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Lethal and sub-lethal effects of fluxametamide and Sero-X on biological parameters of *Neoseiulus californicus* McGregor (Acari: Phytoseiidae)

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ABSTRACT

This study investigates the lethal and sub-lethal effects of the pesticides fluxametamide and Sero-X on the predatory mite, *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae). As a key biocontrol agent, *N. californicus* is crucial in regulating mite pest populations and small insects like thrips. Understanding the effects of these pesticides is essential for maintaining effective biological control strategies. In an integrated pest management (IPM) program, selecting low-risk pesticides that target pests while preserving beneficial organisms is critical. To assess these effects, bioassay tests were conducted under controlled laboratory conditions (25 ± 2 °C, 65 ± 5% RH, and a 16:8 light-dark photoperiod). Lethal bioassay test results showed that the LC_{50} values of fluxametamide and Sero-X on adults of *N. californicus* were 1421 mg/L and 2169 mg/L, respectively. The results showed that exposure to the LC_{30} concentration of either fluxametamide or Sero-X significantly affected the predator's reproduction and developmental parameters. Both treatments reduced life table parameters (r, R_0, λ, and GRR) while increasing generation time (T) compared to the control. Additionally, maternal exposure influenced progeny life history in varied ways. These findings highlight the need for sustainable pest management that minimizes chemical impact while preserving ecological balance, ensuring natural predators remain effective in agriculture.

KEYWORDS

Acaricide, life table parameters, pest control, population parameters, predatory mite

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INTRODUCTION

The two-spotted spider mite (*Tetranychus urticae* Koch; Tetranychidae) is a major polyphagous pest affecting over 1,000 plant species, leading to significant economic losses in food, fiber, and crop production (Van Leeuwen *et al.* 2010, 2015; Bui *et al.* 2018; Wu *et al.* 2019). Its short development time, high fecundity, and adaptability allow populations to grow rapidly, especially under favorable environmental conditions, causing severe damage such as defoliation, chlorophyll depletion, webbing, leaf necrosis, and reduced photosynthesis and transpiration (Badawy *et al.* 2010; Fahim *et al.* 2020).

Biological control, particularly using predatory mites from the Phytoseiidae family, is a widely adopted strategy for managing *T. urticae* populations. This approach is gaining traction in many countries due to its effectiveness, sustainability, and reduced environmental impact (Amoah *et al.* 2016; Akyazi and Liburd 2019). Among these predators, *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) is highly effective in controlling mite populations in agricultural fields and greenhouses (Poletti *et al.* 2007; Vergel *et al.* 2011; McMurtry *et al.* 2013; Glinushkin *et al.* 2019).

Integrating compatible pesticides with biological control agents is a key component of Integrated Pest Management (IPM) strategies (Sáenz de Cabezón Irigaray *et al.* 2007). Biorational acaricides, which are less harmful to beneficial organisms, offer a promising alternative to conventional pesticides (Issa *et al.* 1974; Mochizuki 2003; Amano *et al.* 2004; Hamedi *et al.* 2011; Bozhgani *et al.* 2018).

Fluxametamide (Gracia®) is a newly developed pesticide known for its precision targeting, effectively eliminating pests while minimizing harm to non-target species (Li *et al.* 2019). Similarly, Sero-X®, an eco-friendly botanical pesticide, has been approved for commercial use in Australia to control insect pests in cotton and macadamia. Its insecticidal activity is attributed to cyclotides, a class of insecticidal peptides (Jennings *et al.* 2001; Oguis *et al.* 2020).

Numerous studies have used demographic techniques to assess sublethal effects of pesticides on Phytoseiidae species (Alinejad *et al.* 2014; Sarbaz *et al.* 2017; Bozhgani *et al.* 2018; Havasi *et al.* 2021). In some cases, sublethal pesticide concentrations can contribute to pest management strategies. The life-table technique is particularly effective in evaluating pesticide effects on both target and non-target organisms, providing insights into multiple sublethal impacts on insects and mites (Biondi *et al.* 2013; Cira *et al.* 2017; Nawaz *et al.* 2017; Asgari *et al.* 2020).

Currently, no study has examined the effects of fluxametamide and Sero-X on the biological performance of *N. californicus*. This research aims to evaluate the lethal and sublethal effects of these pesticides on key biological parameters of *N. californicus*. The findings will contribute to optimizing laboratory rearing and acaricide application in greenhouse and field settings.

