

Demographic analysis of sublethal effects of spiromesifen on *Neoseiulus californicus* (Acari: Phytoseiidae)

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ABSTRACT — The sublethal effect of spiromesifen on life table parameters of *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) was evaluated under laboratory conditions 25±1 °C, 65±5% RH and 16:8 (L:D) h. To take both sexes and the variable developmental rate among individuals into consideration, the raw data were analyzed based on the age stage, two sex life table. The estimated values of LC₅, LC₁₀ and LC₁₅ for *N. californicus* are found to be 5.834, 9.529 and 13.267 ppm, respectively. The total preoviposition period of *N. californicus* treated with sublethal concentrations of spiromesifen was not significantly longer than for the control treatment. The minimum total fecundity was recorded for the LC₁₅ concentration of spiromesifen (19.37 eggs/female) that was significantly different to the LC₅ and control treatments. Compared with the control, the intrinsic rate of increase (*r*), finite rate of increase (λ), net reproductive rate (*R*₀) and gross reproduction rates (*GRR*) of mites treated by the dose of the LC₁₅ concentration, decreased significantly but the mean generation time (*T*) was not affected by this sublethal concentration. Our results indicated that increasing concentrations of spiromesifen had significant negative effects on life table parameters of *N. californicus*. These quantities should not be used in combination with *N. californicus* in integrated pest management programs in order to obtain optimal of biological control conclusions.

KEYWORDS — spiromesifen; *Neoseiulus californicus*; life table parameters; sublethal effect

INTRODUCTION

Phytoseiidae is a family of mites which feed on thrips and other mite species. Because of their usefulness as biological control agents, interest in Phytoseiids has steadily increased over the past century (Waite 1998; Van Lenteren 2000). Phytoseiids can postpone or delay egg production during periods where preys are scarce. This allows them to have a longer lifespan and likely serves as an adaptation to environments where prey availability is variable (McMurtry *et al.* 1970). The predatory mite *Neo-*

seiulus californicus (McGregor) is used commercially around the world to control the two-spotted spider mite and several other economically important mites on avocado, citrus, dwarf hops, grapes, raspberries, roses and other ornamentals, strawberries, and several vegetable crops (Castagnoli and Simoni 1991; Gotoh *et al.* 2004).

This predatory mite develops faster when consuming the two-spotted spider mite, *Tetranychus urticae* Koch, than when consuming other prey sources (Jolly 2000). If two-spotted spider mite pop-

ulation is already high, it may be necessary to spray a miticide before releasing *N. californicus* (Calvo *et al.* 2012). Spiromesifen is a novel non-systemic insecticide/acaricide belonging to the new chemical class of spirocyclic phenyl-substituted tetroneic acids. It is especially active against whiteflies (*Bemisia* spp. and *Trialeurodes* spp.) after foliar application and is highly active against *Tetranychus* spp. as an acaricide in many cropping systems such as cotton, vegetables and ornamentals (Nauen *et al.* 2002). But despite of its widespread usage, Kaplan *et al.*, 2012, reported that spiroadiclofen can be harmful for *N. californicus* at field application dosage (Kaplan *et al.* 2012).

Most toxicological studies have focused on measuring the lethal effects of this pesticides on predatory mites (Raudonis 2006; Bostanian *et al.* 2009; Duchovskienė *et al.* 2009; Kaplan *et al.* 2012). But it may be a partial measure of their deleterious effects and so sublethal effects can be very subtle and affect populations at concentrations lower than the traditional concentration and can complete our information about its sublethal effects on physiology and behavior of arthropods (Stark and Banks 2003; Desneux *et al.*, 2007). For this purpose many researchers have used Probit regression line for depicting relationship between probit of mortality and log concentration of pesticide following 24 h of exposure. In addition, evaluation of pesticide effects based solely on females would not exhibit the overall impacts of pesticides, so it's necessary to conduct the specifying sublethal effects on biology of both sexes of the mite (Alinejad *et al.* 2014). Therefore, in this study we apply the age-stage, two-sex life table approach to analyze the life history data and life parameters of *N. californicus* to incorporate the variable developmental rates among individuals and the male population.

