

Research Article

## Influence of low-lethal concentrations of thiamethoxam on biological characteristics of *Neoseiulus californicus* (Acari: Phytoseiidae)

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**Abstract:** For successful implementation of integrated pest management (IPM) programs, having knowledge on lethal and low-lethal effects of pesticides on natural enemies is necessary. The present study evaluated the low-lethal effect of thiamethoxam on life table parameters of the subsequent generation of the predatory mite, *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) fed on *Tetranychus urticae* Koch under laboratory conditions. The low-lethal concentrations LC<sub>5</sub>, LC<sub>10</sub> and LC<sub>20</sub> were determined based on a dose-effect assay. The raw data were analyzed based on age-stage two sex life table analysis. Exposure to the low-lethal concentrations of thiamethoxam had no significant effects on developmental time of offspring of treated mites. Compared with control treatment, the oviposition period of treated mites with LC<sub>5</sub>, LC<sub>10</sub> and LC<sub>20</sub> decreased significantly. The highest and lowest values of total fecundity were obtained at control (35.3 eggs/female/day) and LC<sub>20</sub> (23.6 eggs /female/day), respectively. The net reproductive rate ( $R_0$ ) decreased with increasing dose from LC<sub>5</sub> (22.6 offspring) to LC<sub>20</sub> (15.0 offspring). The intrinsic rate of increase ( $r$ ) and finite rate of increase ( $\lambda$ ), were not affected by increasing concentrations. The mean generation time ( $T$ ) decreased significantly at upper dose (LC<sub>20</sub> = 13.2 d), compared with control (14.7 d). In consequence, the low-lethal concentration influences of thiamethoxam in combination with *N. californicus* in order to design management programs of *T. urticae* are discussed.

**Keywords:** predatory mite, LC<sub>50</sub>, *Tetranychus urticae*, toxicity, life-table

### Introduction

The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae), is one of the most important pests found in

ornamental, agricultural and horticultural crops such as cucumber, bean, eggplant, soybean (Sedaratian *et al.*, 2011; Khanamani *et al.*, 2013; Maleknia *et al.*, 2016). Plant photosynthesis is prevented by feeding of this pest from sap, also producing silk webbing (Huffaker *et al.*, 1969; Nachman and Zemek, 2003). Great efforts are being made every year to cope with this pest (Watson, 1964; Aydemir and Toros, 1990). Chemical control

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is the primary strategy for IPM programs due to its, cost-effectiveness, rapidity and ease of use (Zhao, 2000).

A single chemical control method against pests cannot in itself be successful (Kaplan *et al.*, 2012), but knowledge of the effects of pesticides on biological control agents is necessary for successful implementation of integrated pest management (IPM) programs (Hamed *et al.*, 2010). Spider mites are difficult to control with miticides (Naher *et al.*, 2005) as a result of inaccessibility to lower leaf surface, short lifespan, high reproductive capacity (Cranham and Helle, 1985), which can lead to rapid population growth (Nauen *et al.*, 2001). Biological control using natural enemies is an alternative method to chemical control of these pests in agricultural systems (Lewis *et al.*, 1997; Barbosa, 1998).

Phytoseiid predators are effective natural enemies of spider mites (McMurtry *et al.*, 2013). The predator mite, *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) is a successful species in the control of mites in fields and greenhouses and feeds on Tetranychidae and Tarsonemidae (Castagnoli and Simoni, 1999). This species of phytoseiid, can also feed and reproduce on small arthropod prey or pollen (Khanamani *et al.*, 2017).

*Neoseiulus californicus* prefers to prey on spider mites, however, it also has the ability to prey on other tetranychid species, as well as on other pest mites (Swirski *et al.*, 1970; McMurtry *et al.*, 2013). Numerous studies have shown that the predatory mites by themselves cannot maintain the population of spider mites under the economic injury level, although their effectiveness as predatory mites for biological control of *T. urticae* has been proven (Helle and Sabelis, 1985; Greco *et al.*, 1999, 2005; Alzoubi and Cobanoglu, 2007).

Integration of biological and chemical control is the fundamental tenet and this integration include reducing pesticide use, application of selective pesticides, and

modifying natural enemies to reduce their susceptibility to pesticides (Newsom *et al.*, 1976; Croft, 1990; Greathead, 1995; Biondi *et al.*, 2012; Roubus *et al.*, 2014). However, it is important to reduce the usage of pesticides and select products which have low negative impact on biological control agents (Isman, 2000; Hassan and Van De Veire, 2004). Therefore, the combination of using suitable insecticides, along with biological control agents has been widely recommended as an important part of IPM strategies (Elzen, 2001).

Studies that only consider the lethal effects may underestimate the negative effects of pesticides on natural enemies (Galvan *et al.*, 2005) and chemicals with minimal toxicity to natural enemies have been applied in integrated pest management programs (Croft, 1990). Demographic toxicology has been considered as a better measure of response to toxicants than individual life history traits (Forbes and Calow, 1999). By using 'population growth rate', it is possible to more accurately measure the toxicity of pesticides on useful organisms (Kim *et al.*, 2004). Neonicotinoids are presently well-known for their non-target effects on predatory mites and capability to cause spider mite flare-ups in diverse ecosystems (Raupp *et al.*, 2004; Beers *et al.*, 2005; Szczepanic *et al.*, 2011; Beers and Schmidt, 2014; Duso *et al.*, 2014 *et al.*). At the same time, these insecticides are chemically similar to nicotine, thus they act antagonistically to insect nicotine acetylcholine receptors (Nauen *et al.*, 2003), although they reduce the impacts of insect pests, they can also affect the population levels and dynamics of biological-control agents in agro-ecosystems (Desneux *et al.*, 2007; Biondi *et al.*, 2012; Guedes *et al.*, 2016).

