Article

Side effects of thiacloprid+deltamethrin, pirimicarb and pymetrozine on the black bean aphid parasitoid, *Lysiphlebus fabarum* Marshall (Hymenoptera: Aphidiidae)

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Abstract

Lysiphlebus fabarum Marshall is the main parasitoid of the black bean aphid, *Aphis fabae* Scopoli. Lethal and sublethal effects of thiacloprid+deltamethrin, pirimicarb and pymetrozine were evaluated on the parasitoid under laboratory conditions. Newly emerged females were exposed to dry insecticide residues that were applied on glass plates. Thiacloprid+deltamethrin caused 100% mortality. Pirimicarb produced significant mortality and adverse effects on fecundity, while pymetrozin did not. According to the IOBC (International Organization of Biological Control) standards, thiacloprid+deltamethrin (E = 100%), pirimicarb (E = 62.70%) and pymetrozine (E = 11.86%) were classified as harmful, slightly harmful and harmless, respectively. Life table assays revealed that intrinsic rate of natural increase (r_m) inthiacloprid+deltamethrin (50%) and pirimicarb (12%) were significantly reduced compared to the control group, while pymetrozine had no such effects. Our results showed that pymetrozine was safe for *L. fabarum*, but pirimicarb and thiacloprid+deltamethrin had deleterious effects on this parasitoid. In sum, the present study suggests a relative compatibility between pymetrozine and *L. fabarum*.

Keywords ecotoxicology; IOBC; insecticide; life table parameters.

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1 Introduction

The black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae) is one of the most important pests of several cultivated crops worldwide (Völkl and Stechman, 1998). *Lysiphlebus fabarum* Marshall (Hymenoptera: Aphidiidae) is one of the main endoparasitoid of *A. fabae* in many agro-ecosystems (Raymond et al., 2000;

Nuessly et al., 2004), including those in Iran (Mahmoudi et al., 2010; Rasekh et al., 2010). This parasitoid has the potential to cause drastic reductions in *A. fabae* populations and could be useful in the biological control of this pest species (Völkl and Stechman, 1998).

Hymenopterous parasitoids are well known for their ability to control pests within a range of cropping systems worldwide (Desneux et al., 2005; Han et al., 2014). They can reduce the populations of their hosts by parasitizing up to 90% of the individuals (Hawkins et al., 1997); however, applications of insecticides reduce their beneficial impact (Desneux et al., 2005).In addition to direct mortality, insecticides can cause sublethal effects against natural enemies. The sublethal effects of an insecticide may be expressed as reduction in life span, development rates, fecundity, changes in sex ratio and behavior (Stark et al., 2004; Tran et al., 2004; Biondi et al., 2012; Saber and Abedi, 2013). In the context of Integrated Pest Management (IPM), the use of selective insecticides is essential for the conservation of natural enemies (Desneux et al., 2006; Moens et al., 2012).

The integration of chemical and biological control systems requires knowledge about the impact and selectivity of the insecticides on natural enemies (Stark et al., 2004).One of the widely used methods in studying the side effects of insecticides on natural enemies is a tiered approach through which the initial screening of insecticides is carried out in the laboratory and according to the obtained results, followed by semi-field or field tests (Candolfi et al., 2001). The aim of this method is to evaluate the acute residual toxicity and also the sublethal effects of the insecticides on the reproductive performance (Vogt et al., 2000).Furthermore, demographic toxicological analysis or the life table response experiment (LTRE) is usually considered the best way to evaluate the total effects of insecticides on non-target insects(Stark et al., 2004; Biondi et al., 2013).This approach provides an advantage in that it determines the total effects into a single endpoint, the intrinsic rate of natural increase (r_m) (Stark and Banks, 2003).

The objective of this study was to assess the lethal and sublethal effects of three major insecticides, thiacloprid+deltamethrin, pirimicarb and pymetrozine, on the parasitoid *L. fabarum* using the IOBC (International Organization of Biological Control) and life table experiments.