So, the aim of this work was to evaluate the sublethal effect of spiromesifen on the survival and reproduction of *N. californicus* to determine the compatibility of the mentioned acaricide as an effective pesticide with *N. californicus* can be used to manage *T. urticae* population.

MATERIALS AND METHODS

Colony source and experimental conditions

The colony of *N. californicus* was provided by Giah Bazr Alvand Company, agent of Koppert Company in Tehran and reared in the laboratory on kidney bean plants (*Phaseolus vulgaris* L.) infested with *T. urticae*. The colony of *T. urticae* was collected from infested greenhouses located in Varamin (south eastern of Tehran). The rearing arenas were constructed according to Abou-Setta and Childers (1987) method with some changes. A 1 cm diameter hole was drilled in the center of a 9 cm diameter plastic Petri dish. The first Petri dish was sealed using glue over another 9 cm diameter Petri dish, which was used to hold water. Afterward, a 2 mm diameter hole was created in the upper part of the side wall of the second Petri dish for filling it with water using a squeeze bottle. A narrow wick made from filter paper was inserted to the second Petri dish through the hole of the first Petri dish. The wick supplies water for keeping a bean leaf fresh in the first Petri dish. Then a thin cotton wool layer was located at the bottom of the first Petri dish and was covered with filter paper. An upturned bean leaf was placed on the filter paper and surrounded with saturated cotton to prevent escape of the mites. The rearing arena was covered with a ventilated lid.

Concentration-response bioassay

A commercial formulation of spiromesifen (IUPAC name: [2-oxo-3-(2,4,6-trimethylphenyl)-1-oxaspiro[4-4]non-3-en-4-yl]3,3-dimethylbutanoate) (Oberon[®], Bayer CropScience) was used in the present study. The response of *N. californicus* adults to various concentrations of spiromesifen was assessed using the leaf dip bioassay method (Helle and Overmeer 1985). The experimental arena was the same as the rearing arena but using 6 cm diameter Petri dish. Freshly cut bean leaf discs of 4 cm diameter were dipped into various spiromesifen solutions for 15 seconds and air dried for about 3 hours. The control leaf discs were dipped into distilled water. Each leaf disc was transferred into the experimental arena. Afterward, forty same age (less

than 24-h-old) adult mites (male and female, 20:20) of the same age (less than 24-h-old) were placed on the treated leaf discs for each concentration. The main doses obtained from preliminary tests was performed in four replicates to get sublethal concentrations. Mortality of the mites was assessed after 24 hour of exposure. Abbott's formula was used to estimate the natural mortality (Abbott 1925).

Effect of sublethal concentrations on biological parameters of offspring from treated females

After treatment of bean leaf discs with sublethal concentrations of spiromesifen (LC₅, LC₁₀ and LC₁₅), same aged (24 h old) females were placed on the discs. After 24 hours, the surviving females were separately introduced onto untreated bean leaf discs (20 mm in diameter). Subsequently, after 24 hours, the eggs laid by the treated and untreated females in each experimental arena were saved. All saved eggs were checked daily and development time and survival were recorded. Each predatory mite was fed with four to six immature stages of spider mite daily and enough corn pollen every week. Newly emerged females were coupled with a male for mating. The daily survival of each adult and fecundity of females were recorded and population parameters were calculated.

Data analysis

The sublethal concentrations (LC₅, LC₁₀ and LC₁₅) were determined using a probit procedure. The raw data of the life table parameters were analyzed according to the theory of age-stage, two-sex life table (Chi and Liu 1985) by using a user-friendly computer program, TWOSEX-MS Chart (Chi 2012). The age-stage specific survival rate (S_{xj}), age-specific

survival rate (l_x), age-specific fecundity (m_x), and population parameters were calculated accordingly.