MATERIAL AND METHODS

To conduct the experiments, the stock population of two-spotted spider mites, *T. urticae* was collected from infested greenhouses at the Iranian Research Institute of Plant Protection in Tehran, Iran. The mites were reared on bean plants (*Phaseolus vulgaris* L.) under greenhouse conditions at 25 ± 2 °C, 60 ± 5% RH, and a 16:8 h light-dark photoperiod.

The initial population of *Neoseiulus californicus* was obtained from the Agricultural Zoology Department, Iranian Research Institute of Plant Protection in Tehran, Iran, and reared in the laboratory on bean plants infested with *T. urticae*. Predator-rearing arenas were constructed following the method described by Walzer and Schausberger (1999) and maintained in a growth chamber under the same laboratory conditions. Predatory mites were fed daily using heavily mite-infested bean leaves as a food source.

Pesticides

Sero-X, a non-toxic, bee-friendly, botanical pesticide derived from Butterfly Pea, *Clitoria ternatea* L. (Fabaceae) extract, which contains bio-active peptides called cyclotides. It offers an effective organic alternative to synthetic pesticides by controlling a broad spectrum of insect pests, including mites like the two-spotted mite, while being safe for pollinators and beneficial insects (Oguis *et al.* 2019).

The fluxametamide commercial product (10% SC) was obtained from Nissan Chemical Industries Ltd. (Tokyo, Japan). Fluxametamide is a novel isoxazoline pesticide that selectively antagonizes insect ligand-gated chloride channels, including GABA- and glutamate-gated chloride channels (IC_{50} of 1.95 nM and 225 nM for *Musca domestica* (Dip.: Muscidae) GABACls and GluCls).

Concentration-Response Bioassay

A modified leaf dip method (Helle and Overmeer 1985) was used to evaluate the response of *N. californicus* adults to different concentrations of fluxametamide (0.028 ml/l) and Sero-X (0.045 ml/l), covering a mortality range of 10–90%. Freshly cut bean leaf discs (4 cm diameter) were dipped in aqueous solutions for 15 seconds, air-dried at room temperature for approximately three hours, and then placed in an experimental arena that closely resembled the rearing arena but on a smaller scale. Control leaf discs were dipped in distilled water only.

Twenty adult predatory mites (15 females and 5 males) were placed on treated leaf discs for each of six tested concentrations using a fine, soft-bristled brush. The lethal bioassay test was replicated four times. Mortality rates were recorded 24 hours after exposure, and lethal concentrations were determined using a probit analysis (IBM SPSS Version 19.0). Experiments were conducted under above mentioned controlled laboratory conditions.

Life-table parameters assay

To evaluate the sublethal effects of fluxametamide and Sero-X on *N. californicus*, bean leaf discs were treated with a low-lethal concentration (LC_{30}) using the modified leaf dip method (Helle and Overmeer 1985). Distilled water was used for the control group, and treated discs were air-dried for three hours.

Forty-five age-matched females (less than 24 hours old) were transferred onto treated and untreated bean leaf discs. After 24 hours, surviving females were individually placed on fresh untreated leaf discs (3 cm in diameter). Following another 24 hours, one egg from each female was preserved in its respective experimental arena, with 45 replicates per treatment.

Daily observations were conducted to track egg development time, adult longevity, oviposition days, and fecundity rate until the last mite's death. To study fecundity and reproductive parameters, females were paired with males from the stock colony and placed in Petri dishes. To ensure an adequate food supply, 4–6 prey larvae and nymphs were added multiple times daily (4–5 times) to support the immature and adult stages of *N. californicus*. All Petri dishes were checked daily, and adult mite survival, reproductive duration, adult longevity, fecundity, and population growth parameters were recorded.

Statistical Analysis

To determine acute toxicity and lethal concentrations, concentration-mortality regression was analyzed using the Probit program in IBM SPSS (version 19.0). Life history data were evaluated based on the age-stage, two-sex life table method (Chi and Liu 1985; Chi 1988) using the Two Sex MSChart program (2024 version) (Chi 2021).

The variances and standard errors of population growth parameters were estimated using the bootstrap procedure (Efron and Tibshirani 1993). Additionally, a paired bootstrap test (×100,000) was applied to assess statistical differences in development, fecundity, and population growth parameters across different treatments (Efron and Tibshirani 1993; Huang and Chi 2013).