Thiamethoxam, IUPAC name 3-(2-Chlorothiazol-5-ylmethyl)-5-methyl-(1, 3, 5) oxadiazinan-4-ylidene-N-nitroamine, is a second generation neonicotinoid possessing stomach and contact activity, nervous system and inhibits feeding reflex (Maienfisch *et al.*, 2001; Torres *et al.*, 2003). This

insecticide is presently one of the most effective chemicals for the control of sucking pests (Sharma and Lal, 2002). It is commercially available with the common name Actara® (WG25%). Many studies have investigated the lethal and low-lethal effects of pesticides on phytoseiid mites (Çobanoğlu and Alzoubi, 2008; Hamed *et al.*, 2009; Lima *et al.*, 2013; Alinejad *et al.*, 2014, 2016). However, there are few studies about the low-lethal effects of this insecticide on predatory arthropods such as *N. californicus* (Poletti *et al.*, 2007). Due to the importance of the predator *N. californicus* for integrated pest-management programs (IPM), and the overuse of neonicotinoid insecticides for the control of insect pests, studies to assess the impacts of insecticides on biological and population parameters of this mite are essential to support IPM programs.

Therefore, the present study aimed to understand the low-lethal concentrations of thiamethoxam on pre-imaginal developmental period, adult longevity, fecundity and demographic parameters of *N. californicus*, using the age-stage, two-sex life table to predict this neonicotinoid insecticide potential in combination with one of the effective natural enemies of *T. urticae*.

## Materials and Methods

### Biological material

The initial stock of *N. californicus* (Spical®) was provided from the Giah Bazr Alvand Company, an agent of the Koppert Company (Tehran, Iran) and reared in the laboratory on kidney bean *Phaseolus vulgaris* L. plants infested with *T. urticae*. The two-spotted spider mites were obtained from infested plants in Pakdasht (South Eastern part of Tehran) and were released on the kidney bean plants under greenhouse conditions of  $25 \pm 2$  °C,  $60 \pm 5\%$  RH and a photoperiod of 16:8 (L: D) hr. The predator rearing arenas were made according to McMurtary and Scriven (1965) method and were stored in a growth chamber at  $25 \pm 2$  °C  $65 \pm 5\%$  RH, and 16:8 (L: D) hr. Bean leaves infested with *T. urticae* were added daily to each arena as food source.

### Insecticide solutions

A thiamethoxam-based commercial product, Actara® 25WG (Syngenta Crop Protection), was diluted with distilled water. In toxicity bioassays, the highest tested dose was specified based on the recommended field concentration,  $12.5-50$  g AI Ha<sup>-1</sup>, and other five reduced concentrations (1500, 1660, 1800, 1990, and 2200 µg a.i./ml) were chosen to emulate lower concentrations.

### Concentration-response bioassay

A modified leaf dip method (Helle and Overmeer, 1985) was used to determine the response of *N. californicus* adults to different concentrations of thiamethoxam (the mortality covering the range of 10-90%). Fresh leaf discs of bean (4 cm diameter) were dipped for 15 s into thiamethoxam solutions, and then were dried for 3 hour at room conditions. Control leaf discs were dipped in distilled water only. In the next stage, 20 same-aged (24 h-old) adult predatory mites (male and female) were placed on the treated leaf discs for each concentration (LC<sub>5</sub>, LC<sub>10</sub> and LC<sub>20</sub>) using a soft pointed brush. Mite mortality was assessed after 24 h. The low-lethal concentrations including LC<sub>5</sub>, LC<sub>10</sub>, and LC<sub>20</sub> were determined using a probit procedure (IBM SPSS, Version 19.0). Each concentration was replicated four times. All experiments were conducted in the laboratory at  $25 \pm 2$  °C,  $65 \pm 5\%$  RH and a photoperiod of 16:8 (L: D) hour.

The *T. urticae* population, were maintained in greenhouse conditions at  $25 \pm 2$  °C,  $60 \pm 5\%$  RH, and a photoperiod of 16:8 (L: D) h.

### Life-Table Assay

In order to evaluate the low-lethal effects of thiamethoxam on *N. californicus*, after treatment (modified leaf dip method; Helle and Overmeer, 1985) of bean leaf discs with low-lethal concentrations (including LC<sub>5</sub>, LC<sub>10</sub> and LC<sub>20</sub>), and distilled water, allowed to dry for 3 h. Then forty-five same-aged females (24 h-old) were transferred on the treated and untreated leaf discs of bean. After

24 h, surviving females were separately moved onto the untreated leaf discs (3 cm in diameter). After 24 h, one laid egg was saved in each experimental arena (45 replications for each concentration). In the next procedure, all saved eggs were checked daily and the development time, longevity, oviposition period and fecundity rate until death of the last mite, was recorded. In order to study the fecundity and reproduction parameters, females were coupled with males that were selected from the stock colony in the Petri-dishes. To provide an ample food supply in treatments, 4-6 prey larva and nymph (4-5 times per day) were added as a food source for the immature and adult stages of this predatory mite, respectively. All Petri dishes were checked daily and the information of adult mites such as survival, reproductive durations, adult longevity, fecundity, along with population growth parameters were recorded.

