0022	0.0012	0.8708
$df_{t,e}$	2, 67	2, 67	2, 67	2, 67	2, 67	2, 67	2, 67	2, 67

[a]Means followed by the same letters within a column are not significantly different (Tukey's test; $P < 0.05$). See 'Materials and methods' section for abbreviations.

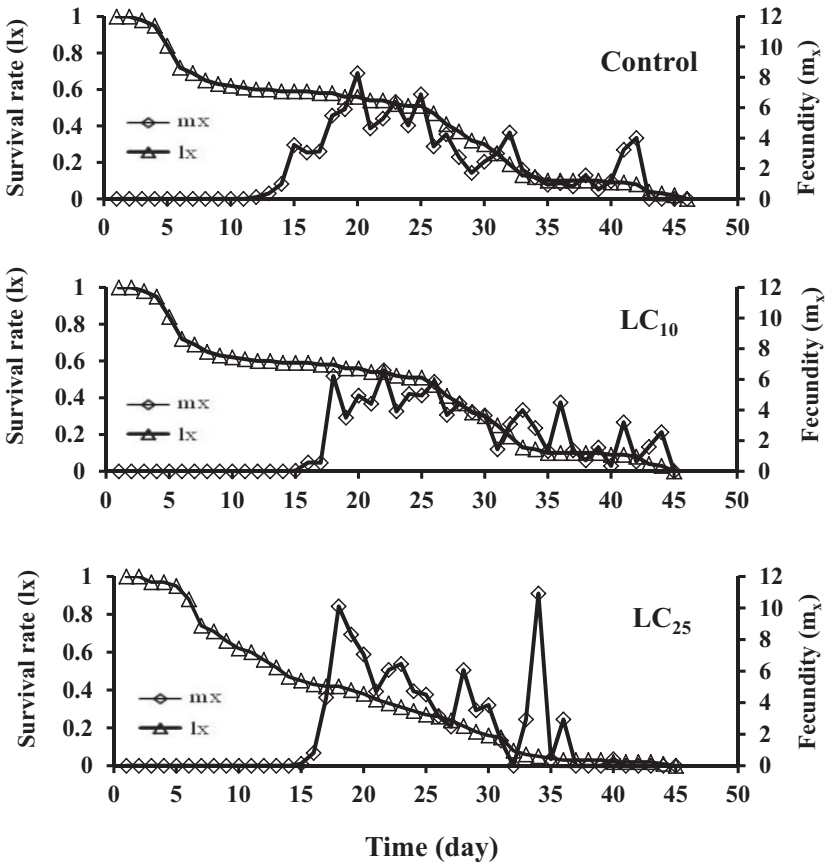

Fig 2. Age-specific survival rate (l_x) and age-specific fecundity (m_x) of *P. xylostella* in offspring when third-instar larvae were fed on fenoxycarb treated leaves for 96 h in the previous generation.

as a consequence of fenoxycarb treatment. We found that developmental time of eggs, larvae, and pupae prolonged significantly in the offspring generation. Extending the development time of pre-adult stages may increase exposure risk to natural enemies (Charleston 2004). It seems that fenoxycarb, like JHs, can usually maintain juvenalizing character by extending the immature stages as stated by other researchers (Mauchamp et al. 1989; Reid et al. 1990, 1994; Letellier et al. 1995; Singh and Johnson 2013). It could be concluded that JHAs act in the same manner as JHs but they are much more chemically stable (Matolcsy et al. 1988). Therefore, fenoxycarb may cause the adult insects to maintain larval characteristics with ultimate failure of normal reproduction. Our findings revealed the adult life span was affected by fenoxycarb. The pre-oviposition period in parents was significantly increased when last instar larvae were fed with treated leaves. Similarly, female longevity of *C. pyricola* (F.) (Homoptera: Psyllidae) (Horton and Lewis 1996) and DBM (Yin et al. 2008) has extended when they treated by fenoxycarb and spinosad in larval stage, respectively. In contrast, adult's longevity was diminished when DBM larvae was treated with hexaflumuron an IGR (Mahmoudvand et al. 2011b).

In this study, we found that fenoxycarb was highly toxic to third-instar larvae of DBM. Larval death might be caused by abnormal amounts of JHA. We also demonstrated that feeding the third-instar larvae with fenoxycarb-treated leaves for 96 h may cause a significant decrease in pupal weight and fecundity in subsequent generation, even the insects were fed with untreated leaves.

High levels of JHAs such as fenoxycarb, when applied to later instars, may cause the final adult stage to maintain larval characteristics and these insects generally cannot reproduce (Sullivan 2000). Fenoxycarb had toxic effects by decreasing the fecundity but increasing the development time. In the other hand, fenoxycarb can effectively influence reproductive physiology, such as intrinsic rate of increase and other biological parameters. However, further studies are necessary to reveal long-term effects of fenoxycarb on DBM.

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