Intrinsic rate of increase was estimated using the iterative bisection method from the Euler-Lotka formula:

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1,$$

with age indexed from 0 (Goodman, 1982). The mean generation time is defined as the length of time that a population needs to increase to R₀-fold of its size (*i.e.* $e^{rT} = R_0$ or $\lambda^T = R_0$) at the stable age-stage distribution and is calculated as $T = (\ln R_0)/r$. Paired bootstrap method in Two-Sex, Age-Stage life table software (2015 version) was used to estimate the means and standard errors with 10,000 replications (Huang and Chi 2013). Bioassay data obtained were subjected to one-way ANOVA ($P \leq 0.05$) after checking for normality. Means were compared by Tukey's test, admitting significant differences at $P \leq 0.05$. SPSS 19.0 software was used for mean comparisons.

RESULTS

Concentration-response bioassay

The LC₅₀ value for treatments with spiromesifen, and its 95% confidence interval, calculated from probit regression, was estimated 53.779 ppm. No mortality was recorded in the controls (Table 1). The sublethal concentrations for *N. californicus* were 5.834 ppm, 9.529 ppm and 13.267 ppm for LC₅, LC₁₀ and LC₁₅, respectively.

TABLE 1: Probit analysis for the concentration-mortality response of spiromesifen on *N. californicus*.

	LC ₅	LC ₁₀	LC ₁₅	LC ₅₀	Slope±SE	χ^2 (df)	<i>p</i>
	5.834	9.529	13.267	53.779	1.705±0.124	70.657(18)	0.0089
Lower	2.586	5.096	7.971	40.048			
Upper	9.34	13.957	18.489	80.13			

*40 individuals per replicate, four replicates per concentration, six concentrations per assay.

Developmental time, longevity and total life span

The effects of different concentrations of spiromesifen on developmental time, longevity and total life span of offspring from the treated females are presented in Table 2. The time required for *N. californicus* eggs to hatch was 1.1 and 1.21 days for the untreated males and females, respectively. Sublethal concentration was not affected egg incubation time either male or female. This nontoxic effect of spiromesifen showed in larval, protonymph and deutonymph periods on both sex. The longevity and total life span of treated male ($df=3$; $F=302.42$; $P=0.0004$) and female ($df=3$; $F=639.13$; $P=0$) treated with LC_{15} concentration of spiromesifen was significantly different from control and other sublethal concentration.

Reproduction

No significant differences between all experimental doses and control were scored in adult pre-oviposition and total pre-oviposition period. Treatment with LC_5 sublethal concentration of spiromesifen

showed no significant change in total fecundity about per female of *N. californicus* nearly like control treatment. By increasing in concentration at LC_{10} , harmful effect of spiromesifen showed relatively in oviposition duration but non-effect in pre-oviposition period. In other word LC_{10} demonstrated an intermediate concentration between non-affecting dosage (LC_5) and affecting dosage (LC_{15}). Applying LC_{15} and LC_{10} concentrations caused significant reduction in mites total fecundity (Table 3).

Population parameters

The life table parameters of offspring related to the treated females are demonstrated in Table 4. The intrinsic rate of increase (r) was varied between 0.17 and 0.24 day^{-1} that the highest one was related to the control treatment. So, the (r) gradually decreased by increasing the concentrations of spiromesifen. The highest and lowest finite rate of increase (λ) was observed in control and LC_{15} concentrations which showed significant difference. The net reproductive rate (R_0) significantly decreased by increas-

TABLE 2: Development time, longevity and total life span (days) of offspring from females of *N. californicus* treated with sublethal concentrations of spiromesifen Mean (\pm SE).

