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SUBLETHAL EFFECTS OF FENAZAQUIN ON BIOLOGICAL PERFORMANCE OF THE TWO-SPOTTED SPIDER MITE, *TETRANYCHUS URTICAE* (ACARI: TETRANYCHIDAE): APPLICATION OF AGE-STAGE, TWO-SEX LIFE TABLES

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ABSTRACT: Estimating sublethal effect of acaricides is a reliable approach in predicting of pesticide impacts. Our study evaluated the sublethal effects of fenazaquin (Pride®) on life table parameters of the subsequent generation of the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) under laboratory conditions at 25–27 ºC, 60±3% RH and a photoperiod of 16:8 (L:D) hours. Adult individuals of *T. urticae* were exposed to different concentrations of fenazaquin and LC_{10}, LC_{20} and LC_{30} were determined based on concentration-response bioassay. The total developmental period of both sexes increased with increasing concentration from LC_{10} to LC_{30}. Fenazaquin treatments gradually reduced the longevity and total life span in both sexes and this reduction increased with increasing concentration. All reproductive periods declined in a concentration-dependent manner. Or data indicated a significant reduction in the intrinsic rate of increase (r_m), the net reproductive rate (R_0) and the finite rate of increase (λ) of the mites treated with LC_{20} and LC_{30} concentrations. Moreover, the shortest mean generation time (T) belonged to the LC_{30} treatment, which caused the longest doubling time (DT). Understanding of sublethal effects of fenazaquin can be useful in designing management programs for *T. urticae*.

KEY WORDS: Acari, fenazaquin, Life-table parameters, *Tetranychus urticae*, Sublethal effects

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is one of the most important pest species responsible for significant yield losses in many horticultural, ornamental, and agronomic crops worldwide (Stumpf and Nauen 2002; Eken and Hayat 2009). It feeds by sucking on the contents of plant cells and damage occurs such as webbing, fine stippling, leaf yellowing, leaf drop and even plant death (Mondal and Ara 2006).

Acaricides play a major role in management of phytophagous mite populations such as *T. urticae* (Van de Vrie et al. 1972). Fenazaquin is a broad-spectrum, non-systemic acaricidal compound that is effective in controlling phytophagous mites which infest a variety of crops, namely fruits and vegetables (Solomon et al. 1993). This compound can affect metabolism, inhibiting the mitochondrial electron transport chain by binding with complex I at coenzime site Q0 (Dekeyser 2005).

Lethal dose estimates are less useful in predicting pesticide impact in the field. Therefore, life table response experiments have been proposed as more reliable than the lethal dose estimates in predicting pesticide impacts in the field since they account for the total effect of a pesticide, providing a measure of its effect on population growth rate (Marcic 2005; Li et al. 2006). However, the data on sublethal effects of acaricides on a subsequent generation should be considered, when evaluating the effectiveness of pesticides. A number of studies focus on sublethal effects of acaricides on life table parameters of *T. urticae* (Marcic 2005; Marcic 2003; Castagnoli et al. 2005), but none of them exactly determine the sublethal effects of fenazaquin on life history, survival and reproduction of offspring of treated *T. urticae*. In addition, evaluation of pesticide effects based solely on females would have not expressed the overall impacts of pesticides. Therefore, in order to evaluate the overall effects of the pesticides on predators, specifying sublethal effects on both sexes is necessary. Therefore, in this study, we estimated demographic parameters with respect to both sexes based on the age-stage, two-sex life table theory (Chi and Liu 1985).

Therefore, the aim of the present study was to estimate the life table parameters of *T. urticae* offspring of the treated females with three concentrations of fenazaquin, including LC_{10}, LC_{20} and LC_{30}, using demographic toxicological analysis. Furthermore, an understanding of sublethal effects of fenazaquin can be helpful in designing management programs for *T. urticae*.