2 Materials and Methods

2.1 Insect culture

A thelytokous colony of parasitoid *L. fabarum* was established from mummified *A. fabae* collected from broad bean fields in the Tarom region, Zanjan Province, Iran. The parasitoid was reared on *A. fabae* fed on potted broad bean, *Viciafaba* var. Sarakhsi.

Synchronous cohorts of wasps were produced by exposing third instar (3-day-old) nymphs of *A. fabae* fed on broad bean leaves which were placed on 7% Agar gel to 3-day-old females of *L. fabarum* in a 5:1 ratio in ventilated petri dishes (9 cm diameter).After a 6 h exposure, aphids were removed and placed on potted broad bean plants in ventilated plastic nymphal rearing cages (15 cm diameter × 25 cm height) until mummies were formed. When mummies appeared, they were removed from broad bean plants and then placed in plastic cylinders (5 cm diameter ×3.5 cm height) until the adult wasps emerged. Whereupon, adult females were released into ventilated rearing cages (7 cm diameter× 5 cm height) and provisioned with diluted honey (as droplets on a cotton wool).Cultures of both insects were maintained and experiments were conducted in a growth chamber at 21 ± 1°C, 60 ± 5% RH and a 16:8 h (L:D) photoperiod.

2.2 Insecticides

The tested insecticides were applied at the recommended field rates for the control of aphid: thiacloprid+deltamethr in 500 ml a.i./ha (Proteus, 11% OD, Bayer crop Science, Germany) (for pea), pirimicarb250g a.i./ha (Pirimor, 50% WG, Syngenta Crop Protection A.G., Switzerland)(for broad bean) and

pymetrozine250g a.i./ha (Chess, 50% WG, Syngenta Crop Protection A.G., Switzerland) (for brassica). Fresh insecticide solutions were prepared with distilled water.

2.3 Chemical tests

The experiments were carried out following the method of Moens et al. (2012).

2.3.1 IOBC assay

Newly emerged *L. fabarum* females (less than 24h old) were exposed to dry residues of insecticides on glass plates (10 cm diameter). Appropriate amounts of each insecticide were diluted with 100 ml of distilled water to provide the recommended field concentrations, which were 0.5, 0.25 and 0.25 ml a.i.l^{-1} for thiacloprid+deltamethrin, pirimicarb and pymetrozine, respectively. One side of each glass plate was sprayed with 1 ml of aqueous solution of each insecticide using a Potter tower (BURKARD MFG. CO. LTD) at 14 mbar pressure. The spraying resulted in a homogeneous spray coverage of 0.92 ml aqueous solution deposit per cm^2. In the control, the glass plates were sprayed with distilled water. The plates were left to dry for 1 h, after which they were joined with an Plexiglas cylinder (10 cm diameter × 3 cm height) to form a drum cell. Each cylinder had three holes (2 cm diameter) covered with nylon gauze which allowed for ventilation. Batches of fifteen newly emerged females (less than 24h old) were confined in the treated drum cells and were given droplets of honey-water (20% honey in tap water) on the nylon gauze as a food source. There were five replicates per treatment.

Twenty four hours after exposure, the mortality of the parasitoids was recorded. The females surviving exposure after 24h were placed individually in ventilated plastic cylinders (25 cm diameter × 50 cm height) containing twenty, third instar nymphs of *A. fabae* fed on potted broad bean plants daily for oviposition in new cages until death of the females. The survival of each female parasitoid and its fecundity (the number of blackish colored aphids) were recorded daily.

2.3.2 Life table assay

The technique used to assess the effects of the insecticides on the life table of *L. fabarum* was the same as described in previous section. Approximately 300 (20 batches of fifteen) newly emerged *L. fabarum* females (less than 24 h old) were treated with the recommended field concentrations of the insecticides.

One day after exposure, the drum cells were dismantled and mortality was checked. Twenty four hours after exposure, surviving females were transferred to a glass box (15 cm diameter × 5 cm height) and 40 of females were randomly selected. Each female was placed individually in a plastic cylinder (25 cm diameter × 50 cm height) containing twenty, third instar nymphs of *A. fabae* fed on potted broad bean plants daily for oviposition in new cages until death of the females. The survival and fecundity (the number of blackish coloured aphids) of each female parasitoid were recorded daily for their whole life span.