Sex	Control	Treatment		
		LC ₅	LC ₁₀	LC ₁₅
Male				
Egg duration (day)	1.10±0.09 ^a	1.20±0.13 ^a	1.24±0.12 ^a	1.18±0.12 ^a
Larva duration (day)	1.00±0.0 ^a	1.00±0.0 ^a	1.00±0.0 ^a	1.00±0.0 ^a
Protonymph (day)	1.20±0.13 ^a	1.39±0.16 ^a	1.33±0.14 ^a	1.54±0.15 ^a
Deutonymph (day)	1.29±0.15 ^a	1.20±0.13 ^a	1.42±0.14 ^a	1.36±0.15 ^a
Longevity (day)	19.70±1.65 ^a	17.19±1.07 ^{ab}	14.52±0.96 ^{bc}	12.46±0.65 ^c
Total life span (day)	24.30±1.71 ^a	21.99±1.71 ^a	19.52 ±1.10 ^{ab}	17.55 ±0.64 ^b
Female				
Egg duration (day)	1.21±0.08 ^a	1.13±0.10 ^a	1.29±0.11 ^a	1.31±0.11 ^a
Larva duration (day)	1.00±0.0 ^a	1.00±0.0 ^a	1.00±0.0 ^a	1.00±0.0 ^a
Protonymph (day)	1.21±0.08 ^a	1.27±0.09 ^a	1.35±0.11 ^a	1.24±0.11 ^a
Deutonymph (day)	1.26±0.09 ^a	1.31±0.10 ^a	1.23±0.10 ^a	1.31±0.11 ^a
Longevity (day)	26.52±1.13 ^a	25.45±0.84 ^a	21.11 ±1.09 ^b	16.81 ±1.20 ^c
Total life span (day)	31.23±1.15 ^a	30.18±0.85 ^a	26.00±1.08 ^b	21.69 ±1.32 ^c

Means within a row followed by the same letter are not significantly different. The SEs were estimated by using 10,000 bootstraps and compared by using paired bootstrap test based on CI of differences.

TABLE 3: Mean (\pm SEM) reproductive period and total fecundity of offspring from females of *N. californicus* treated with sublethal concentrations of spiromesifen and distilled water (control).

	Treatment			
	Control	LC ₅	LC ₁₀	LC ₁₅
Oviposition (day)	16.53 \pm 0.09 ^a	16.49 \pm 0.83 ^a	13.41 \pm 0.89 ^b	11.00 \pm 1.03 ^b
APOP (d)*	1.34 \pm 0.10 ^a	1.36 \pm 0.10 ^a	1.47 \pm 0.12 ^a	1.37 \pm 0.12 ^a
TPOP (d)**	6.04 \pm 0.07 ^a	6.09 \pm 0.10 ^a	6.35 \pm 0.23 ^a	6.24 \pm 0.20 ^a
Total fecundity (offspring)	36.11 \pm 1.68 ^a	34.22 \pm 1.66 ^a	26.53 \pm 1.67 ^b	19.37 \pm 1.75 ^c

*APOP, adult pre-ovipositional period, **TPOP, total pre-ovipositional period. Means within a row followed by the same letter are not significantly different. The SEs were estimated by using 10,000 bootstraps and compared by using paired bootstrap test based on CI of differences.

ing the concentrations of spiromesifen specially in LC₁₀ and LC₁₅ treatments. The gross reproductive rates (GRR) was 26.71 and 14.72 offspring in control and LC₁₅ respectively that showed its reduction by increasing concentration of acaricide. Although the mean generation time (T) in control and other treatments, had not significant differences.