MATERIAL AND METHODS

Origin and rearing of mites

A stock population of *T. urticae* was obtained from infested greenhouses of Varamin (central part of Iran) and reared continuously on bean plants (*Phaseoulus vulgaris* var. Khomein) under laboratory conditions at 25–27 ºC, 60 ± 3% RH and a photoperiod of 16:8 (L:D) hours.

Chemical tested

Fenazaquin, IUPAC name 4-tert-butylphenethyl quinazolin-4-yl-ether, commercial formulation Pride® 20% SC, Behavar, Iran.

Concentration-response bioassay

The commercial formulation of fenazaquin was serially diluted in six concentrations covering the range of 20–90% mortality. Concentration-response bioassay was conducted using a modified leaf dip method (Helle and Overmeer 1985) at 25–27ºC, 60±3% RH and a photoperiod of 16:8 (L:D) hours. The experimental arena consisted of a Petri dish with a thin layer of wet cotton at the bottom and filter paper placed on it to prevent the leaf from drying. The Petri dishes were covered with a ventilated lid. Twenty-four hour old adult females and males of *T. urticae* were used in this experiment. The bean leaf discs (4 cm diameter) were dipped in fenazaquin solutions for 15 seconds and allowed to be dried for about 3 h. The control leaf discs were dipped in distilled water. Each leaf disc was transferred into the experimental arena and surrounded with saturated cotton to prevent escape of the mites. Then, forty adult mites of the same age (less than 24-h-old) were placed on the treated leaf discs for each concentration. Each experiment was replicated five times. The number of dead mites was counted after 24 h. Individuals that became entangled in wet cotton wool were excluded from the analysis. The criterion for mortality was an inability of mites to walk when lightly prodded. Abbot formula was used to estimate the natural mortality (Abbott 1925) which did not exceed 10%. The concentrations of the acaricide were chosen based on initial range-finding tests. The sublethal concentrations including LC_{10} (40 µg ml^{-1}), LC_{20} (60 µg ml^{-1}) and LC_{30} (100 µg ml^{-1}) were determined using probit analysis.

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

To evaluate the sublethal effect of fenazaquin on offspring of treated mites, bean leaf discs were treated with sublethal concentrations including LC_{10}, LC_{20} and LC_{30} and allowed to dry for 3 h. One hundred and fifty same-aged (24 hours old) mated females were used for each treatment. After 24 hours, the surviving females were separately placed on clean and bean leaf discs (20 mm in diameter). After 24 hours, the eggs laid by the treated and untreated females in each experimental arena were saved. All saved eggs (75 to 100 eggs) were checked daily and development time and survival of them were recorded. Newly emerged females were associated with a male for mating. The daily survival of each adult and fecundity of females were recorded and population parameters were calculated with respect to both sexes.

Data analysis

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; Chi 1988) by using a user-friendly computer program, TWOSEX-MS Chart (Chi 2012). 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); and the population growth parameters (the intrinsic rate of increase (r); the finite rate of increase (λ); the net reproductive rate (R_0); the gross reproductive rate (GRR); the mean generation time (T) and the doubling time (DT)) were calculated accordingly. The intrinsic rate of increase is estimated by using iterative bisection method from:

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

with age indexed from 0 to ω (Goodman 1982). In the age-stage, two-sex life table, the l_x and m_x are calculated as:

$$l_x = \sum_{j=1}^{k} s_{xj} \quad (2)$$

and

$$m_x = \frac{\sum_{j=1}^{k} s_{xj} f_{xj}}{\sum_{j=1}^{k} s_{xj}} \quad (3)$$

where k is the number of stages.