2.4 Data collection procedure

The data related to the mortality and fecundity were subjected to Analysis of variance (ANOVA) and the means were compared using LSD test ($P < 0.05$) (SPSS, 2004). To provide a single value, summarizing potential deleterious effects of the insecticide tested, the toxic effects of each insecticide were expressed as the reduction coefficient (E_x) for insecticide x using the following formula (Biondi et al., 2012):

$$E_x = 100\left\{1-\left[\left(1-\frac{E_{mx}}{100}\right)\left(1-\frac{E_{fx}}{100}\right)\right]\right\} \quad (1)$$

where, E_{mx} is the corrected mortality (Abbott, 1925) and E_{fx} is the corrected parasitoid reproductive capacity estimated using the formula:

$$E_{fx} = 100 - \frac{F_x 100}{F_c} \quad (2)$$

where F_x is the mean parasitoid reproductive capacity (fecundity or eggs laid by parasitoid) for insecticide x and F_c is the parasitoid reproductive capacity (fecundity) recorded in control group (untreated group).The values E_x were then classified and interpreted according to the standard of the International Organization for Biological Control (IOBC) which includes four categories: (1) harmless: E_x< 30 %; (2) slightly harmful: 30 % < E_x< 80 %; (3) moderately harmful: 80 % < E_x< 99 % and (4) harmful: E_x> 99 % (Biondi et al., 2012).

Daily schedules of mortality and fecundity were integrated into a life table format (Chi and Liu, 1985) and used to calculate the demographic parameters. The age-specific survival rate (L_x) and the age-specific fecundity (m_x) were calculated using the following formulas, respectively (Chi and Liu, 1985):

$$L_x = \sum_{j=1}^{\beta} S_{xj} \quad (3)$$

$$m_x = \frac{\sum_{j=1}^{\beta} S_{xj} f_{xj}}{\sum_{j=1}^{\beta} S_{xj}} \quad (4)$$

where, S_{xj} is the age-stage specific survival rate, β is the number of stages and f_{xj} is the age-stage specific fecundity (x = age and j = stage).

The intrinsic rate of increase (r_m) was calculated according to the following formula (Chi and Liu, 1985):

$$\sum_{x=0}^{y} L_x m_x e^{-rx} = 1 \quad (5)$$

where y is the oldest age class and x is the age of each female at each age interval.

The other main life table parameters including gross reproduction rate (*GRR*), net reproductive rate (R_0), finite rate of increase (λ) and mean generation time (T) were computed using the following formulas, respectively:

$$GRR = \sum m_x \quad (6)$$

$$R_0 = \sum L_x m_x \quad (7)$$

$$\lambda = e^{r_m} \quad (8)$$

$$T = \frac{\ln R_0}{r_m} \quad (9)$$

The values for the demographic parameters (L_x, m_x, *GRR*, R_0, r_m, λ and T) were calculated by the jackknife method using the TWOSEX-MS Chart program (Chi, 2005).The jackknife pseudo-values for the demographic parameters for each treatment were subjected to analysis of variance (ANOVA) and means were compared by LSD test ($P < 0.05$) (SPSS, 2004).

3 Results

3.1 IOBC assay

The mean mortality for thiacloprid+deltamethrin and pirimicarb was significantly higher than that in the control group (F = 196.446;df = 3,16; p<0.05); however, there were no significant differences between pymetrozine and the control (Table 1). Pymetrozine had no significant effect on the fecundity (the total number of mummies produced per surviving female) but pirimicarb caused a significant reduction (F = 12.793; df = 2,159;p<0.05).

Comparing the total effects (E) of the insecticide revealed that thiacloprid+deltamethrin (E = 100%), pirimicarb (E = 62.70%) and pymetrozine (E = 11.86%) were classified as harmful, slightly harmful and harmless, respectively, based on the IOBC classification.

Table 1 Effects of thiacloprid+deltamethrin, pirimicarb and pymetrozine on the mortality and fecundity of *Lysiphlebus fabarum* in the adult stage (Mean ± SEM).