Survival and fecundity

Figure 1 Shows the daily and age-stage survival curves of both untreated and treated individuals of *N. californicus* with sublethal concentrations of spiromesifen. The total lifetime for the untreated mites was 38 days, while it was 38, 31 and 28 days for LC₅, LC₁₀ and LC₁₅ treatments, respectively. In addition, examination of lx in treated mites revealed more mortality in immature stages in contrast to control. The age-stage survival rate (S_{xj}) illustrates the probability that a new born will sur-

vive to age x and stage j (Figure 2). There are significant overlaps between curves of different stages and different sublethal concentrations. The highest female and male survival rate was observed at control treatment and effect of LC₅ showed same trend like survival rates of control. By increasing in concentration (LC₁₀ and LC₁₅), survival rates declined in compare with what was in control. The maximum values of mx were approximately 1.68 eggs/female/day for the mites treated by LC₅ sublethal concentration, which was occurred on day 9 of the life span (Figure 3). In comparison, maximum value of 1.75 eggs/female/day was observed on day 12 of the life span for the untreated mites. But maximum values of mx were approximately 1.4 and 1.2 eggs/female/day which was occurred on days 10 and 18 of the life span for the mites contacted with LC₁₀ and LC₁₅ concentrations, respectively.

TABLE 4: Mean (\pm SEM) population parameters of offspring from females of *N. californicus* treated with sublethal concentrations of spiromesifen or distilled water (control).

Parameters	Treatment			
	Control	LC ₅	LC ₁₀	LC ₁₅
Intrinsic rate of increase, r (day ⁻¹)	0.24 \pm 0.013 ^a	0.23 \pm 0.014 ^a	0.20 \pm 0.018 ^{ab}	0.17 \pm 0.019 ^b
Finite rate of increase, λ (day ⁻¹)	1.27 \pm 0.01 ^a	1.26 \pm 0.01 ^{ab}	1.22 \pm 0.02 ^{bc}	1.18 \pm 0.02 ^c
Net reproductive rate, R_0 (offspring)	20.76 \pm 2.96 ^a	18.79 \pm 2.84 ^a	11.26 \pm 2.17 ^b	7.75 \pm 1.63 ^b
Mean generation time, T (day)	12.33 \pm 0.25 ^a	12.27 \pm 0.31 ^a	12.02 \pm 0.37 ^a	11.74 \pm 0.43 ^a
Gross reproductive rate, (offspring)	26.71 \pm 3.06 ^a	25.05 \pm 2.96 ^{ab}	17.40 \pm 2.63 ^{bc}	14.22 \pm 2.22 ^c

Means within a row followed by the same letter are not significantly different. The SEs were estimated by using 10,000 bootstraps and compared by using paired bootstrap test based on CI of differences.

