

# Acute and population level toxicity of imidacloprid and fenpyroximate on an important egg parasitoid, *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae)

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**Abstract** One focus of integrated pest management (IPM) is the use of biological and chemical control in an optimal way. The availability of selective pesticides is important as is information about both lethal and sublethal effects of pesticides on biocontrol agents. Acute and sublethal effects of imidacloprid and fenpyroximate exposure were studied on adult stage of egg parasitoid *Trichogramma cacoeciae* Marchal and the emergence rate and life table parameters were determined. The adult wasps were exposed to field recommended concentration (FRC) of the pesticides on glass plates. Field rates of imidacloprid and fenpyroximate caused 100 and 32% adult mortality, respectively. Based on concentration–response experiments, the  $LC_{50}$  values of imidacloprid and fenpyroximate were 6.25 and 1,949 ppm, respectively. The effect of imidacloprid and fenpyroximate on larvae, prepupae and pupae of the parasitoid was tested by exposing parasitized eggs of *Sitotroga cerealella* Olivier or *Cydia pomonella* L. to the FRC. Imidacloprid and fenpyroximate reduced adult emergence by 10.7 and 29%, respectively, when *S. cerealella* eggs were used as the host and 10.9 and 24.9%, respectively, when *C. pomonella* eggs were used as the host. Population parameters of emerged adults from treated pre-imaginal stages by FRC of the pesticides were also studied. The parameters were longevity and progeny production of emergent adults and also intrinsic rate of increase ( $r_m$ ), generation time ( $T$ ) and doubling time ( $DT$ ). Longevity and progeny production of the emergent adults was not affected by pesticide exposure in comparison to the control. In addition, none of population parameters such as

$r_m$ ,  $T$  and  $DT$  were affected by pesticide exposure. The intrinsic rate of increase for the control, fenpyroximate and imidacloprid exposed populations were 0.388, 0.374, and 0.372 female offspring per female per day, respectively. Overall, results of this study suggest a relative compatibility between fenpyroximate and *T. cacoeciae*, but imidacloprid showed deleterious effects on adults of the parasitoid.

**Keywords** *Trichogramma cacoeciae* · Biological control · Selectivity · Side effect · Life table parameters

## Introduction

Some species of egg parasitoids of genus *Trichogramma* are important biological control agents that have been reared and released for controlling insect pests of corn, rice, cotton, sugar-beet, tomatoes, vegetables, and orchards (Hassan 1993; Smith 1996). Studies on *Trichogramma* have been carried out in more than 50 countries. Commercially, around 32 million hectares are subject to inundative release of this parasitoid each year (Smith 1996). Despite intensive research, the use of insecticides to control multiple pest problems reduces the action of *Trichogramma* (King et al. 1986; Meierrose and Araujo 1986). Because of the negative consequences associated with pesticide use, chemical control and biological control with *Trichogramma* have long been considered incompatible. Smith (1996) believes that with a few exceptions, the use of *Trichogramma* and toxic insecticides in the same crop must be carefully planned, as the two are generally incompatible. The utilization of selective insecticides is a reasonable strategy in pest management, because it favors the conservation of natural enemies in the agroecosystem

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(Carvalho et al. 2003). IPM should use biocontrol agents and selective pesticides in an optimal way. In this regard the availability of selective pesticides is needed (Croft 1990), thus it is important to assess both lethal (Croft 1990) and sublethal effects of pesticides (Desneux et al. 2007).

Traditionally, measurement of the acute toxicity of a pesticide towards beneficial arthropods has relied largely on the determination of an acute median lethal dose or lethal concentration. 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). Sublethal effects are defined as effects on individuals that survive exposure to a pesticide (the pesticide dose/concentration can be sublethal or lethal) (Desneux et al. 2007). Studies of the sublethal effects of pesticides on natural enemies often aim to assess the suitability of the pesticide for IPM. To reduce non-target effects of pesticides on natural enemies, selectivity tests are performed with the aim of choosing a pesticide with a high degree of lethal toxicity against the target pests and minimal nontarget lethal toxicity (Croft 1990). However, given the potential importance of sublethal effects on natural enemies, pesticide choice should also consider those with minimal sublethal effects on key components of beneficial efficiency (Desneux et al. 2006).