### Statistical analysis

The population growth parameters (net reproductive rate [ $R_0$ ], intrinsic rate of natural increase [ $r$ ], finite rate of increase [ $\lambda$ ], and mean generation time [ $T$ ]) (Fathipour and Maleknia, 2016), also the age-stage specific survival rate ( $s_{xj}$ ) (where  $x$  = age in days and  $j$  = stage); the age-specific survival rate ( $l_x$ ); the age specific fecundity ( $m_x$ ); age-stage fecundity of female ( $f_{xj}$ ) of *N. californicus* were calculated with age stage, two-sex life table (Chi and Liu, 1985; Chi, 1988) using the computer program of TWO-SEX\_Ms Chart program (Chi, 2016). Comparisons of statistical differences among means of parameters related to development, as well as fecundity with the Tukey-Kramer procedure was carried out using SAS (SAS Institute, 2002). The means of the latter parameters in population growth parameters between different treatments were compared using paired bootstrap test (Riahi *et al.*, 2017; Khanamani *et al.*, 2017).

## Results

### Concentration-response bioassay

The regression equation of concentration-mortality was  $Y = -1.52 + 2.03X$  [ $Y$  = mortality (probit),  $X$  = concentration ( $\mu\text{g/ml}$ )]. As shown in Table 1, the estimated  $LC_{50}$  for the predatory mite was 1822  $\mu\text{g a.i./ml}$  while no mortality was recorded for the control (Table 1). In addition, the values of  $LC_5$ ,  $LC_{10}$ , and  $LC_{20}$  were 1449, 1525 and 1622  $\mu\text{g a.i./ml}$ , respectively.

### Development time, longevity and total life span

Effects of different concentrations of thiamethoxam on development time of male and female *N. californicus* are shown in Table 2. The time required for *N. californicus* eggs to hatch was 1.18 and 1.24 days for the untreated males and females, respectively ( $F = 0.1$ ;  $df = 3, 36$ ;  $P = 0.96$  for male,  $F = 0.13$ ;  $df = 3, 112$ ;  $P = 0.94$  for female). The number of days to complete larval stage in male and female were not significantly affected by low-lethal concentrations (male:  $F = 0.41$ ;  $df = 3, 36$ ;  $P = 0.74$ , Female:  $F = 0.69$ ;  $df = 3, 112$ ;  $P < 0.56$ ). Protonymphal (male:  $F = 0.45$ ;  $df = 3, 36$ ;  $P = 0.71$ , Female:  $F = 0.23$ ;  $df = 3, 112$ ;  $P = 0.87$ ) and deutonymph (male:  $F = 0.1$ ;  $df = 3, 36$ ;  $P = 0.95$ , Female:  $F = 0.01$ ;  $df = 3, 112$ ;  $P = 0.99$ ) stage duration of males and females were not significantly different among the treatments. Longevity ( $F = 907.3$ ;  $df = 3, 112$ ;  $P < 0.0001$ ) and total life span ( $F = 261.3$ ;  $df = 3, 112$ ;  $P < 0.0001$ ) of treated females was significantly different from the control. Low-lethal concentrations ( $LC_5$ ,  $LC_{10}$  and  $LC_{20}$ ) significantly reduced longevity and total lifespan of both sexes compared to control treatment. The longest and the lowest female adult longevity (longest: 27.28 d for control; lowest: 19.07 d for  $LC_{20}$ ), as well as total life span (longest: 32.10 d for control; lowest: 24.07 d for  $LC_{20}$ ) were observed in control and  $LC_{20}$  treatment, respectively (Table 2).

**Table 1** Probit analysis for the concentration–mortality response of thiamethoxam on adult females and males of *Neoseiulus californicus*.

Probit values	LC <sub>5</sub>	LC <sub>10</sub>	LC <sub>20</sub>	LC <sub>50</sub>	LC <sub>90</sub>	Slope ± SE	df	$\chi^2$	P	n
LC value	1449	1525	1622	1822	2190	2.06 ± 0.35	4	1.67	0.64	480
95% upper limits	1505	1575	1664	1865	2290					
95% lower limits	1374	1460	1570	1790	2119					

20 individuals per replicate, four replicates per concentration, six concentrations per assay.

**Table 2** Mean (± SE) female and male development time of *Neoseiulus californicus* for control and different concentrations of thiamethoxam.