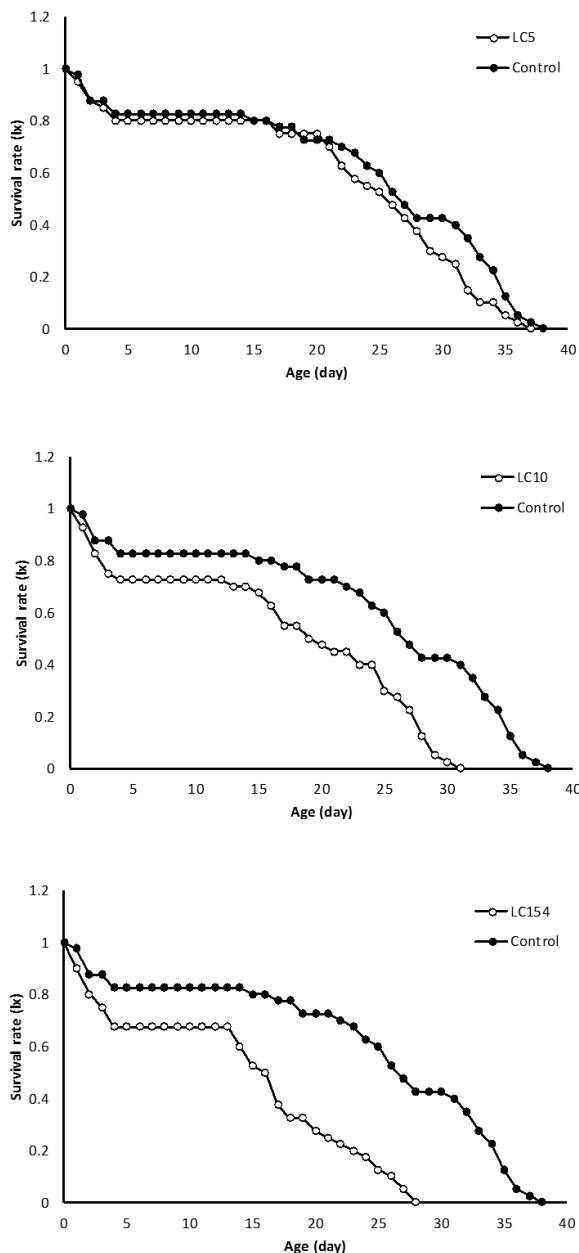


FIGURE 1: Age-specific survival rate (lx) of offspring of the treated and untreated females of *Neoseiulus californicus*.

DISCUSSION

It has been reported that spiromesifen acts on mite and whitefly development probably by interfering with lipid biosynthesis (Nauen *et al.* 2002). There is no information on the sublethal effects of spiromesifen on biological characteristic of *N. californicus*, in-

cluding development time, fecundity, mortality at different life stages and adult emergence. In present study, although the concentrations (LC_5 , LC_{10} , LC_{15}) used were lower than what was recommended rate for two spider mite. Moreover, application of sublethal concentrations of spiromesifen had no significant adverse effects on all immature stages of both sexes of *N. californicus*. Our results are consistent with Hamed *et al.* (2010) that mentioned application of sublethal concentrations of abamectin had no significant effect on larval period of *Phytoseiulus plumifer*.

In spite of no significant effect of sublethal concentrations of spiromesifen on premature stages, this acaricide decreased adult longevity and total life span of both sexes along with increasing of dosages. This result was confirmed in bioassay related to Alinejad *et al.* (2014) concerning effects of sublethal concentrations of fenazaquin on *A. swirskii*. Although application of sublethal concentrations of spiroticlofen had no negative effect on these parameters of *A. swirskii* (Alinejad *et al.* 2016). Moreover, Hamed *et al.* (2011) had shown the same results like ours on *P. plumifer* by increasing of abamectin concentrations as well as they had confirmed this subject about fenpyroximate, previously (Hamed *et al.* 2009).

LC_{10} and LC_{15} sublethal concentrations caused reduction significantly in the total fecundity period of *N. californicus*. Also Park *et al.* (2011) and Alinejad *et al.* (2014) noted the similar trend for pyridaben and fenazaquin, respectively. In this manner Ibrahim and Yee (2000) reported that the exposure to sublethal concentration of abamectin reduced the mean fecundity per female of *Neoseiulus longispinosus* (Evans) to almost half of the untreated females. But they had inconsistent conclusion about application of sublethal concentrations of spiroticlofen. Differences in susceptibility of phytoseiid species and tested acaricides could be responsible for the conflicting results.

In addition to research on the effects of pesticides on predators, Marcic *et al.* (2010) showed that Spiromesifen at concentrations of 180, 18 and 1.8 mg/l significantly reduced fecundity, fertility and population growth of *T. urticae*. In contrary, Barati