The finite rate of increase (λ), the mean generation time (T) and the net reproductive rate (R_0) were calculated as follows:

$$\lambda = e^{r} \quad (4)$$

$$T = \frac{\ln R_0}{r} \quad (5)$$

Table 1.
Probit analysis for the concentration-mortality response of fenazaquin on adult females and males of *Tetranychus urticae.*

	No.*	No. concentration	χ^2	*df*	P	LC_{50} (μg ml^{-1})	Slope ± SE
Adult individuals	720	6	3.35	4	0.50	236	1.43 ± 0.31

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

Table 2.
Influence of different concentrations of fenazaquin on developmental time, adult longevity and total life span (days ± SEM) of *Tetranychus urticae.*

Sex	Control	LC_{10}	LC_{20}	LC_{30}
Male				
Development time (day)	9.62 ± 0.1[b]	10 ± 0.08[a]	10.07 ± 0.07[a]	10.16 ± 0.13[a]
Longevity (day)	–	7.27 ± 0.57[b]	6.92 ± 0.97[b]	5.28 ± 0.86[b]
Total life span (day)	20.72 ± 0.69[a]	16.92 ± 0.97[b]	15.35 ± 0.85[b]	15.13 ± 0.73[b]
Female				
Development time (day)	9.59 ± 0.01[c]	9.95 ± 0.04[b]	10.06 ± 0.03[ab]	10.27 ± 0.06[a]
Longevity (day)	11.51 ± 1.73[a]	8.45 ± 0.79[b]	8.32 ± 0.05[b]	7.86 ± 0.6[b]
Total life span (day)	21.10 ± 0.76[a]	18.59 ± 0.66[b]	18.41 ± 0.79[b]	17.92 ± 0.08[b]

Note: Mean values followed by the same letter in the same row are not significantly different (P < 0.05, Duncan after one-way ANOVA).

$$R_0 = \sum_{x=0}^{\infty} l_x m_x \quad (6)$$

The GRR was calculated as GRR = Σm_x.

Statistical analysis

The means and standard errors of the population parameters were estimated by using the Bootstrap procedure (Efron and Tibshirani 1993; Huang and Chi 2013; Khanamani et al. 2013). Because bootstrapping uses random resampling, a small number of replications will generate variable means and standard errors. To generate less variable results, 10000 replications were used in this study. Differences between means were compared with the Tukey-Kramer procedure (Dunnett 1980).

RESULTS

Concentration-response bioassay

Estimated LC_{50} for *T. urticae* was 239 μg ml^{-1}. No mortality was recorded in the controls (Table 1).

Development time, longevity and total life span

The effect of different concentrations of fenazaquin on development of offspring of the treated females is presented in Table 1. Statistically significant differences were scored in the developmental duration of both sexes (F = 6.03; df = 3, 166; P < 0.0001 for female, F = 15.27; df = 3, 86; P = 0.0009 for male). The longest development time for male and female was 10.16 and 10.27 days, respectively at LC_{30}. Significant differences were observed in the longevity of both sexes in comparison with the control. The longevity of male and female ranged from 5.28 to 11.1 and 7.86 to 11.5 days, respectively (F = 6.08; df = 3, 86; P = 0.0006 for male and F = 11.9; df = 3, 166; P < 0.0001 for female). Maximum values for total life span of male and female were 20.72 and 21.10 days for the mites treated with distilled water (F = 6.08; df = 3, 86; P < 0.0001 for male and F = 11.09; df = 3, 166; P = 0.0043 for female).

Reproduction

The mean oviposition period was significantly affected by sublethal concentrations (F = 11.35; df = 3, 166; P = 0.0159) (Table 2). The oviposition period ranged from 6.52 to 9.25 days. The shortest pre-oviposition (F = 3.70; df = 3, 166; P < 0.0001), oviposition and post-oviposition (F = 3.54; df = 3, 166; P = 0.0131) periods were 1.04, 6.52 and 0.5 days, respectively at LC_{30} treatment. In addition, means for the total fecundity per female decreased significantly at LC_{20} and LC_{30} (F = 3.66; df = 3, 166; P = 0.0137).