Parameter	Control	LC <sub>5</sub>	LC <sub>10</sub>	LC <sub>20</sub>
<b>Male</b>				
Egg duration (day)	1.18 ± 0.12 <sup>a</sup>	1.20 ± 0.13 <sup>a</sup>	1.25 ± 0.16 <sup>a</sup>	1.27 ± 0.14 <sup>a</sup>
Larva duration (day)	1.09 ± 0.09 <sup>a</sup>	1.10 ± 0.10 <sup>a</sup>	1.12 ± 0.12 <sup>a</sup>	1.00 ± 0.00 <sup>a</sup>
Protonymph (day)	1.17 ± 0.12 <sup>a</sup>	1.40 ± 0.16 <sup>a</sup>	1.37 ± 0.18 <sup>a</sup>	1.37 ± 0.15 <sup>a</sup>
Deutonymph (day)	1.27 ± 0.14 <sup>a</sup>	1.30 ± 0.15 <sup>a</sup>	1.39 ± 0.17 <sup>a</sup>	1.37 ± 0.16 <sup>a</sup>
Male longevity (day)	20.27 ± 0.45 <sup>a</sup>	19.00 ± 0.47 <sup>a</sup>	15.88 ± 0.55 <sup>b</sup>	14.64 ± 0.43 <sup>c</sup>
Total life span (day)	25.00 ± 0.49 <sup>a</sup>	24.00 ± 0.49 <sup>a</sup>	21.00 ± 0.76 <sup>b</sup>	19.64 ± 0.45 <sup>c</sup>
<b>Female</b>				
Egg duration (day)	1.24 ± 0.08 <sup>a</sup>	1.24 ± 0.08 <sup>a</sup>	1.30 ± 0.09 <sup>a</sup>	1.30 ± 0.09 <sup>a</sup>
Larva duration (day)	1.03 ± 0.03 <sup>a</sup>	1.07 ± 0.05 <sup>a</sup>	1.13 ± 0.06 <sup>a</sup>	1.07 ± 0.05 <sup>a</sup>
Protonymph (day)	1.23 ± 0.08 <sup>a</sup>	1.24 ± 0.08 <sup>a</sup>	1.29 ± 0.09 <sup>a</sup>	1.32 ± 0.09 <sup>a</sup>
Deutonymph (day)	1.31 ± 0.09 <sup>a</sup>	1.31 ± 0.09 <sup>a</sup>	1.30 ± 0.12 <sup>a</sup>	1.32 ± 0.11 <sup>a</sup>
Female longevity (day)	27.28 ± 0.11 <sup>a</sup>	26.07 ± 0.13 <sup>b</sup>	22.70 ± 0.13 <sup>c</sup>	19.07 ± 0.11 <sup>d</sup>
Total lifespan (day)	32.10 ± 0.18 <sup>a</sup>	30.93 ± 0.25 <sup>b</sup>	27.73 ± 0.25 <sup>c</sup>	24.07 ± 0.19 <sup>d</sup>

Means followed by the same letters in the same row are not significantly different (Tukey-kramer,  $P \leq 0.05$ ).

### Reproduction

Reproductive periods and total fecundity of offspring of the treated females is shown in Table 3. There was no significant effect on adult pre-oviposition period (APOP) ( $F = 1.94$ ,  $P = 0.12$ ,  $df = 3, 112$ ) as well as total pre-oviposition period (TPOP) ( $F = 0.77$ ,  $P = 0.51$ ,  $df = 3, 112$ ) of *N. californicus* associated with thiamethoxam (Table 3). The mean total fecundity for LC<sub>5</sub> was 34.38 offspring/individual and was closer to control (35.31 offspring/individual) while LC<sub>20</sub> was significantly lower (23.61 offspring/individual)

( $F = 159.86$ ,  $P < 0.0001$ ,  $df = 3, 112$ ) than the other treatments. The treatment with different concentration of thiamethoxam demonstrated a significant change in the oviposition period, compared with the control treatment, such that there was a variation from 13.57 (LC<sub>20</sub>) to 21.86 (for control) days in higher concentration and un-treated mites ( $F = 1291.2$ ,  $P < 0.0001$ ,  $df = 3, 112$ ) (Table 3).

### Population growth parameters

The life-table parameters of offspring of treated females are shown in Table 4. The gross

reproduction rate (*GRR*) varied from 17.64 (for LC<sub>20</sub>) to 27.18 (for control) offspring/individual (Table 4). The lowest value of *GRR* as well as *R*<sub>0</sub> (net reproductive rate) was obtained for the mites exposed to the LC<sub>20</sub> treatment. The intrinsic rate of increase (*r*) and finite rate of increase ( $\lambda$ ) were not significant. The mean generation time was longest in control (14.74 d); followed by LC<sub>10</sub> (13.95 d) and LC<sub>20</sub> (13.28 d) treatments (Table 4).

### Survival and Fecundity

Age-specific survivorship (*l<sub>x</sub>*), age-specific fecundity (*m<sub>x</sub>*), and age-stage fecundity of female (*f<sub>xj</sub>*) *N. californicus* at different concentrations of thiamethoxam are shown in Figure 1. Total lifetime for the untreated mites

was 35 days, 33, 31 and 26 days for LC<sub>5</sub>, LC<sub>10</sub> and LC<sub>20</sub> treatments, respectively (Fig. 1). In addition, the maximum values of *m<sub>x</sub>* were approximately 1.61 eggs/female/day for mites treated with LC<sub>5</sub> treatment, which was on day 25 of the lifespan (Fig. 1). Maximum value of *m<sub>x</sub>* for untreated mites, was 1.42 eggs/female/day that was observed on day 24 of life span. However, maximum values of *m<sub>x</sub>* for LC<sub>10</sub> and LC<sub>20</sub> treatments were approximately 1.60 and 1.38 eggs/female/day respectively, which occurred on days 21 and 14 (Fig. 1). The age-stage specific survival rates (*s<sub>xj</sub>*) of *N. californicus* in treatments are plotted in Figure 2. Overlap between different stages of developmental periods, was observed among the individuals (A-D) (Fig. 2).