RESULTS

Concentration-response bioassay

The LC_{50} values of fluxametamide and Sero-X for both sexes of *N. californicus* were determined as 1421 mg/L and 2169 mg/L, respectively. No mortality was observed in the control group (Table 1). The lethal concentration of the tested pesticides was significantly different because there was no overlap between the fiducial limits at LC_{50} rates.

Table 1. Probit analysis for the concentration-mortality response of *N. californicus* adults to fluxametamide and Sero-x.

Treatment	n	df	P-value	X^2	LC_{30} (Lower-Upper)	LC_{50} (Lower-Upper)
fluxametamide	480	4	0.47	2.49	1256 (1197–1303)	1421 (1375–1467)
Sero-x	480	4	0.40	2.92	1334 (1090–1545)	2169 (1910–2454)

Concentrations are reported in units of ml/L with a 95% confidence limit.
Twenty individuals per replicate, four replicates per concentration, six concentrations per assay.

Effects of fluxametamide and Sero-x on development time and adult longevity

Fluxametamide and Sero-X significantly affected the duration of developmental stages and adult longevity of *N. californicus* mites (Table 2). Adult longevity was significantly decreased by the pesticides compared to the control. The LC_{30} concentration of either tested chemical significantly reduced the lifespan of *N. californicus* compared to untreated mites (Table 2). At LC_{30} treatments, the lifespan (mean number of days from egg to death) of *N. californicus* females was notably shorter than that of the control group.

Table 2. Effects of LC_{30} of fluxametamide and Sero-x on development time, longevity, and total life span (day ± SE) of *Neoseiulus californicus*.

Parameter	Control	Fluxametamide	Sero-x
Male			
Developmental time (day)	4.73 ± 0.19b	5.12 ± 0.36a	5.12 ± 0.31 a
Adult Longevity (day)	20.27 ± 0.45a	13.40 ± 0.37c	14.88 ± 0.55b
Total life span (day)	25.11 ± 0.49a	18.51 ± 0.52c	20.02 ± 0.42b
Female			
Developmental time (day)	4.83 ± 0.21a	4.87 ± 0.12a	4.97 ± 0.18a
Adult Longevity (day)	27.28 ± 0.11a	17.57 ± 0.17c	19.52 ± 0.11b
Immature survival rate	91.2 ± 1.8a	72.5 ± 2.3b	78.3 ± 2.0b
Total life span (day)	32.11 ± 0.18a	22.39 ± 0.19c	24.48 ± 0.23b

The standard errors were calculated using the bootstrap procedure with 100,000 samples. The means followed by different letters in the same row are significantly different using the paired bootstrap test at a 5% significance level

Effects of fluxametamide and Sero-x on reproductive parameters

The sublethal effects of fluxametamide and Sero-X on reproductive periods and fecundity of *N. californicus* are summarized in Table 3. The oviposition period was significantly reduced at LC_{30} concentrations of fluxametamide and Sero-X. Pre-oviposition periods (APOP) and Total Pre-oviposition periods (TPOP) in *N. californicus* were also shortened in the treatments. Additionally, total fecundity decreased in response to fluxametamide and Sero-X exposure. Figure 1 shows age-specific survival (l_x), age-specific fecundity (m_x), and age-stage specific fecundity (f_{xj}) of *Neoseiulus californicus* exposed to three treatments.