Treatment	Mortality (%)	Fecundity (number)
thiacloprid+deltamethrin	100.0 ± 0.0 a*	_
pirimicarb	54.68 ± 4.24 b	23.42 ± 1.74 a
pymetrozine	18.76± 2.51 c	31.12 ± 1.19 b
Control	14.65 ± 2.43 c	33.65 ± 1.12 b

* Means within a column followed by different letters are significantly different (α= 0.05; LSD test).

3.2 Life table assay

The age-specific survival rate (L_x) (Fig. 1) and the age-specific fecundity (m_x) (Fig 2.) did not vary significantly among treatments (F = 0.243; df = 3,76;p = 0.866 and F = 1.173; df = 3,76;p = 0.325, respectively). Some demographic parameters of *L. fabarum* were affected by applying the insecticides (Table 2). The value of gross reproductive rate (*GRR*) in thiacloprid+deltamethrin was significantly lower than other insecticides as well as the control treatments (F = 31.304; df = 3,156; p < 0.05); however, there were no significant differences between *GRR* obtained in pirimicarb and pymetrozine treatments with that of the control.The values of the net reproductive rate (R_0), the intrinsic rate of increase (r_m) and finite rate of increase (λ) in thiacloprid+deltamethrin and pirimicarb were significantly lower than those of the control (F = 47.535; df = 3,156; p < 0.05, F = 145.864; df = 3,156; p < 0.05 and F = 139.334; df = 3,156; p < 0.05, respectively). However, there were no significant differences between pymetrozine and the control group, whereas the difference between thiacloprid+deltamethrin and pirimicarb was significant. The mean generation time (T) did not vary significantly among treatments (F = 1.500; df = 3,156; p = 0.217).

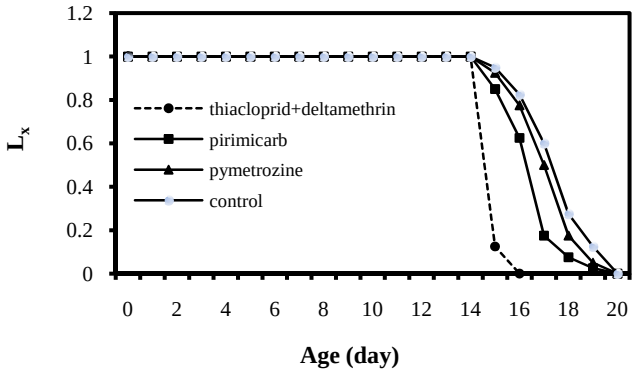

Fig. 1 Effects of thiacloprid+deltamethrin, pirimicarb and pymetrozine on age-specific survival rate (L_x) of *Lysiphlebus fabarum* in the adult stage.

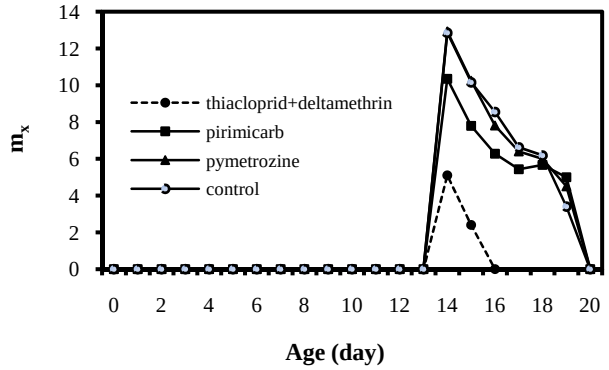

Fig. 2 Effects of thiacloprid+deltamethrin, pirimicarb and pymetrozine on age-specific fecundity (m_x) of *Lysiphlebus fabarum*in the adult stage.

Table 2 Effects of thiacloprid+deltamethrin, pirimicarb and pymetrozine on life table parameters of *Lysiphlebus fabarum*in the adult stage (Mean ± SEM).