Ecotoxicological analysis based on population growth rates result in more accurate assessments of the impact of pesticides and other toxicants because measurement of population growth rates combine lethal and sublethal effects, which are not measured by lethal dose/concentration estimates ( $LD_{50}/LC_{50}$ ) (Stark and Banks 2003). Sublethal effects may be manifested as reductions in life span, development rates, fecundity, changes in sex ratio, and changes in behavior (Croft 1990; Stark and Banks 2003). Sublethal effects on fertility, fecundity, developmental rate, survival, and sex ratio can be detected by estimating the  $r_m$  (Desneux et al. 2006). Life-table experiments provide a more accurate measure of toxic effect than do lethal concentration estimates (Forbes and Calow 1999) and have been used successfully to evaluate side effects of pesticides on several natural enemies (Acheampong and Stark 2004; Stark et al. 2004).

The aim of this study was to assess the lethal and sublethal effects of imidacloprid and fenpyroximate on an important egg parasitoid, *Trichogramma cacoeciae* Marchal. *T. cacoeciae* has great potential and has played significant roles in controlling different lepidopterous pests such as codling moth *Cydia pomonella* (L.) in apple orchards (Almatni et al. 2002; Sakr et al. 2002) and field crops (Abdelgader and Hassan 2002). *T. cacoeciae* is also a suitable indicator species for testing the side effects of pesticides on *Trichogramma* genus (Hassan 1998). I was interested in the total effects of imidacloprid and fenpyroximate because they are widely used to protect fruits and

crops against a variety of insect and mite pests, respectively. Imidacloprid is a major insecticide that is applied against thrips, aphids and whiteflies on variety of crops such as cotton, sugar beet and citrus trees in Iran (Rakhshani 2002). Fenpyroximate is a commonly used acaricide against *Tetranychid* spider mites in orchards and several crops in Iran (Khanjani 2004; Talebi-Jahromi 2007). Knowing the total effects of commonly used pesticides on *T. cacoeciae* is very important when considering their effects upon IPM based control. The objective of this study was to assess the acute and sublethal effects (Desneux et al. 2007) of two major pesticides, imidacloprid and fenpyroximate on adult and preimaginal stages of *T. cacoeciae*, and finally estimate subsequent population level effects (Stark and Banks 2003).

## Materials and methods

### Insect cultures

The *T. cacoeciae* colony used in all experiments originated from parasitized *C. pomonella* eggs collected from a plum tree orchard located in Darmstadt, Germany. This species is thelytokous (100% female offspring) (Hassan 1998). *Trichogramma* wasps were reared on *Sitotroga cerealella* Olivier eggs (obtained from Insectary AMW Nutzlinge GmbH, Darmstadt) for 8 generations in an incubator at  $25 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  RH and a photoperiod of 16:8 (L:D) h. The wasps were fed on a diet of honey–gelatin (gelatin is a water soluble protein). The quality of the parasitoid may be compromised after rearing *Trichogramma* for many generations on an atypical host. Approaches taken to counter this effect include periodically switching the parasitoids to a different host (Smith 1996). Every 3 months, the population of the parasitoid was transferred on *Cydia pomonella* eggs for one generation.

The codling moth pupae were obtained from the Zoological Institute at the Technical University in Darmstadt and maintained in a plastic cylindrical container (20 cm high and 15 cm in diameter) to emerge and oviposit on plastic films. The eggs of the codling moth, *C. pomonella* were used for exposing pre-imaginal stages.

### Chemicals

Imidacloprid (Confidor<sup>®</sup> 200SL, Bayer, Germany) and fenpyroximate (Naja<sup>®</sup> 5EC, Zeneca Agro, Belgium) were obtained as formulated products. Imidacloprid is a systemic chloronicotinyl pesticide, belonging to the class of neonicotinoid insecticides. It acts as a neurotoxin and interferes with the transmission of nerve impulses in insects by binding to specific nicotinic acetylcholine receptors

(Talebi-Jahromi 2007). Since imidacloprid is efficacious at low levels it can be applied at lower concentrations (350 ppm) for controlling several homopteran and other insect pests on cotton, sugar-beet, vegetables, and fruit trees (Rakhshani 2002). Fenpyroximate is a contact broad-spectrum phenoxy-pyrazole acaricide that is applied against *Tetranychid* spider mites on citrus, apple, and pear orchards and also on cotton and vegetables at 500–1,000 ppm in Iran (Rakhshani 2002). Its mode of action is to interfere with mitochondrial electron transport (Talebi-Jahromi 2007).