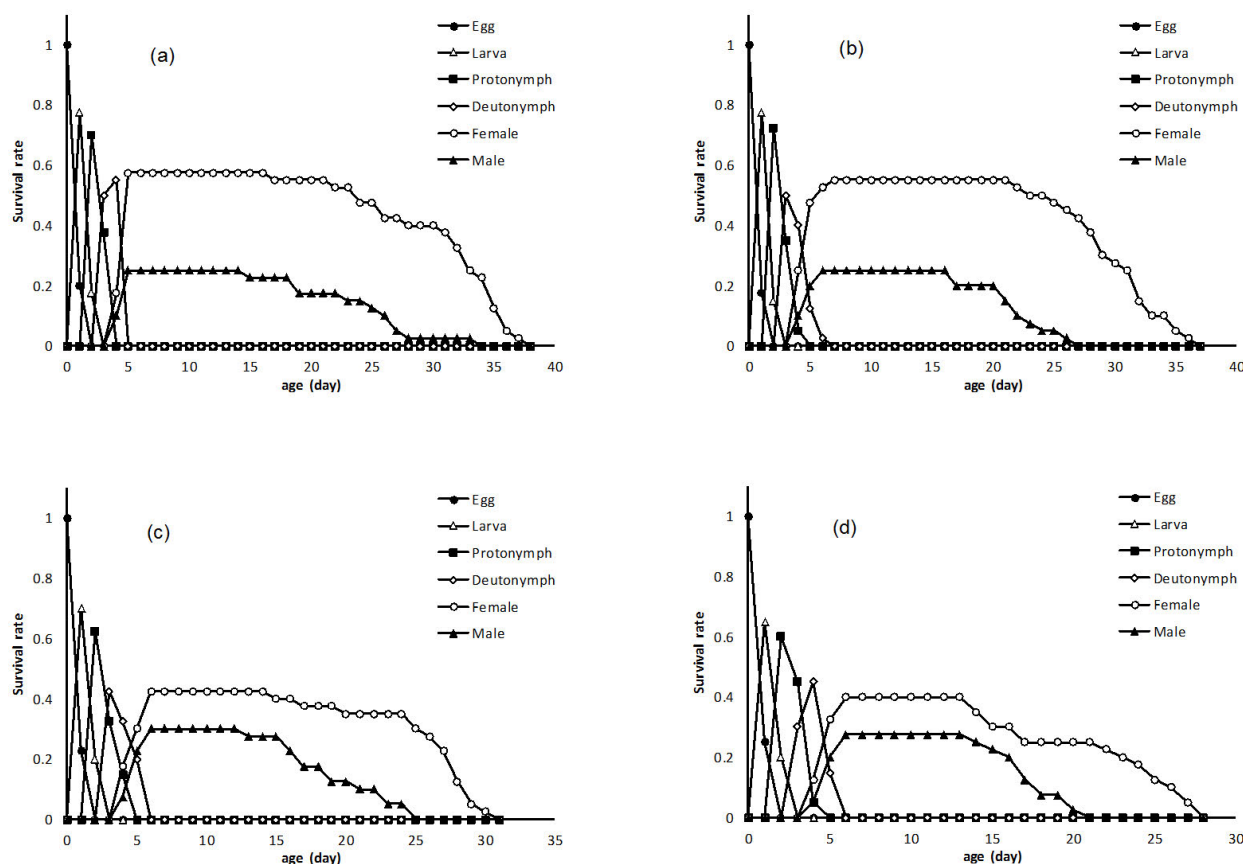


FIGURE 2: Age-stage specific survival rate (S_{xj}) of offspring of the treated and untreated females of *Neoseiulus californicus*; control (a), LC_5 (b), LC_{10} (c), LC_{15} (d).

and Hejazi (2015) indicated that all neonicotinoids tested increased the fecundity of *T. urticae* because of changes in physiology of the mites. So the use of acaricides against mites in the presence of predators requires necessary precautions. Consequently, use of spiromesifen in crops should be limited while this predator is important for biological control to get a better result. It is necessary to pay attention to the direct toxicity of spiromesifen on *N. californicus* for considering this acaricide in IPM program. The sublethal effects of pesticides on life table parameters of phytophagous and predatory mites were previously reported by several researchers (Alinejad *et al.* (2014), Ghaderi *et al.* (2013), Hamed *et al.* (2010), Ibrahim and Yee (2000) and Marcic (2007) demonstrated decline trend for population parameters of *A. swirski*, *P. persimilis*, *P. plumifer*, *N. longispinosus* and *T. urticae*, when treated by sub-

lethal concentrations of fenazaquin, fenpyroximate, abamectin and spiroadiclofen, respectively). Our results revealed that LC_{15} sublethal concentration of spiromesifen had significant effect on population parameters as confirmed in previous parts and are corroborated by Ibrahim and Yee (2000), Alinejad *et al.* (2014), Hamed *et al.* (2011) and Ghaderi *et al.* (2013).