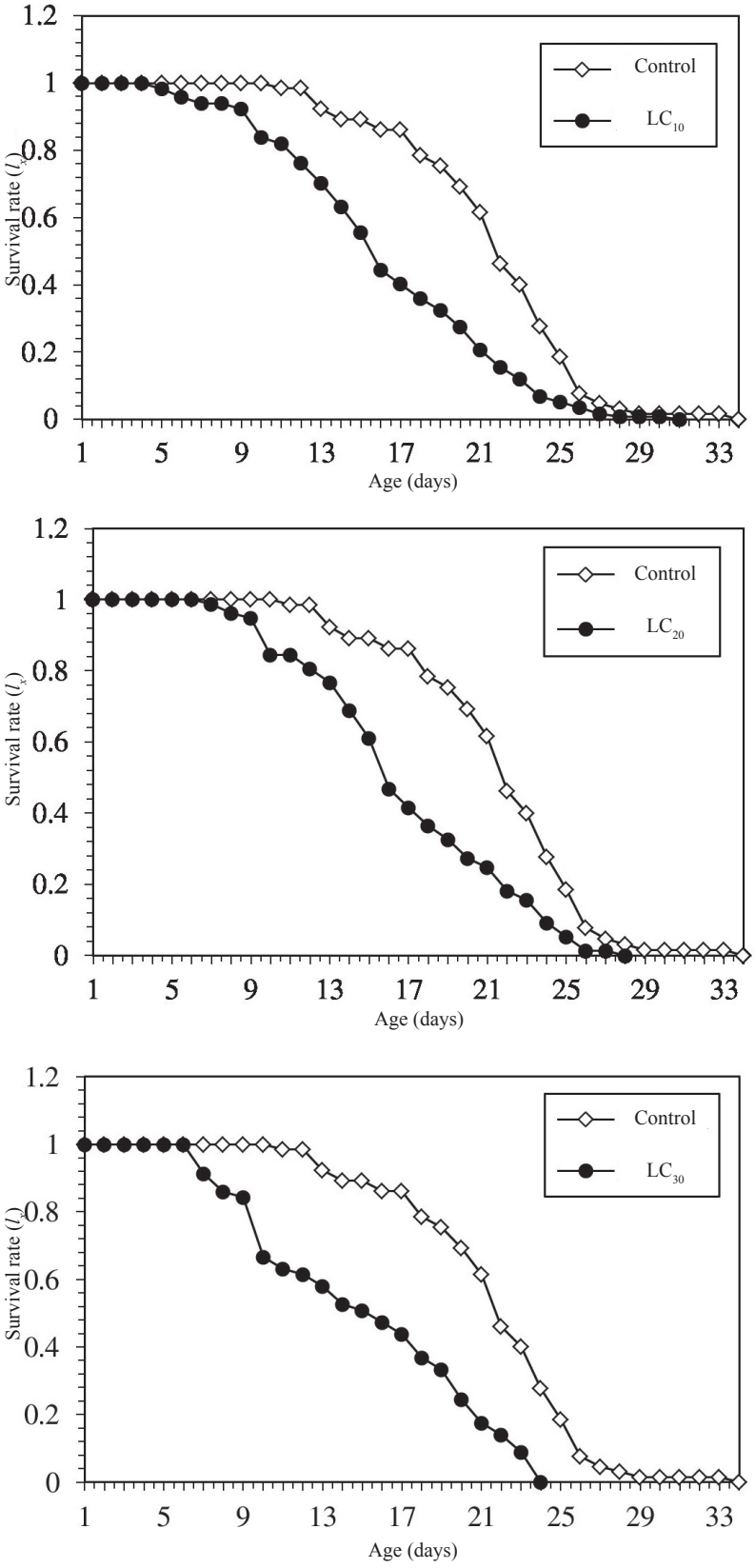

Fig. 1. Comparison of the water check and sublethal concentrations of fenazaquin on survivorship (l_x) of *T. urticae.*

Population parameters

The life table parameters of offspring of the treated females are predicted in Tables 3, 4. With exception of LC_{10}, significant reductions were detected in the intrinsic rate of increase (r) and net reproductive rate (R_0). The shortest mean generation time (T) was estimated 13.69 days at LC_{30}

Table 3.
Reproductive period and total fecundity of *Tetranychus urticae* treated with different concentrations of fenazaquin (means ± SEM).