**Table 1** Mean ( $\pm$  SE) reproductive period and total fecundity of offspring from females of *Neoseiulus californicus* treated with low-lethal concentrations of thiamethoxam and distilled water (CK).

Parameter	Control	LC <sub>5</sub>	LC <sub>10</sub>	LC <sub>20</sub>
Oviposition period (day)	21.86 $\pm$ 0.08 <sup>a</sup>	20.66 $\pm$ 0.09 <sup>b</sup>	17.42 $\pm$ 0.11 <sup>c</sup>	13.57 $\pm$ 0.12 <sup>d</sup>
APOP (day) <sup>1</sup>	2.28 $\pm$ 0.08 <sup>a</sup>	2.28 $\pm$ 0.08 <sup>a</sup>	2.23 $\pm$ 0.07 <sup>a</sup>	2.52 $\pm$ 0.12 <sup>a</sup>
TPOP (day) <sup>2</sup>	7.12 $\pm$ 0.17 <sup>a</sup>	7.14 $\pm$ 0.21 <sup>a</sup>	7.27 $\pm$ 0.22 <sup>a</sup>	7.51 $\pm$ 0.23 <sup>a</sup>
Total fecundity (offspring/individual)	35.31 $\pm$ 0.37 <sup>a</sup>	34.38 $\pm$ 0.43 <sup>a</sup>	29.62 $\pm$ 0.49 <sup>b</sup>	23.61 $\pm$ 0.38 <sup>c</sup>

Means followed by the same letters in the same row are not significantly different (Tukey-Kramer,  $P \leq 0.05$ ).

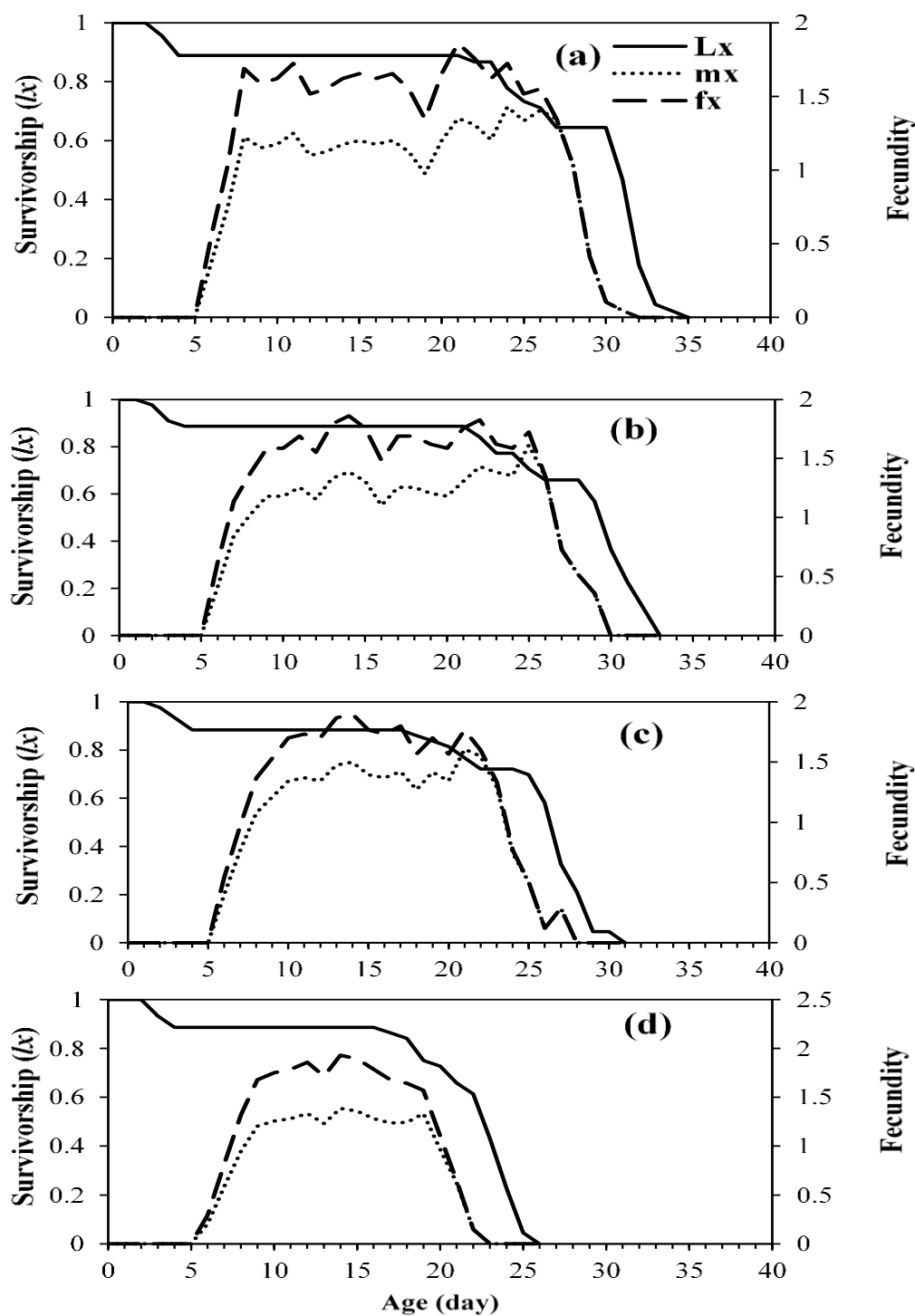
<sup>1</sup> APOP: Adult pre-oviposition period, <sup>2</sup> TPOP: Total pre-oviposition period.