#### Preimaginal development bioassay

*S. cerealella* and *Cydia pomonella* eggs were used to investigate the impact of the pesticides on preimaginal stages of *T. cacoeciae* developing within the host eggs. The fresh *Sitotroga* eggs were glued using Traganth<sup>®</sup> gum glue (Merck, DAB 7) in circles on stripes of white papers to prepare egg discs. The egg discs (ca.  $140 \pm 20$  eggs) were offered to about ten young adults of *T. cacoeciae* in a glass tube for 24 h. The wasps remaining on eggs at the end of this period were gently brushed off with a camel's-hair brush. The number of wasps used was enough to guarantee close to 100% of parasitism and the number of eggs were enough to prevent superparasitism (Saber et al. 2004). The parasitized egg discs were prepared at 3 day intervals to provide 3, 6 and 9 day old pre-imaginal stages. These days correspond to larval, prepupal and pupal stages of *Trichogramma*, respectively (Cônoli et al. 1998; Suh et al. 2000).

Appropriate amounts of each pesticide were diluted with 100 ml of distilled water to provide the recommended field concentrations (350 and 1,000 ppm for imidacloprid and fenpyroximate, respectively). Randomly taken parasitized egg discs were dipped in pesticide solution for 5 s (Carvalho et al. 2003; Cônoli et al. 1998; Hewa-Kapuge et al. 2003; Saber et al. 2004). This procedure ensured that all parasitized eggs were sufficiently and similarly exposed to the pesticides. The control groups were submerged in distilled water only. The treated egg discs were allowed to dry for 3 h under laboratory conditions. Each egg disc was then transferred to a small glass tube (nine by 75 mm). The glass tubes containing the eggs were then plugged with cotton wool and held at  $25 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  RH, and a photoperiod of 16:8 (L:D) h. Under these conditions, adults generally emerged from eggs 10 to 11 days after the initial parasitism. A final assessment of emergence was made 14 days after initial parasitism by removing the eggs chorion from the vials and visually inspecting the eggs for emergence holes and adults. The total number of eggs, the number of black eggs (an indication of parasitization) and the emerged wasps were recorded. The eggs with dead

adults, or the eggs with partially chewed exit holes with dead adults remaining inside, were categorized as failed to emerge. Eggs that failed to yield wasps were dissected to determine whether they had reached advanced developmental stages. Eight randomly chosen parasitized egg discs were used in each pesticide treatment and the trial was repeated three times. The experiment was designed as a multifactorial design. The factors were treatment (three levels: control, imidacloprid and fenpyroximate), instar treated (three levels: larvae, prepupae and pupae) and host (two levels: *C. pomonella* eggs and *S. cerealella* eggs). An arcsine square root transformation was performed on percent emergence before analyzing data. The emergence data were subjected to a three-way (treatment\*instar treated\*host) analysis of variance (ANOVA). To determine which treatments differed after the ANOVA test, posthoc tukey tests (Sokal and Rohlf 1995) were undertaken. Preparing of the pre-imaginal stages and exposure to insecticidal treatments for *C. pomonella* were conducted similar to *S. cerealella* procedure.

#### Life table parameter measurements

Adult parasitoids that emerged from *Cydia* eggs previously were treated with either the pesticides at the pupal stage were used for the life table studies because the parasitoids likely received the maximum possible insecticide residues when chewing through host egg shells to emerge (Hsieh and Allen 1986; Orr et al. 1989). Vials containing treated eggs at the pupal stage were monitored daily for emerged parasitoids. More than 100 emerged young adults (<24 h old) from eight replicates of each treatment were transferred to small glass vials (9 × 75 mm) individually and then 25 of them were selected randomly. Each female parasitoid was presented one *Sitotroga* egg disc (ca.  $140 \pm 20$  eggs) and a small drop of gelatin-honey mixture as food. The egg discs were changed daily and mortality of the wasps was recorded. The parasitized egg discs were maintained at the rearing conditions described above and allowed to emerge and die. The total numbers of eggs, black eggs (parasitized eggs), emerged wasps, and eggs containing dead adults were recorded.

Daily schedules of mortality and fecundity were integrated into a life table format (Carey 1993) and used to calculate the net reproductive rate ( $R_0$ ), mean generation time ( $T$ ), and intrinsic rate of increase ( $r_m$ ). The Jackknife technique was used to calculate the variance of the  $r_m$  and other life table parameter estimates (Maia et al. 2000).