	Treatment			
Parameters	Control	LC_{10}	LC_{20}	LC_{30}
Pre-oviposition (day)	1.44 ± 0.07[a]	1.32 ± 0.05ab	1.16 ± 0.04[ab]	1.04 ± 0.09[b]
Oviposition (day)	9.25 ± 0.73[a]	7.04 ± 0.75[b]	6.98 ± 0.51[b]	6.52 ± 0.58[b]
Post-oviposition (day)	1.08 ± 0.13[a]	1.03 ± 0.17[a]	0.7 ± 0.15[ab]	0.5 ± 0.08[b]
Total fecundity (offspring)	62.47 ± 6.1[a]	46.89 ± 4.75[ab]	40.33 ± 5.71[b]	38.7 ± 4.89[b]

Note: Mean values followed by the same letter in the same row are not significantly different ($P < 0.05$, Duncan after one-way ANOVA).

Table 4.
Population parameters (means ± SEM) of *Tetranychus urticae* treated with different concentrations of fenazaquin.

	Treatment			
Parameters	Control	LC_{10}	LC_{20}	LC_{30}
Intrinsic rate of increase, r (day^{-1})	0.232 ± 0.009[a]	0.210 ± 0.009[ab]	0.199 ± 0.008[b]	0.187 ± 0.013[b]
Net reproductive rate, R_0 (offspring)	36.14 ± 5.17[a]	25.12 ± 3.81[ab]	23.64 ± 3.23[b]	16.98 ± 3.56[b]
Mean generation time, T (day)	15.87 ± 0.17[a]	15.33 ± 0.26[a]	15.11 ± 0.22[a]	13.69 ± 0.80[b]
Doubling time, DT (day)	3.60 ± 0.121[c]	4.02 ± 0.129[bc]	4.36 ± 0.123[b]	4.87 ± 0.224[a]
Finite rate of increase, λ (day^{-1})	1.262 ± 0.01[a]	1.234 ± 0.01[ab]	1.220 ± 0.01[b]	1.206 ± 0.01[b]

Note: Mean values followed by the same letter in the same row are not significantly different ($P < 0.05$, Duncan after one-way ANOVA).

treatment. Moreover, LC_{30} had the longest doubling time (DT) among the treatments. Furthermore, the finite rate of increase (λ) at LC_{20} and LC_{30} were significantly lower than those in the control and LC_{10}.

Survival and fecundity

Figures 1 and 2 show daily survival and fecundity curves of offspring of the treated females with sublethal concentrations of fenazaquin. Total lifetime for the untreated mites was 34 days, while it was 31, 28 and 25 days for LC_{10}, LC_{20} and LC_{30} treatments, respectively. In addition, examination of l_x in untreated mites revealed no mortality in the immature stages, with 100% chance of reaching adulthood. In contrast, mites treated by LC_{10}, LC_{20} and LC_{30} concentrations showed higher mortality in immature stages, with 83%, 80% and 67% chances of reaching adulthood, respectively.

The maximum values of m_x were 7, 9 and 5 eggs/individual for the mites treated with LC_{10}, LC_{20} and LC_{30} concentrations, which was occurred on 17, 16 and 14 days of the life span. In comparison, maximum value of 5 eggs/individual was observed on 32 days of the life span for untreated mites.

The age-stage survival rate (s_{xj}) represents the probability that an egg of *T. urticae* will survive to age x and stage j (Fig. 3). Because of variation in the development time among individuals, there is obvious stage overlapping in all treatments (A–D). The highest female survival rate was observed at LC_{20} treatment and 62% of eggs normally survive to the adult stage. In contrast, the highest male survival rate belonged to the control (41%).