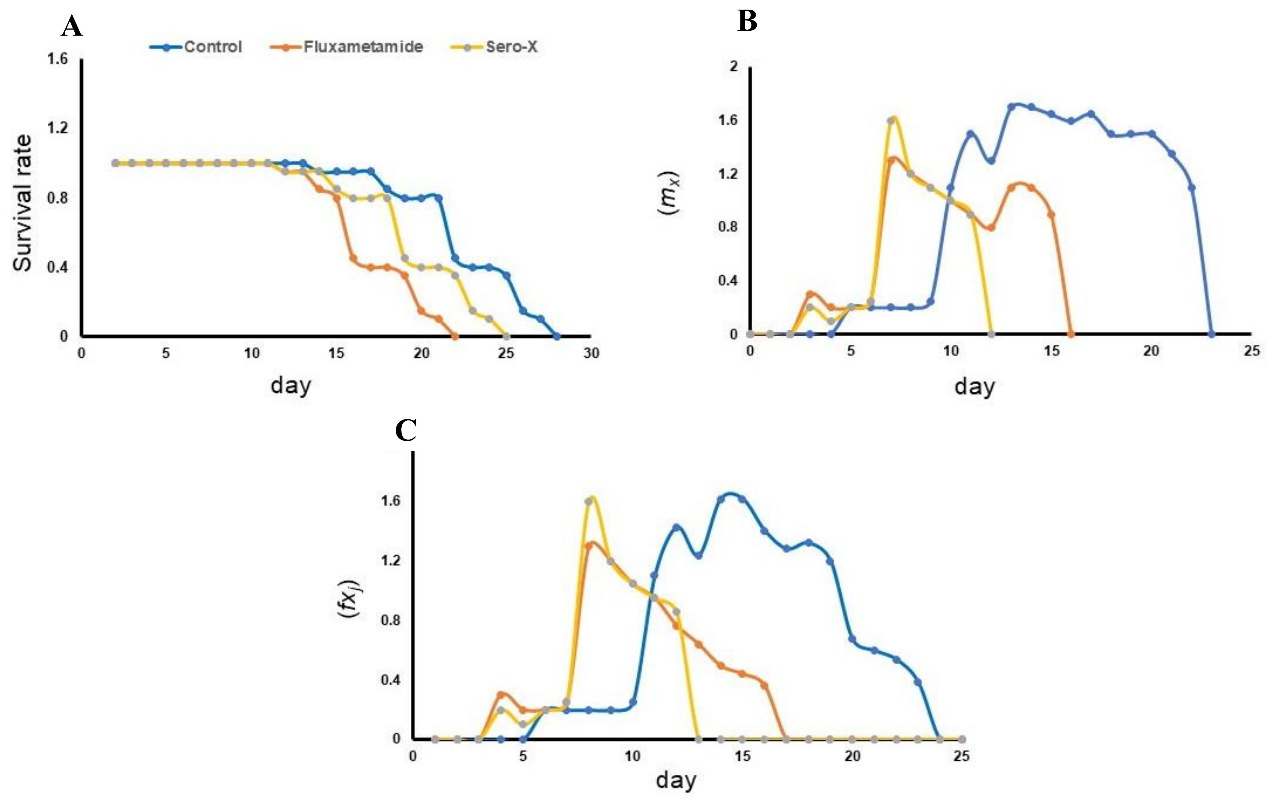

Figure 1. Age-specific survival (l_x) **(A)**, Age-specific fecundity (m_x) **(B)**, age-stage specific fecundity (f_{xj}) **(C)** of *Neoseiulus californicus* under three treatments.

Table 3. Sublethal effect of LC_{30} of fluxametamide and Sero-x on reproductive parameters of *N. californicus*.

Parameter	Treatment		
	Control	Fluxametamide	Sero-x
Oviposition days	13.59 ± 0.18 b	11.89 ± 0.16 c	21.86 ± 0.08 a
APOP (day)	2.31 ± 0.09 a	2.46 ± 0.10 a	2.28 ± 0.08 a
TPOP (day)	7.28 ± 0.22 a	7.29 ± 0.14 a	7.10 ± 0.17 a
Total fecundity	22.48 ± 0.32 b	19.29 ± 0.35 c	35.31 ± 0.37 a

APOP: adult pre-oviposition period; TPOP: total pre-oviposition period.
Means with similar letters in each row do not have a significant difference at the 5% probability level (Paired bootstrap test). Standard errors were calculated using the bootstrap method with 100,000 repetitions.

Effects of fluxametamide and Sero-X on population growth parameters

Fluxametamide and Sero-X influenced the population growth parameters of *N. californicus* mites (Table 4). Gross reproduction rate (*GRR*), net reproduction rate (R_0), intrinsic rate of population increase (*r*), finite rate of population increase (λ), and mean generation time (*T*) decreased under LC30 treatments of fluxametamide and Sero-X. These findings highlight the potential of fluxametamide and Sero-X to significantly suppress *N. californicus* population growth (Table 4).

Table 4. Sublethal effects of LC_{30} value of fluxametamide and Sero-X on life table parameters of *N. californicus*.

Parameter	Control	Fluxametamide	Sero-X
GRR **(offspring/individual)**	27.18 ± 2.16^{a}	14.63 ± 1.32^{c}	18.15 ± 1.41^{b}
R_0 **(offspring/individual)**	22.75 ± 2.53^{a}	12.27 ± 1.42^{c}	15.52 ± 1.61^{b}
r **(day^{-1})**	0.212 ± 0.01^{a}	0.199 ± 0.01^{c}	0.206 ± 0.009^{b}
λ **(day^{-1})**	1.23 ± 0.01^{a}	1.22 ± 0.01^{b}	1.22 ± 0.01^{b}
T **(day)**	14.72 ± 0.25^{a}	12.58 ± 0.15^{c}	13.27 ± 0.24^{b}

The standard errors were calculated using the bootstrap procedure with 100,000 samples.
The means followed by different letters in the same row are significantly different using the paired bootstrap test at a 5% significance level.