A square root transformation was performed on population parameters including  $T$ ,  $DT$ ,  $r_m$ ,  $m_x$  data before analysis. The mentioned data were subjected to analysis of variance and means compared using Tukey poshoc test ( $P < 0.05$ ) (Meyer et al. 1986; Maia et al. 2000).

## Adult bioassays

Stock solutions of formulated pesticide were prepared at a concentration reflecting twice the recommended field rate. Aliquots were taken from each stock solution and mixed with water to prepare 6 concentrations 21, 15, 10, 7, 5 and 4 ppm for imidacloprid and 3,000; 2,600; 2,300; 2,000; 1,700 and 1,500 ppm for fenpyroximate determined after an initial concentration-setting experiment. Glass plates (13 × 13 cm) were sprayed with 3 ml of an aqueous suspension of the pesticides using a Potter Spray Tower (BURKARD MFG. CO. LTD, UK). Control plates were sprayed with water. This resulted in homogeneous spray coverage of  $1.28 \pm 0.11 \mu\text{l}$  (mean  $\pm$  SE) fluid per square centimeter. The plates were placed in laboratory for 1 h and allowed to dry completely. The exposure cages were assembled after drying the plates. The exposure cage consisted of an aluminum frame and two glass plates as floor and ceiling (Hassan 1974). Each of the three sides of the frame contained six ventilation holes, covered with black netting. Two openings on the fourth side of the frame were used to introduce the *Trichogramma* wasps and food. After assembly, the cages were transferred to a climate controlled ( $25 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  RH, and 16:8 h photoperiod) room. To prevent the possible accumulation of pesticide fumes in the cages, the cages were connected to suction pumps through a tube ventilation system. Fifty-five to 192 young (<24 h old) *T. cacoeciae* adults were introduced into each exposure cage by emergence tubes. Two exposure cages were used for each pesticide concentration and each bioassay test was replicated three times. The parasitoids in the test cages were supplied with honey placed on a small strip of paper as food. The number of dead wasps in each cage was counted 24 h after initial exposure to the chemicals residue. Insects that appeared extremely lethargic or unable to maintain equilibrium at this time also were recorded as dead.

The result of each trial were tested for goodness of fit using PROC GENMOD procedures (SAS institute 2002) and the data were analyzed using PROC PROBIT (SAS Institute 2002) to compute  $\text{LC}_{10}$ ,  $\text{LC}_{50}$  and  $\text{LC}_{90}$  values on a standard and log scale with associated 95% fiducial limits.

A comparison was made based on the Hazard Quotient (HQ) approach which is calculated by dividing the estimated exposure value (maximum application rate (g a.i./ha)) by the toxicity value ( $\text{LR}_{50}$  = Median Lethal Rate (g a.i./ha)) (Campbell et al. 2000; Candolfi et al. 2001). The other comparison was made by Risk Quotient (RQ) which is the ratio between the field rate and  $\text{LC}_{50}$  of the beneficial (Preetha et al. 2009).