DISCUSSION

The recommended field rate of fenazaquin application for *T. urticae* control is 400 µg ml^{-1} according to instructions on the label (Behavar, Iran). In the current study, although the concentrations used were lower than what was recommended, some of them considerably affected population growth of the two-spotted spider mite. The total developmental period of both sexes increased with increasing concentrations from LC_{10} to LC_{30}. Fenazaquin treatments gradually reduced the longevity and total life span of both sexes with increasing concentration. Similar trends for developmental time and longevity were recorded for the same species when treated by another member of the METI acaricides (tebufenpyrad)

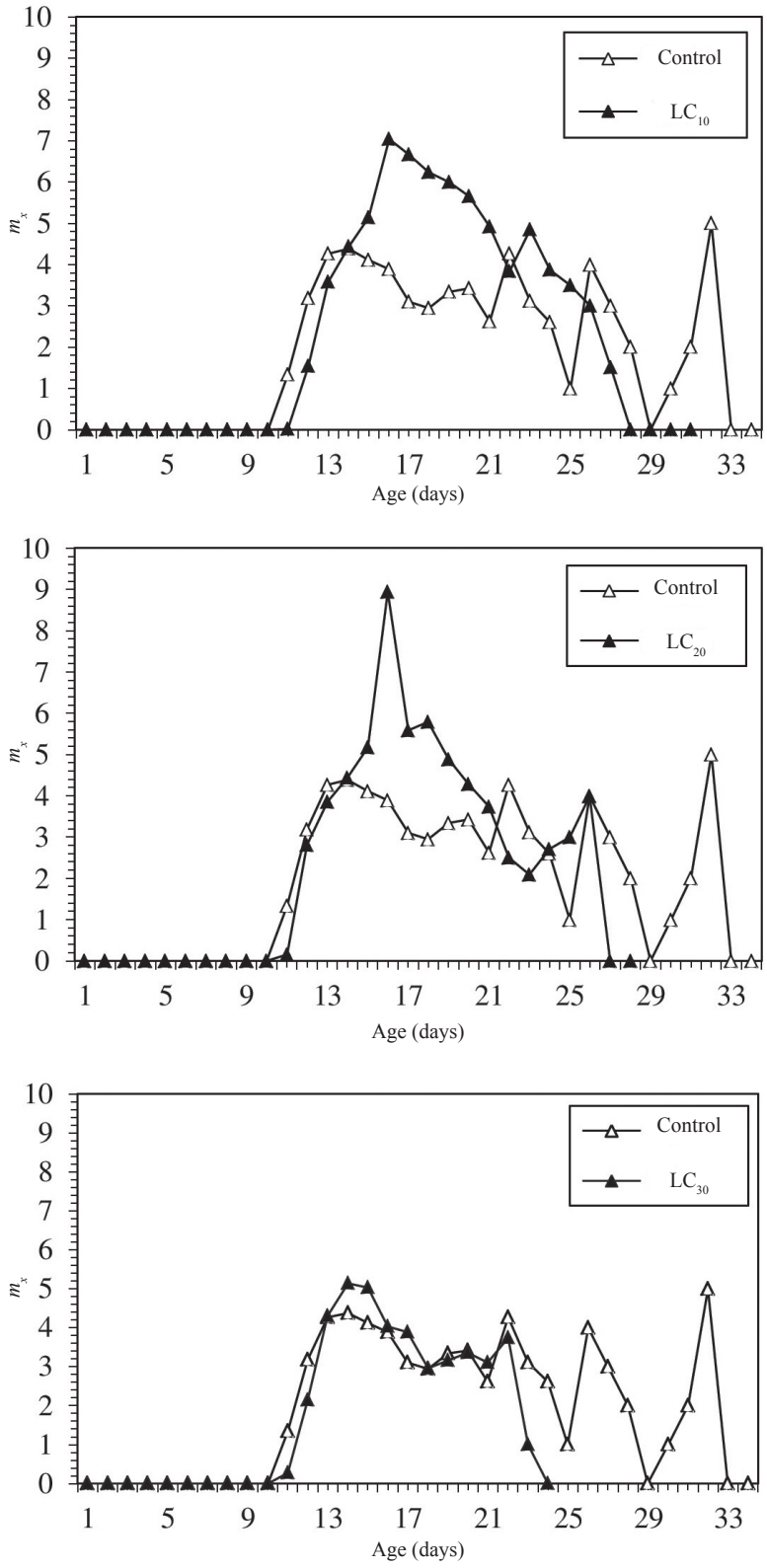

Fig. 2. Comparison of control and sublethal concentrations on fecundity (m_x) of *T. urticae.*