**Table 2** Life table parameters (mean  $\pm$  SE) of *Neoseiulus californicus* at different concentrations of thiamethoxam and control treatment.

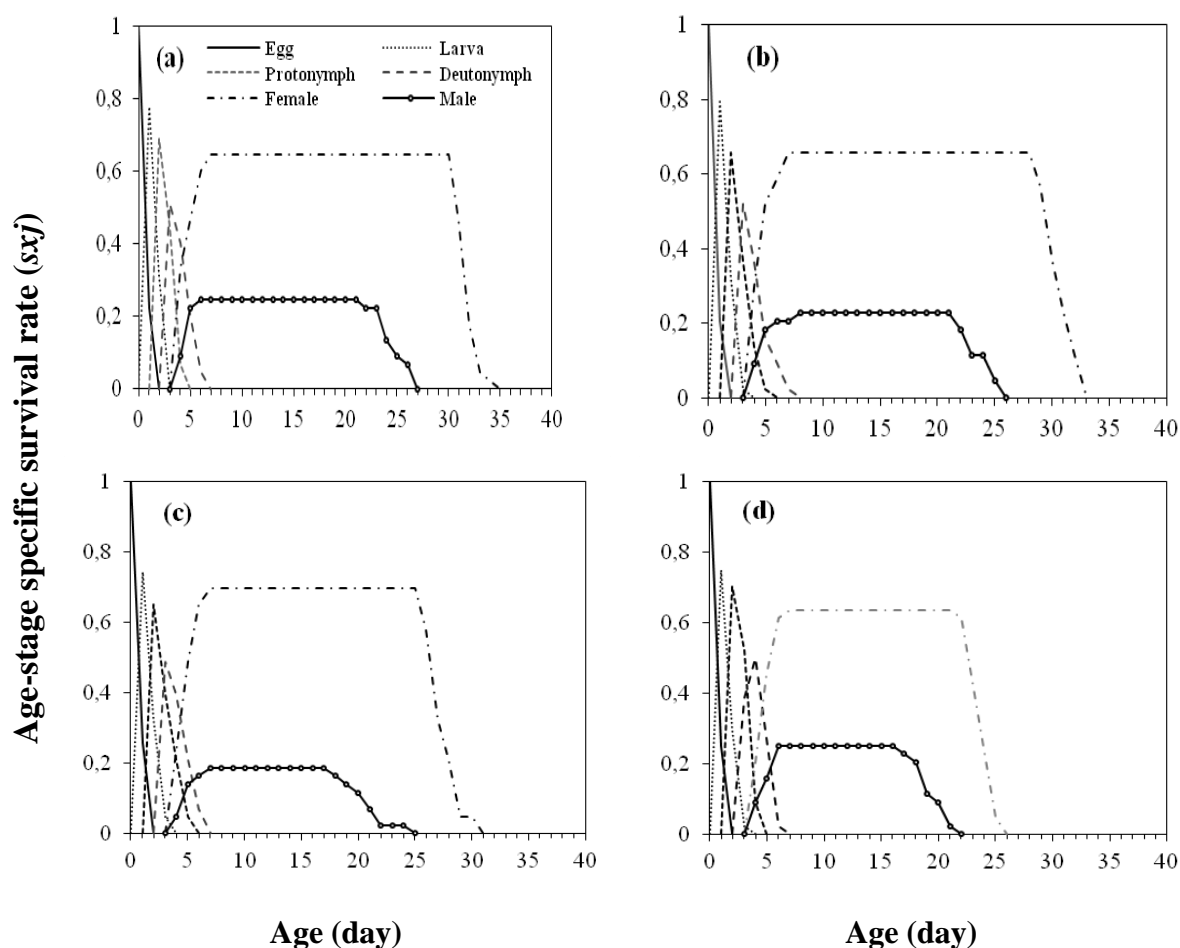
Parameters	Control	LC <sub>5</sub>	LC <sub>10</sub>	LC <sub>20</sub>
<i>r</i> (day <sup>-1</sup> )	0.2116 $\pm$ 0.010 <sup>a</sup>	0.2136 $\pm$ 0.009 <sup>a</sup>	0.2166 $\pm$ 0.009 <sup>a</sup>	0.2036 $\pm$ 0.010 <sup>a</sup>
$\lambda$ (day <sup>-1</sup> )	1.2357 $\pm$ 0.012 <sup>a</sup>	1.2382 $\pm$ 0.012 <sup>a</sup>	1.2419 $\pm$ 0.011 <sup>a</sup>	1.2259 $\pm$ 0.012 <sup>a</sup>
<i>R</i> <sub>0</sub> (offspring/individual)	22.7400 $\pm$ 2.527 <sup>a</sup>	22.6500 $\pm$ 2.468 <sup>a</sup>	20.6500 $\pm$ 2.101 <sup>a</sup>	15.0200 $\pm$ 1.719 <sup>b</sup>
<i>GRR</i> (offspring/individual)	27.1800 $\pm$ 2.162 <sup>a</sup>	27.1100 $\pm$ 2.016 <sup>a</sup>	24.7900 $\pm$ 1.676 <sup>a</sup>	17.6400 $\pm$ 1.593 <sup>b</sup>
<i>T</i> <sup>*</sup> (day)	14.7400 $\pm$ 0.258 <sup>a</sup>	14.5800 $\pm$ 0.273 <sup>a</sup>	13.9500 $\pm$ 0.265 <sup>a</sup>	13.2800 $\pm$ 0.213 <sup>b</sup>

The SE were estimated by using 100,000 bootstraps. The means followed by the same letter in each row are not significantly different using paired bootstraps test at the 5% significance level.