## Results

### Preimaginal development bioassay

Adult *T. cacoeciae* emergence from *C. pomonella* and *S. cerealella* eggs was affected by imidacloprid and fenpyroximate ( $F = 76.5$ ;  $\text{df} = 2, 143$ ;  $P < 0.0001$ ) (Table 1). The timing of the exposure to each pesticide relative to the parasitoid development stage affected emergence ( $F = 19.48$ ;  $\text{df} = 2, 143$ ;  $P < 0.0001$ ) (Table 1). However, the emergence rate was not affected by the type of egg host ( $F = 0.05$ ;  $\text{df} = 1, 143$ ;  $P < 0.83$ ) (Table 1). The adult parasitoid emergence rate from *C. pomonella* parasitized eggs treated with imidacloprid and fenpyroximate was 85.3 and 73%, respectively. Similarly, the adult emergence rate in *S. cerealella* parasitized eggs treated with imidacloprid and fenpyroximate was 86.8 and 68.99%, respectively. The interactions between treatments and time of exposure ( $F = 6.29$ ;  $\text{df} = 4, 143$ ;  $P < 0.0001$ ) and also interactions between time of exposure and host ( $F = 29.22$ ;  $\text{df} = 2, 143$ ;  $P < 0.0001$ ) were significant. This indicates that the time of pesticide exposure relative to the parasitoid development stage in the host body had a significant effect on emergence rate of the parasitoid. Overall, parasitized *Sitotroga* eggs exposed to either imidacloprid or fenpyroximate at the pupal stage yielded the lowest percent emergence (Table 1). Imidacloprid and fenpyroximate had almost the same effects on the emergence rate of the parasitoid when *Cydia* eggs were used as the host. *T. cacoeciae* emergence from *C. pomonella* eggs was affected by the pesticides at larval ( $F = 4.63$ ;  $\text{df} = 2, 20$ ;  $P < 0.024$ ) prepupal ( $F = 6.21$ ;  $\text{df} = 2, 20$ ;  $P < 0.009$ ), and pupal ( $F = 5.1$ ;  $\text{df} = 2, 20$ ;  $P < 0.018$ ) stages (Table 1). Emergence of *T. cacoeciae* from *S. cerealella* eggs was also affected by imidacloprid and fenpyroximate at larval ( $F = 33.36$ ;  $\text{df} = 2, 23$ ;  $P < 0.0001$ ), prepupal ( $F = 3.96$ ;  $\text{df} = 2, 23$ ;  $P < 0.035$ ), and pupal ( $F = 100.57$ ;  $\text{df} = 2, 23$ ;  $P < 0.0001$ ) stages (Table 1). Overall, the emergence rate was reduced by a greater amount following exposure to fenpyroximate than following exposure to imidacloprid in both host eggs.

### Adult $\text{LC}_{50}$ bioassay

The  $\text{LC}_{50}$  values indicated that the acute toxicity of imidacloprid (1.25  $\mu\text{g}$  a.i./ml) on *T. cacoeciae* was higher than that of fenpyroximate (97.45  $\mu\text{g}$  a.i./ml) (Table 2). Twice the field recommended concentration (FRC) of fenpyroximate had lethal effect on just 50% of the population (Table 2). HQ values for imidacloprid and fenpyroximate were 437.5 and 0.2, respectively. Risk quotient (RQ) rate were 56 and 0.51 for imidacloprid and fenpyroximate, respectively.

**Table 1** Effect of imidacloprid and fenpyroximate on emergence rate of *Trichogramma cacoeciae* from *Sitotroga cerealella* and *Cydia pomonella* parasitized eggs exposed to field rate of the pesticides at three pre-imaginal developmental stages

Host	Treatment	Mean percentage of adult parasitoid emergence from parasitized host eggs treated at different pre-imaginal stages (days after parasitization)			Mean emergence in treatments (%)	Mean reduction in percentage of emergence (%)
		Larvae (3)	Prepupae (6)	Pupae (9)		
<i>S. cerealella</i>	Imidacloprid	94.4 ± 0.78 a A	96.24 ± 1.04 a A	69.8 ± 3.88 b B	86.8 ± 2.8 b	10.71
	Fenpyroximate	80.7 ± 2.5 b A	89.18 ± 3.15 a A	37.1 ± 3.31 c B	68.99 ± 5.04 c	29.03
	Control	98.2 ± 0.78 a A	96.59 ± 1.49 a A	96.85 ± 0.83 a A	97.21 ± 0.62 a	–
<i>C. pomonella</i>	Imidacloprid	82.9 ± 5.3 ab A	89.6 ± 5.3 a A	83.4 ± 5.4 b A	85.3 ± 2.99 b	10.96
	Fenpyroximate	76.3 ± 7.02 b A	64.2 ± 9.1 b B	78.2 ± 4.9 b A	72.6 ± 4.3 c	24.22
	Control	97.85 ± 1.42 a A	92.8 ± 2.82 a A	96.73 ± 1.24 a A	95.8 ± 1.18 a	–

Means in a column for each host followed by *different small letters*, or in a row by *different capital letters* are significantly different (by Posthoc tests, Tukey test,  $P < 0.05$ )

**Table 2** Acute toxicity of imidacloprid and fenpyroximate to adult egg parasitoid *Trichogramma cacoeciae*

Pesticide	$n^a$	$\chi^2$	Slope ± SE	HQ <sup>b</sup>	Lethal concentrations (ppm) <sup>c</sup> or (µg a.i./ml)		
					LC <sub>10</sub> (95% FL)	LC <sub>50</sub> (95% FL)	LC <sub>90</sub> (95% FL)
Imidacloprid	2,714	26.1	3.15 ± 0.62	437.5	2.45 (0.83–3.73) [0.49 (0.17–0.75)]	6.25 (4.38–7.68) [1.25 (0.88–1.54)]	15.96 (12.17–30) [3.2 (2.4–6)]
Fenpyroximate	2,038	0.86	1.45 ± 1.56	0.2	254.65 [12.7]	1,949 [97.45]	14,910 [745.5]