(Marcic 2005). In comparison, according to Marcic (2003), clofentezine (a tetrazine compound and growth inhibitor) treatments decreased both longevity and fertility of the two-spotted spider mite but no effect was found on the developmental time at 27.5±1.5 ºC and 65–85% RH. This difference can mainly be attributed to differences in the pesticide's mode of action or experimental conditions.

The results also demonstrated that all reproductive periods declined in a concentration-dependent manner. The lowest total fecundity and

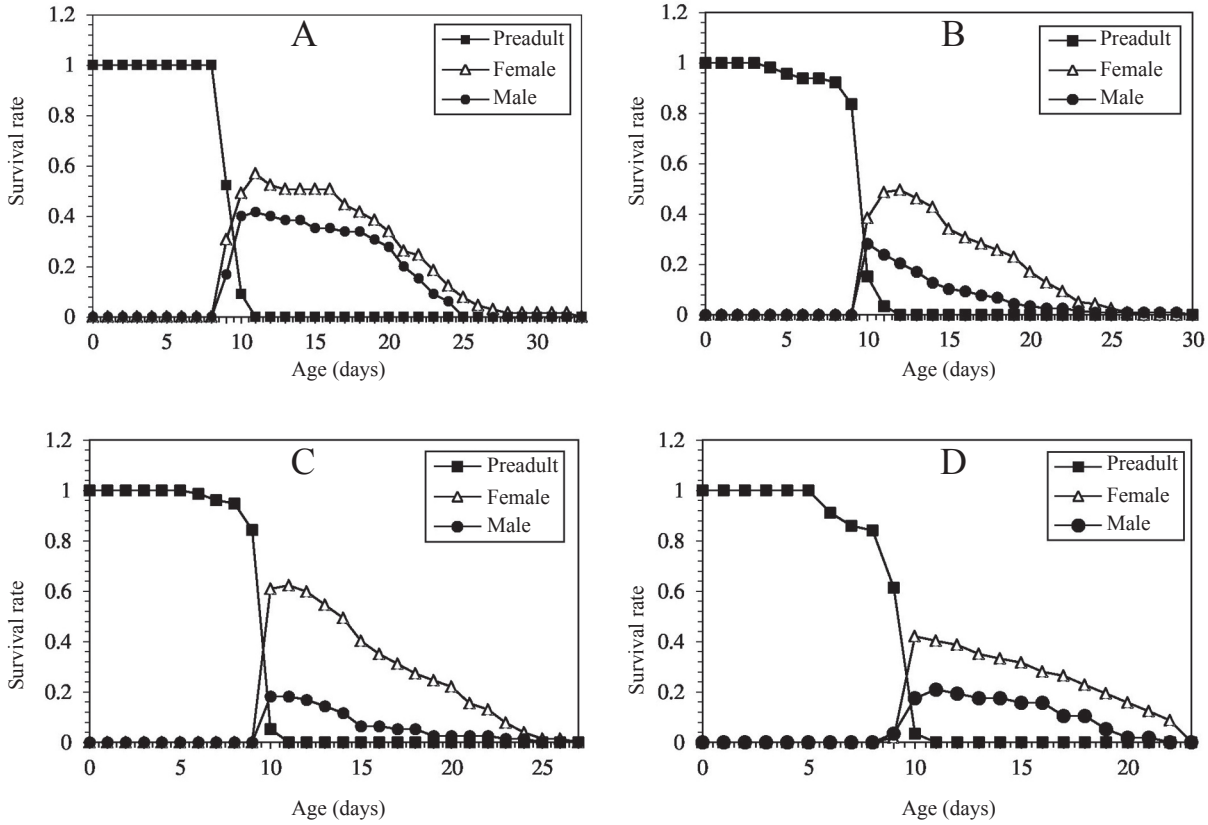

Fig. 3. The Age-stage specific survival rate (*sxj*) of *T. urticae* treated with different concentrations of fenazaquin: control (A), LC_{10} (B), LC_{20} (C), LC_{30} (D).