Abbreviations: *r*: intrinsic rate of increase;  $\lambda$ : finite rate of increase; *R*<sub>0</sub>: net reproductive rate; *GRR*: Gross reproductive rate; *T*: mean generation time.



**Figure 1** Age-specific survivorship ( $l_x$ ), age-stage fecundity of female ( $f_{xj}$ ), and age-specific fecundity ( $m_x$ ) of *Neoseiulus californicus* for control and different concentrations of thiamethoxam: (a) Control, (b) LC<sub>5</sub>, (c) LC<sub>10</sub>, (d) LC<sub>20</sub>.



**Figure 2** Age-stage-specific survival rate ( $s_{xj}$ ) of *Neoseiulus californicus* for control and different concentrations of thiamethoxam: (a) Control, (b)  $LC_5$ , (c)  $LC_{10}$ , (d)  $LC_{20}$ .

## Discussion

IPM program betterments need a comprehension of how pesticides/insecticides impress natural enemies of the pests that are being targeted. Insecticides may influence insects directly and/or via exposure to low-lethal concentrations (Guedes *et al.*, 2016). Various studies have been conducted on the effects of various pesticides on biological parameters of two-spotted spider mite and predatory mites (Nadimi *et al.*, 2009; Alinejad *et al.*, 2015; Ganjisaffar and Perring, 2017; Havasi *et al.*, 2018). However, no evidence is available with regard to low-lethal concentration ( $LC_5$ ,  $LC_{10}$  and  $LC_{20}$ ) of thiamethoxam on biological parameters of *N.*

*californicus*. Determining the effects of pesticides on natural enemies can be useful in appropriate selection of these compounds for integrated pest management programs (Golmohammadi and Hejazi, 2014).

According to our results, thiamethoxam treatment had no significant effect on developmental time of different immature stages (egg, larvae, protonymph and deutonymph) of *N. californicus*, which is in accordance with Villanueva and Walgenbach (2005) who concluded that low-lethal doses of acetamiprid (115 ppm), thiamethoxam (37 ppm) and imidacloprid (60 ppm) had no significant effects on pre-adult duration of *N. fallacis* Garman. In our study, longevity and



total lifespan were declined for both sexes of the *N. californicus* after treatment with two sublethal ( $LC_{10}$  and  $LC_{20}$ ) concentrations of thiamethoxam (Table. 2), which is consistent with results provided by Döker *et al.* (2015) that showed a similar trend for immature survival and high mortality in *Iphiseius degenerans* Berlese (Acari: Phytoseiidae) after exposure to acetamiprid and thiamethoxam. Other studies have also confirmed the adverse effect of imidacloprid and/or thiamethoxam on *G. occidentalis* (Bostanian *et al.* 2009), *N. fallacis* (Bostanian *et al.*, 2010) and *I. degenerans* (Döker *et al.*, 2015), respectively.

It was especially noteworthy that the oviposition period and fecundity of *N. californicus* were affected by experimental treatments ( $LC_{10}$  and  $LC_{20}$ ) which corroborates the results reported for another neonicotinoid insecticide, namely acetamiprid, which lowered drastically the fecundity of female *Galendromus occidentalis* (Nesbitt) by > 75% and *Amblyseius swirskii* Athias-Henriot (Beers and Schmidt, 2014; Fytrou *et al.*, 2017). To the contrary, the fecundity of whitefly parasitoid *Encarsia inaron* (Walker) treated with low-lethal (62.5 and 23.37 ppm) concentration of imidacloprid, had a higher fecundity compared to a control treatment (Sohrabi *et al.*, 2012).

In our opinion, this discrepancy between the results presumably occurred due to different examined concentrations of pesticide and the species differences in physiological responses to the insecticides.

Based on the obtained results, neither the pre-oviposition nor total pre-oviposition period showed significant variation. Our result was not in agreement with Xiao *et al.* (2016) who illustrated an increase trend for the pre-oviposition period of seven-spotted ladybird beetle, *Coccinella septempunctata* L., when treated by 0.484 and 4.837 mg l<sup>-1</sup> of imidacloprid.

Life history parameters were affected by the low-lethal concentrations of thiamethoxam in some cases and in some others they were not, and that is why demographic search is an invaluable method of chemical toxicity against

arthropods since such studies provide further understanding of the effect of the pesticide on insect (Stark and Banks, 2003).