Lethal concentrations and 95% fiducial limits (FL) were estimated using logistic regression (SAS Institute 2002)

<sup>a</sup> The total number of adult wasps used for bioassay test

<sup>b</sup> Hazard Quotient (which is calculated by dividing the estimated exposure value (maximum application rate (g a.i./ha)) by the toxicity value (LR<sub>50</sub> = Median Lethal Rate (g a.i./ha)) (Campbell et al. 2000; Candolfi et al. 2001)

<sup>c</sup> ppm relate to commercial product

### Life table parameters

The mean longevity for *T. cacoeciae* the emerged from treated host eggs at pupal stage was not affected by exposure to imidacloprid or fenpyroximate ( $F = 0.45$ ;  $df = 2, 74$ ;  $P < 0.64$ ) (Table 3). Although the control group had higher longevity compared to the treatments (Table 3). Survivorship was higher in control group compared to the treatments during the first 12 days of adult survival time (Fig. 1). This survivorship is important because most offspring were produced in first 10 days after emergence (Fig. 2). The mean number of female offspring per female ( $m_x$ ) ( $F = 0.14$ ;  $df = 2, 74$ ;  $P < 0.87$ ) was not differ compared to control. None of other population parameters of *T. cacoeciae* such as intrinsic rate of increase

( $r_m$ ) ( $F = 0.02$ ;  $df = 2, 74$ ;  $P < 0.98$ ), generation time ( $T$ ) ( $F = 0.08$ ;  $df = 2, 74$ ;  $P < 0.92$ ) and doubling time ( $DT$ ) ( $F = 0.12$ ;  $df = 2, 74$ ;  $P < 0.89$ ) were affected by either imidacloprid or fenpyroximate (Table 3). The intrinsic rate of increase for the control, fenpyroximate and imidacloprid-exposed populations were 0.388, 0.374, and 0.372 female offspring per female per day, respectively (Table 3).

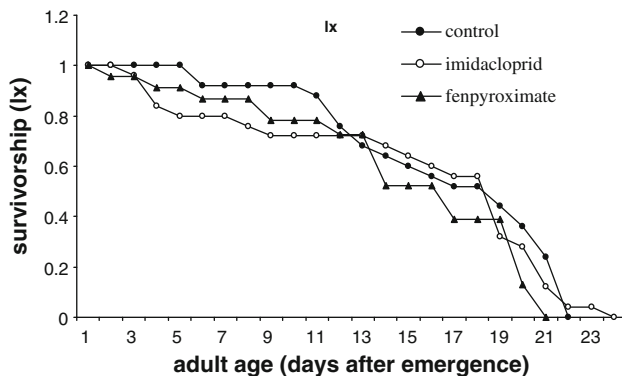
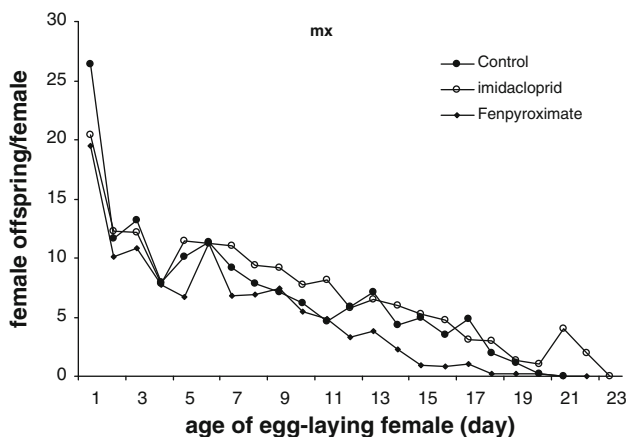
### Discussion

The present study showed that field rate (field recommended concentration) of imidacloprid (350 ppm) severely affected the adult stage of the parasitoid and resulted in

**Table 3** Effects of imidacloprid and fenpyroximate on life table parameters of *T. cacoeciae* emerged from parasitized *C. pomonella* eggs previously exposed to field rate of the pesticides at pupal stage