Treatment	*GRR* (offspring)	R_0 (offspring/individual)	r_m (d^{-1})	λ (d^{-1})	*T* (d)
thiacloprid+deltamethrin	7.5 ± 0.8 a*	5.4 ± 0.4 a	0.112 ± 0.004 a	1.119 ± 0.005 a	15.05 ± 0.02a
pirimicarb	45.4 ± 5.7 b	22.4 ± 1.9 b	0.197 ± 0.005 b	1.218 ± 0.006 b	15.79 ± 0.07 a
pymetrozine	48.7 ± 3.6 b	32.9 ± 2.4 c	0.219 ± 0.004 c	1.245 ± 0.005 c	15.12 ± 0.08a
Control	47.6 ± 2.1 b	35.7 ± 2.5 c	0.223 ± 0.003 c	1.249 ± 0.004 c	16.09 ± 0.06a

* Means within a column followed by different letters are significantly different (α= 0.05; LSD test).

4 Discussion

The present study was performed to determining the tested insecticides effects on mortality, fecundity and life table parameters of the parasitoid *L. fabarum* and providing applied information for IPM managers for further researches in field and semi-field studies.

Our results showed that thiacloprid+deltamethrin, pirimicarb and pymetrozine caused 100, 54.7 and 18.8% mortality, respectively. According to the IOBC classification of insecticide toxicity, these insecticides were classified as harmful, slightly harmful and harmless, respectively. Former studies were performed to determine the lethal and sublethal effects of these insecticides on different biocontrol agents that comparing their results with ours could have interesting findings.

Thiacloprid+deltamethrin has been previously classified as harmful (IOBC class 4) to the lady beetle *Hippodamia variegata* Goeze, notably because of high mortality (100%) induced in larval and adult stages (Almasi et al., 2013). Thiacloprid as a neonicotinoid insecticide, act on the acetylcholine receptors of insects nervous system (Rouhani et al., 2013); while, deltamethrin modify electrical activity of the nervous system as a pyrethroid (Rehman et al., 2014). It could be deduced that, gathering the effects of both neonicotinoid and pyrethroid compounds, makes the proteus a really harmful insecticide for different natural enemies.

Several studies have reported that pirimicarb caused high mortality when adult parasitoids were exposed to dry residues or directly spray method (Desneux et al., 2004; Moens et al., 2012), but no effects (Symington., 2003; Moens et al., 2012) or only sublethal effects (Umoru and Powell, 2002) were observed when the parasitoids were developed in treated aphids. These studies indicate that adult parasitoids are more susceptible to pirimicarb in comparison with pupae. Indeed, aphid parasitoids could be protected against insecticides during the pupal stage in the mummified host aphid (Desneux et al., 2006). However, our results showed the significant reduction in population increase indices due to treatment of *L. fabarum* by pirimicarb that revealed its reductive effects on the parasitoid reproductive ability. Further studies are needed to determine the exact effect of pirimicarb on reproductive organs or habitats of *L. fabarum*.

The present study showed that pymetrozine induced low acute toxic effects on *L. fabarum*. Pymetrozine has repeatedly been reported to be harmless a wide spectrum of important beneficial arthropods (Torres et al., 2003; Medina et al., 2007; Liu et al., 2012). Because pymetrozine show very specific mode of action and has feeding inhibitory effects against sap sucking insect pest (Acheampong and Stark, 2004); accordingly, it is considered compliant with IPM.

The current study showed that insecticides had different effects on fecundity. Pymetrozine had no adverse effect on fecundity of *L. fabarum*. Similarly, Jansen et al. (2011) reported that the maximum recommended field rate of pymetrozine had no significant effect on fecundity of *Aphidius rhopalosiphi*e Stefani-Perez. However, pirimicarb significantly reduced the fecundity rate of the parasitoid in compare with control. Umoru and Powell (2002) reported that pirimicarb reduced the capacity of parasitism of *Diaeretiella rapae* when females were treated during the pupal stage. Reductions in fecundity associated with pesticides may be due to both physiological and behavioral effects. Pesticides can disrupt the very precise coordination between the insect nervous and hormonal systems, resulting in a breakdown in the complex series of behavioral (e.g., host searching) and physiological (e.g., vitellogenesis or ovulation of mature eggs) events related to oviposition (Desneux et al., 2007).