The age specific fecundity and survival curves demonstrated that sublethal concentrations of spiromesifen caused significant reduction in these parameters of *N. californicus*, to compare with the control. Like the study of Alinejad *et al.* (2014) and Ibrahim and Yee (2000) in which the sublethal concentrations of fenazaquin and abamectin were examined on *A. swirskii* and *N. longispinosus* respectively.

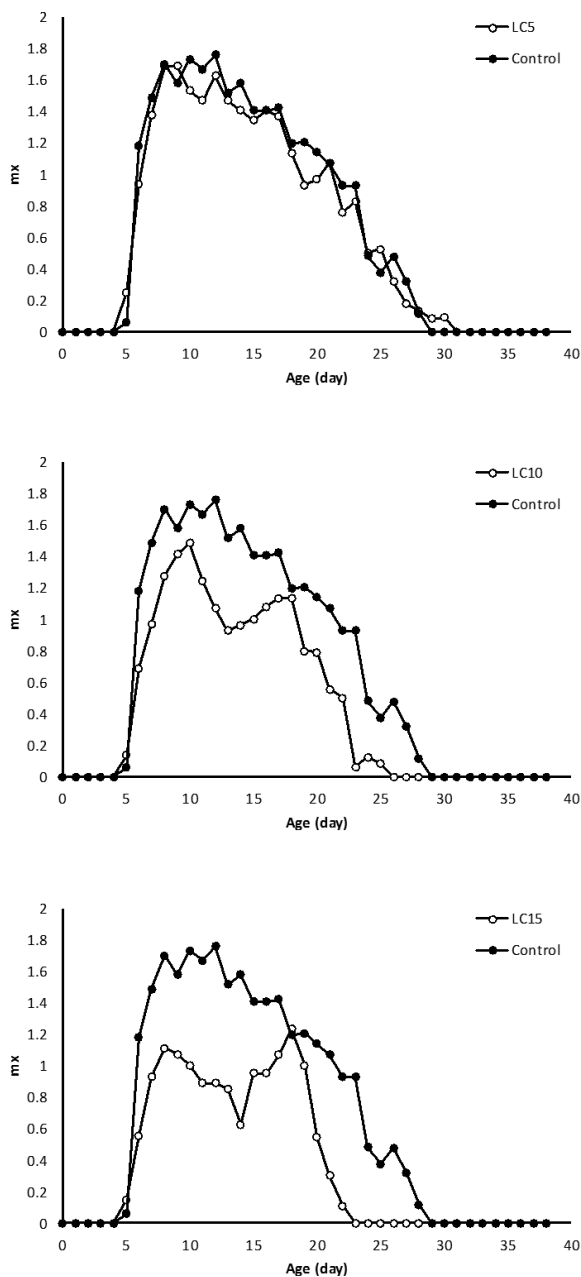


FIGURE 3: Age-specific fecundity (mx) of the offspring of the treated and untreated females of *Neoseiulus californicus*.

Conclusively, this study has revealed that treating *N. californicus* with increasing lethal concentration of spiromesifen decreased fecundity and population numbers of *N. californicus* under laboratory conditions. However, it's necessary to consider the direct toxicity of spiromesifen on *N. californicus* in

IPM programs. Final judgment on the effect of high sublethal concentration of spiromesifen on *N. californicus* fecundity and life table parameters should be postponed until the results are obtained from semi-field and field trials. If results are similar, the use of these chemicals in IPM programs requires necessary precautions.

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