DISCUSSION

Improving Integrated Pest Management (IPM) programs requires a thorough understanding of how pesticides and insecticides affect the natural enemies of target pests. These chemicals can impact the pests and their natural enemies either directly or indirectly, including through sublethal exposure (Guedes *et al.* 2016). Resistance to pesticides in phytoseiid mites has been linked to variations in their biological traits (Salman and Ay 2014).

Identifying the sublethal effects of pesticides on natural enemies is crucial for optimizing their use and improving insecticide assessments (Stark and Banks 2003). Additionally, selecting efficient biological control agents is a key step in developing sustainable pest management programs (Gerson *et al.* 2003).

In this study, we evaluated the lethal and sublethal effects of fluxametamide and Sero-X on *N. californicus*, a significant phytoseiid predator of *T. urticae*, under controlled laboratory conditions. Several studies have examined the impact of pesticides on the biological parameters of predatory mites (Alinejad *et al.* 2014; Ganjisaffar and Perring, 2017). However, no prior studies have evaluated the effects of low-lethal concentrations (LC30) of fluxametamide and Sero-X on the biological parameters of *N. californicus*. Understanding these effects can help refine IPM strategies by selecting pesticides that minimize harm to beneficial organisms (Golmohammadi and Hejazi 2014).

Direct bioassay studies on Sero-X against *Tetranychus urticae* or phytoseiid mites are not explicitly available, but interest in organic pest control suggests such products are assessed for their compatibility with biological control agents and environmental impact. Bioassays for acaricides like fluxametamide examine their lethal and sublethal effects on *T. urticae* while also evaluating their impact on beneficial predatory mites to support integrated pest management. Predators like *Phytoseiulus macropilis* (Banks) and

Amblyseius species are commonly studied for non-target effects. Sustainable options like Sero-X are valued for their ability to control pests while minimizing harm to natural enemies, though more research is needed to confirm specific bioassay results (Amin *et al.* 2009; Schmidt-Jeffris *et al.* 2021; Saber *et al.* 2024; Kewedar *et al.* 2025).

The results of this study showed negative effects of fluxametamide and Sero-X on the developmental stages of *N. californicus* females, consistent with findings related to hexithiazox and pyridaben in *Phytoseiulus persimilis* Athias-Henriot and *N. californicus*, respectively (Sanatgar *et al.* 2011; Ghadim Mollaloo *et al.* 2018), as well as spirodiclofen on *Amblyseius swirskii* Athias-Henriot (Alinejad *et al.* 2014).

Differences in populations, experimental methods, pesticide formulations, concentrations, and modes of action may explain variations in results. These factors can contribute to inconsistencies observed across studies. Mensah *et al.* (2013) reported that Sero-X® was derived from a plant used as cattle fodder, containing several compounds without prior commercial applications or available toxicological data. Additionally, adult longevity and total lifespan of *N. californicus* exposed to fluxametamide and Sero-X were decreased significantly compared to the control group. These findings align with those reported for *A. swirskii* (Alinejad *et al.* 2014) and *Amblyseius cucumeris* (Oudemans) (Cheng *et al.* 2018) following exposure to fenazaquin and acetamiprid, respectively.

Based on data analysis, fluxametamide and Sero-X treatments did not have effect on pre-oviposition periods but significantly reduced the oviposition period. The oviposition period, defined as the time from the start to the end of egg-laying (Chen *et al.* 2018), was significantly affected by experimental treatments (LC_{30}). Notably, the oviposition period and fecundity of *N. californicus* were reduced in response to LC_{30} concentrations. The results of this study demonstrated that fluxametamide and Sero-X significantly impacted total fecundity and oviposition in females of this phytoseiid species, aligning with findings by Park *et al.* (2011) and Havasi *et al.* (2023).

Life table studies help assess how various factors affect pest and natural enemy population dynamics (Kakde *et al.* 2014). In this study, life table analysis revealed a decline in all population characteristics of *N. californicus* following treatment with LC_{30} concentrations of fluxametamide and Sero-X.