In addition to direct mortality induced by pesticides, their sublethal effects must be considered for a complete analysis of their impact (Desneux et al., 2006). Moreover, demographic toxicology is usually considered to be an important tool for the accurate assessment of the total effects of an insecticide (Stark and Banks, 2003).

The results of sublethal effects showed that life table parameters such as *GRR*, R_0, r_m and λ were reduced by thiacloprid+deltamethrin exposure. In a similar study, Almasi (2011) compared the effects of thiacloprid+deltamethrin and pymetrozine exposures to the predator *H. vaiegata* in larval stage. He found that thiacloprid+deltamethrin severely reduced all life table parameters in comparison with pymetrozine. Proteus® with the combination of the two different active ingredients, thiacloprid and deltamethrin, provides fast knockdown effect and long-lasting residual control against aphids and other sucking pests (Talebi-Jahromi, 2007). The high susceptivity of *L. fabarum* to thiacloprid+deltamethrin may be related to the type of insecticide, higher rates of its penetration through the integument and lower rate of decomposition (Mardani et al., 2016). Obtained results also showed that thiacloprid+deltamethrin caused the maximum reduction in survival rate of adult *L. fabarum*; it means that this insecticide reduces the time in which the parasitoid could infect its host and laid more eggs that increases its population in the next generation (Mardani et al., 2016).

Pirimicarb significantly affected some life table parameters including R_0, r_m and λ. Similarly, Kheradmand et al. (2012) found that field application concentration of pirimicarb significantly reduced R_0, r_m and λ parameters of the aphid parasitoid, *D.rapae* McIntosh. The susceptivity of *L. fabarum* to pirimicarb could be related to the method of application, the parasitoid species and time of exposure.

Acheampong and Stark (2004) and Kheradmand et al. (2012) studied the effects of the applications of field-recommended dose of pymetrozine on several biological and demographic parameters of adult *D. rapae*. They found that pymetrozinehad no adverse effects on life table parameters of this wasp. In agreement with these findings, our results showed that pymetrozine had no adverse effects on life table parameters of *L. fabarum*. Thus, according to short-term and long-term effects ofthis insecticide will likely have low toxicity to the parasitoid and could be an appropriate candidate in integrating chemical and biological control in IPM programs.

In a similar study, Mardani et al. (2016) investigated the lethal and sublethal effects of thiacloprid+deltamethrin, pirimicarb and pymetrozine on some biological and demographic parameters of *L. fabarum* at the mummy stage. Their results showed that thiacloprid+deltamethrin was moderately harmful, but pymetrozine and pirimicarb were as harmless to the preimaginal stage of the parasitoid, according to the IOBC toxicity classification. They also found that thiacloprid+deltamethrin had the most adverse effect on life table parameters (*GRR*, R_0, r_m, λ and *T*) of *L. fabarum*; while, pirimicarb and pymetrozine had no adverse effect on r_m and λ of the parasitoid. Their study confirms our results that thiacloprid+deltamethrin have higher adverse effects on *L. fabarum* than other two insecticides. On the other hand, this insecticide showed high toxicity against both preimaginal and adult stages of *L. fabarum*.

The present study indicated that the field rate of pirimicarb adversely affected the adult stage of *L. fabarum*, but Mardani et al. (2016) showed that the developmental stages of the parasitoid inside the mummified host apparently remain unaffected by pirimicarb. According to these results, in case of release of *L. fabarum* adults in IPM, pretreatment of crops by primicarb may be deleterious to the parasitoid. By contrast, carefully timed applications of this insecticide may have a role in IPM program that rely on a buildup of *L. fabarum*. Therefore, applications of primicarb to coincide with mummy stage may result in minimal side effects on *L. fabarum*.

In sum, our results suggest that pymetrozine is more compatible than other insecticides tested in this study for simultaneous application with *L. fabarum* in IPM programs. On the contrary, thiacloprid+deltamethrin and pirimicarb are not safe to the adult stage of this parasitoid and should not be used in situations where release of *L. fabarum* adults is undertaken or when the natural population of this parasitoid are mostly in adult stage. Further studies needed to be done under more realistic semi-field and field conditions to assess the potential impact of these insecticides on *L. fabarum*, more precisely.

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