In our study, the value obtained for gross reproductive rate (*GRR*) in the control treatment (27.18 offspring/individual), was similar to the value found by Khanamani *et al.* (2017) (27.69 offspring/individual) for *N. californicus*. There was a significant decrease in net reproduction rate ( $R_0$ ), gross reproductive rate (*GRR*) and mean generation time (*T*) parameters in higher concentration ( $LC_{20}$ ) of thiamethoxam. Our data is supported by those of Rahmani (2016) and Rahmani and Bandani (2013) who concluded that thiamethoxam treatment ( $LC_{30}$ ), caused significant decrease in  $R_0$  of important predator, *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) and aphid predator, *Hippodamia variegata* Goeze (Coleoptera: Coccinellidae).

No significant differences was reported for intrinsic rate of increase (*r*) and finite rate of increase ( $\lambda$ ) of *N. californicus* exposed to different concentrations of thiamethoxam, which agrees with the findings of Zarandi *et al.* (2017) for *Iphiseiodes zuluagai* (Denmark and Muma) treated with imidacloprid and thiamethoxam.

The adult survival and age-specific fecundity curves demonstrated that sublethal concentrations of thiamethoxam caused reduction in survival and fecundity of offspring compared with the control. The reduced values for survival and fecundity have been reported in previous studies for *P. persimilis* and *N. fallacis*, when treated with thiamethoxam (Bostanian *et al.* 2010; Pozzebon *et al.* 2011). Similar to the results obtained by our work, in laboratory tests Stavrinides and Mills (2009) found that the survival rate of *Galendromus occidentalis* (Nesbitt) treated by imidacloprid (56.25mg/l of active ingredient), had a decreasing trend compared to control. These differences may be due to different predatory species.

In this work, the parameter of  $S_{xj}$  varied after treating individuals with thiamethoxam. For example, according to the curve of the age-stage specific survival rate ( $s_{xj}$ ), increasing lethal concentrations led to an increase in

mortality. Thus, relative numbers alive ( $s_{xj}$ ) were reduced by the LC<sub>10</sub> followed by a considerable decrease in LC<sub>20</sub> among both sexes male and female.

To conclude, it seems that the pesticides can be considered as an economic, labor-saving, and effective tool of pest management (Damalas and Eleftherohorinos, 2011) but IPM programs are complex and variable, and there is more work to be conducted to exactly understand these control strategies (Ullah, 2017). In general, the less of the pesticide may be used in combination with *N. californicus* in an IPM program of *T. urticae* (Roush, 1989, Dent, 2000).

Based on these results, we not only elucidated the low-lethal effects of thiamethoxam on the natural enemy *N. californicus*, but also contributed to better understanding of the interaction of this insecticide and *N. californicus*, and how natural enemies respond to environmental xenobiotic. Further behavioral and physiological studies are necessary to help identify in their field compatibility for two-spotted spider mite management and in order to develop biological pest control programs.

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## تأثیر غلظت‌های کم‌کشنده تیماتوکسام بر پارامترهای بیولوژیکی *Neoseiulus californicus* (Acari: Phytoseiidae)

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**چکیده:** به منظور اجرای موفقیت آمیز برنامه‌های مدیریت تلفیقی آفات (IPM)، آگاهی در مورد اثرات کشنده و کم‌کشنده سموم بر دشمنان طبیعی امری ضروری می‌باشد. پژوهش حاضر به بررسی اثر زیرکشنده تیماتوکسام بر پارامترهای جدول زندگی نسل بعدی کنه شکارگر *Neoseiulus californicus* شرایط آزمایشگاهی پرداخته است. غلظت‌های زیرکشننده LC<sub>5</sub>، LC<sub>10</sub> و LC<sub>20</sub> براساس روش دز اثر تعیین شد. داده‌های خام به دست آمده براساس جدول زندگی دوجنسی سن-مرحله زیستی، تجزیه و تحلیل شد. قرار گرفتن در معرض غلظت‌های زیرکشننده تیماتوکسام تأثیر معنی‌داری بر زمان مراحل رشدی کنه‌های تیمار شده نداشت. در مقایسه با تیمار شاهد، دوره تخم‌ریزی کنه‌های تیمار شده با غلظت‌های LC<sub>5</sub>، LC<sub>10</sub> و LC<sub>20</sub> به‌طور قابل توجهی کاهش یافت. بیش‌ترین و کم‌ترین مقدار باروری کل به ترتیب در غلظت شاهد (۳/۳ تخم/ماده/روز) و غلظت LC<sub>20</sub> (۲۳/۶ تخم/ماده/روز) به دست آمد. نرخ تولیدمثل خالص ( $R_0$ ) با افزایش دز از LC<sub>5</sub> (۲۲/۶ نتاج) به LC<sub>20</sub> (۱۵/۰ نتاج) کاهش یافت. نرخ ذاتی افزایش جمعیت ( $r$ ) و نرخ متناهی افزایش جمعیت ( $\lambda$ )، با افزایش غلظت تحت تأثیر قرار نگرفت. متوسط طول زندگی یک نسل ( $T$ ) در غلظت بالای LC<sub>20</sub> (۱۳/۲ روز) در مقایسه با تیمار شاهد (۱۴/۷ روز) کاهش معنی‌داری داشت. در نتیجه، تأثیرات غلظت زیرکشننده تیماتوکسام در ترکیب با شکارگر *N. californicus* به منظور طراحی برنامه‌های مدیریت کنه تارتن دولکه‌ای مورد بحث قرار گرفته است.

**واژگان کلیدی:** کنه شکارگر، LC<sub>50</sub> *Tetranychus urticae*، سمیت، جدول زندگی