The highest r_m for *N. californicus* was observed in the control group (0.21 day^{-1}). This value was higher than those reported under exposure to various pesticides, including spiromesifen (0.15) (Sarbaz *et al.* 2017), acetamiprid in *A. swirskii* (0.12) (Shahbaz *et al.* 2019), and abamectin (0.0854 day^{-1}) and pyridaben (0.0951 day^{-1}) in *Phytonemus pallidus* (Banks) (Acari: Tarsonemidae) (Hedayati *et al.* 2019).

Sanatgar *et al.* (2011) and Maroufpoor *et al.* (2016) reported that hexythiazox and spirodiclofen significantly reduced r and λ parameters in *P. persimilis* and *N. californicus*. In the present study, R_0 and GRR of *N. californicus* populations were significantly altered when exposed to low-lethal concentrations.

The data from this study revealed that low-lethal concentrations of the tested pesticides had adverse effects on the survivorship, demographic traits, and biological parameters of *N. californicus*. The full impact of fluxametamide and Sero-X could not be determined from laboratory results, highlighting the need for semi-field and field experiments. Further studies are necessary to develop a compatible formulation of fluxametamide and Sero-X that optimizes the reproductive efficiency of phytoseiid mites.

CONCLUSION

This study showed that fluxametamide and Sero-X significantly impacted *Neoseiulus californicus*, reducing adult longevity, fecundity, and population growth parameters. The decline in reproductive success suggests that careful selection of pesticides is essential for maintaining effective biological control in IPM programs. While laboratory results provide valuable insights, further semi-field and field studies are necessary to refine pesticide formulations and ensure minimal disruption to predator populations while maintaining effective pest control.

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تأثیرات کشنده و زیرکشنده فلوکسامتامید و سرو-ایکس بر پراسنجه‌های زیستی *Neoseiulus californicus* McGregor (Acari: Phytoseiidae)

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دریافت
۲۱ اردیبهشت ۱۴۰۴

پذیرش
۱۶ بهمن ۱۴۰۴

انتشار
۲۶ فروردین ۱۴۰۵

دبیر تخصصی
ا. ترابی

چکیده

این پژوهش اثرهای کشندگی و زیرکشندگی دو آفت‌کش فلوکسامتامید و سرو-ایکس را بر هرنای شکارگر *Neoseiulus californicus* بررسی می‌کند. این هرنا که یکی از عوامل مهم کنترل زیستی است، نقش مهمی در تنظیم جمعیت هرناهای آفت و حشرات کوچکی مانند تریپس‌ها دارد. آگاهی از اثرهای این آفت‌کش‌ها برای حفظ اثربخشی برنامه‌های مهار زیستی ضروری است. در برنامه مدیریت تلفیقی آفات (IPM) ، انتخاب آفت‌کش‌های کم‌خطر که آفات را هدف قرار دهند اما به موجودات مفید آسیب نزنند، اهمیت زیادی دارد. برای ارزیابی این اثرها، آزمون‌های زیست‌سنجی در شرایط کنترل‌شده آزمایشگاهی (۲ ± ۲۵ درجه سلسیوس، ۵ ± ۶۵ درصد رطوبت نسبی و دوره نوری ۱۶:۸ ساعت) انجام شد. نتایج آزمون‌های کشندگی نشان داد که مقادیر LC_{50} برای فلوکسامتامید و سرو-ایکس در بالغ‌های *N. californicus* به ترتیب برابر با ۱۴۲۱ و ۲۱۶۹ میلی‌گرم در لیتر بود. نتایج نشان داد که قرارگیری در معرض غلظت LC_{30} هر دو آفت‌کش اثرات معنی‌داری بر تولیدمثل و پراسنجه‌های رشدی این شکارگر داشت. هر دو تیمار پراسنجه‌های جدول زندگی (r، R_0، λ و GRR) را کاهش داده و زمان نسل (T) را نسبت به شاهد افزایش دادند. افزون بر این، قرارگیری ماده‌ها در معرض این مواد شیمیایی به روش‌های مختلفی بر دورهٔ زندگی نسل بعد تأثیر می‌گذارد. نتایج این مطالعه بر نیاز به مدیریت آفات پایدار تأکید می‌کند که با حداقل کردن تاثیر ترکیبات شیمیایی و حفظ تعادل اکولوژیکی اطمینان حاصل شود که دشمنان طبیعی در کشاورزی همچنان کارآمد باقی می‌مانند.

واژگان کلیدی: کنه‌کش، پراسنجه‌های جدول زندگی، مهار آفت، پراسنجه‌های جمعیت، هرنای شکارگر

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