Treatment	Longevity day $\pm$ SE	Mean female progeny per female ( $m_x$ ) $m_x \pm$ SE	Generation time ( $T$ ) day $\pm$ SE	Intrinsic rate of increase ( $r_m$ ) $r_m \pm$ SE	Doubling time ( $DT$ ) day $\pm$ SE
Imidacloprid	13.4 $\pm$ 1.38 a	121.6 $\pm$ 11.98 a	12.87 $\pm$ 0.04 a	0.372 $\pm$ 0.0003 a	1.87 $\pm$ 0.002 a
Fenpyroximate	13.5 $\pm$ 0.73 a	114.32 $\pm$ 7.27 a	12.58 $\pm$ 0.03 a	0.375 $\pm$ 0.0003 a	1.85 $\pm$ 0.004 a
Control	14.76 $\pm$ 1.01 a	129.6 $\pm$ 8.21 a	12.55 $\pm$ 0.01 a	0.388 $\pm$ 0.0002 a	1.79 $\pm$ 0.001 a

Means within a column followed by different letters are significantly different (by posthoc tests, Tukey test,  $P < 0.05$ )

**Fig. 1** Survivorship of *T. cacoeciae* emerged from *Cydia pomonella* eggs exposed to field rate of imidacloprid and fenpyroximate at pupal stage**Fig. 2** Daily female offspring produced by *T. cacoeciae* emerged from *Cydia pomonella* eggs treated with field rate of imidacloprid and fenpyroximate at pupal stage

100% mortality within 24 h. The field rate of fenpyroximate (1,000 ppm), however, caused just 32% mortality within 24 h after the initial exposure. These pesticides are used in cotton fields, apple and citrus orchards in Iran at the mentioned rates (Rakhshani 2002). Based on the risk quotient, which is the ratio between the field rate and  $LC_{50}$  of the beneficial considered (Preetha et al. 2009), imidacloprid was very toxic to *T. cacoeciae*. The risk quotient

for fenpyroximate and imidacloprid was 0.51 and 56, respectively. The ratio showed that imidacloprid had 109 times more risk. And also based on Hazard Quotient (HQ) which is the ratio between maximum application rate and toxicity value ( $LR_{50}$ ) (Candolfi et al. 2001; Campbell et al. 2000), imidacloprid had higher HQ compared to fenpyroximate (Table 2). HQ value was very low ( $HQ < 1$ ) for fenpyroximate means that it had not significant adverse lethal effects on adults. If products tested at the maximum field rate showed no effect, then it could be assumed that if an  $LR_{50}$  test was performed it would generate an  $LR_{50}$  value that was higher than the maximum field rate. Thus, a HQ can be generated from these single rate laboratory test by dividing the maximum field rate by the  $LR_{50}$  (assumed to be more than field rate) and thereby giving a HQ of  $< 1$  (Campbell et al. 2000; Candolfi et al. 2001). The HQ value for fenpyroximate was 0.2 and means that this pesticide had low toxicity to the parasitoid. In the other studies also imidacloprid showed high toxicity to *T. chilonis* (Preetha et al. 2009) and to *Trichogramma nr. brassicae* (Hewakapuge et al. 2003) with low  $LC_{50}$  values. A higher HQ (see Campbell et al. 2000 and Candolfi et al. 2001) for any pesticide shows that it has more deleterious effects on the adult stage of the target parasitoid. Imidacloprid application should be avoided in the before mentioned fields and orchards when inundative release of *T. cacoeciae* is undertaken or when the natural population of the parasitoid are mostly in adult stage.

The emergence rate of the adult parasitoid was affected by both imidacloprid and fenpyroximate (Table 1). Imidacloprid and fenpyroximate reduced the mean emergence rate of the adults by 10.8 and 26%, respectively (Table 1). The majority of *T. cacoeciae* treated at the pre-imaginal stages complete development within host eggs, but some of them are not able to chew the eggshells and fail to emerge (Saber et al. 2004). Some mortality is also observed while emerging adults chew the host egg chorion. Plewka et al. (1975) reported that the insecticides tested did not pass through the chorion of *S. cerealella* eggs, but survival of *T. evanescens* Westwood adults was only affected when they tried to leave the eggs. The pre-imaginal stages of the

parasitoids developing within host bodies appear to be well protected from many pesticides (Singh and Varma 1986; Brar et al. 1991; C onsoli et al. 1998; Suh et al. 2000; Desneux et al. 2004; 2005; 2007; Preetha et al. 2010). Preetha et al. (2010) found that imidacloprid has no adverse effect on emergence rate of *T. chilonis* Ishii at field rate and the emergence rate is about 90%. The emergence rate was reduced slightly by the pesticides tested in this study; however, the emergence rate was still high in both pesticides treatments (Table 1). It should also be noted that the parasitized host eggs were completely dipped in pesticide solution. Consequently, the eggs received a large amount of the chemicals. Under field conditions parasitized host eggs would probably receive a lower concentration, and many eggs such as those oviposited on the underside of the leaves may not be exposed to the pesticides. For some insecticides, the effect on emergence appears to be related to the progression of pre-imaginal development at the time of exposure (C onsoli et al. 1998; Varma and Singh 1987). In some studies, the timing of exposure to insecticides had no impact on emergence rate of *Trichogramma exiguum* Pinto and Platner (Suh et al. 2000). Saber et al. (2004) indicated that the timing of exposure to azadirachtin affects the emergence rate of *T. cacoeciae* differently based on the host. Varma and Singh (1987) reported that in general, the disruptive effect of insecticides on *Trichogramma brasiliensis* Ashmead emergence decreases as the development of the parasitoid progresses. Orr et al. (1989) proposed that the developmental stage of parasitoids at the time of insecticide application appears to be important because it determines the time allowed for pesticide degradation before the emergence of parasitoids. In present study, the timing of the treatment relative to the three pre-imaginal developmental stages had a significant effect on emergence. When the pupal stage of *T. cacoeciae* using *S. cerealella* eggs as host was exposed to the pesticides, the emergence rate was lower compared to the other earlier stages. When *C. pomonella* eggs were used as the host, fenpyroximate reduced the emergence rate at the prepupal stage more than the other stages. These results suggest that many factors such as time of exposure, type of host, type of chemical, commercial formulation and method of exposure may affect the rate of emergence.

In present study, female adult longevity, fecundity, number of female offspring per female and population life table parameters such as intrinsic rate of increase ( $r_m$ ), generation time ( $T$ ) and doubling time ( $DT$ ) were used to assess sublethal effects of imidacloprid and fenpyroximate. When interpreting data on emergence rates from insecticide treated host eggs, it is also important to consider the fitness of the parasitoids that emerge (Smilanick et al. 1996). This study showed that both pesticides did not affect the assessed parameters (Table 3).

Fenpyroximate was slightly toxic to *T. cacoeciae* adults even following exposure at field rate (the field rate killed just 33% of exposed adults) (Table 2). It had lower side effects on pre-imaginal developmental stages, emergence rate, and even life table parameters of emerged adults (Tables 1, 3). These results indicate that fenpyroximate might be compatible with natural or released populations of *Trichogramma* wasps. Abdelgader and Hassan (2002) showed that fenpyroximate was slightly toxic to *T. cacoeciae* when pre-imaginal stage and adults were sprayed by field rate of the chemical.

Imidaclopride was highly toxic to *T. cacoeciae* adults. Similar results (i.e., moderate to high toxicity) have been found in *T. nr. brassicae* (Hewa-Kapuge et al. 2003) and *T. chilonis* (Preetha et al. 2009). Imidaclopride reduces the capacity of parasitism of *Trichogramma pretiosum* when females are treated during the pupal stage (Carvalho et al. 2003). However, in this study the developmental stages of the parasitoid inside the hosts' eggs apparently remain unaffected by imidacloprid. Also it has been report that the field rate of thiacloprid (another neonicotinoid insecticide) does not adversely affect the preimaginal stages of *T. cacoeciae* and the fitness of parasitoids emerging from treated host eggs in comparison to untreated parasitoids (Schuld and Schmuck 2000). Thus, in case of augmentative release of *T. cacoeciae* in IPM, pretreatment of crops by imidacloprid 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 build up of *T. cacoeciae*, because the insecticide does not show detrimental effects when applied to *T. cacoeciae* inside the hosts' eggs. Applications of imidacloprid to coincide with periods after peak egg laying by hosts may result in minimal side effects on *T. cacoeciae*. Semifield and field studies aiming to assess efficacy of combined use of pesticide and *Trichogramma* is needed to obtain more applicable results under field conditions.

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