oviposition period belonged to the LC_{30} treatment, which illustrates that the potential of treated mites for population recovery would be slow. These results are in harmony with those reported by Marti´nez-Vilar et al. (2005) for *T. urticae* treated with azadirachtin at the highest tested concentration (80 ppm). On the contrary, Sáenz-de-Cabezón et al. (2006) discussed that application of different dosages of the chitin synthesis inhibitor, triflumuron, had no effect on fecundity of *T. urticae*. This difference is likely to be related to the mode of action of acaricides. In other words, triflumuron inhibits the chitin synthesis, while fenazaquin abides the mitochondrial electron transport chain.

The age-specific fecundity and survival curves indicate that sublethal concentrations of fenazaquin caused reduction in survival and fecundity of *T. urticae*. At all experimental concentrations, the chances of reaching adulthood decreased with increasing concentration. Compared with the control, the day of maximum reproduction in treated mites was shifted, which probably caused limitations in reproduction and survivorship. In accordance with Marcic et al. (2011), sublethal effects of spirotetramat on age-specific fecundity of spider mites revealed that the females treated with 200 mg/L laid no eggs, while the females treated with 20 mg/L and 2 mg/L laid considerably reduced numbers of eggs compared to females in the control. In this regard, the authors reported that exposure to sublethal concentrations of spirotetramat declined the fecundity and survival of treated mites in comparison to the control, which confirms our results. Moreover, according to Kavousi et al. (2009), overlapping occurred between different stages of *T. urticae*, which is totally in agreement with our explained results. The current authors argued that the age-stage, two-sex life table has the potential for revealing the stage differentiation of *T. urticae* due to the variable developmental rates among individuals.

Several studies have indicated that life-table parameters of phytophagous mites were affected by sublethal concentrations of pesticides (Marti´nez-Vilar et al. 2005; Li et al. 2006; Sáenz-de-Cabezón et al. 2006; Marcic et al. 2010; Marcic et al. 2012). The data acquired in our study indicated a significant reduction in the intrinsic rate of increase (*r*) for the mites treated with LC_{20} and LC_{30} concentrations. The calculated *r* in this

study for untreated mites was 0.232 day^{-1}, which was similar to the results obtained by Sedaratian et al. (2011) for the spider mites on Tms soybean genotype (0.233 day^{-1}). Furthermore, Marcic (2007) noted that the values of *r* for *T. urticae* treated with 6 and 12 mg/l spirodiclofen were 0.21 and 0.14 day^{-1}, respectively, which were nearly equivalent to those obtained in our study. It had been reported previously (Marcic 2003) that spider mites survived clofentezine treatment as 'early' (0–24 h old) eggs expressed significantly higher *r* values than untreated ones. This effect may be interpreted as hormoligosis, which is the stimulation of reproductive physiology by sublethal doses of pesticides. However, according to our results, we can not detect the effect of hormoligosis. Probably this difference is due to the different mode of action of acaricides or the concentrations used.

Li et al. (2006) reported that net reproductive rates (R_0) of *T. viennensis* treated by various concentrations of clofentezine decreased sharply at higher doses (LC_{25} and LC_{50}), while slight reductions at LC_{10} were observed in each life stage tested. Similarly, our estimations demonstrated that R_0 of treated mites declined severely at LC_{20} and LC_{30}, while slight reductions at LC_{10} were recorded.

The finite rate of increase (λ) observed in this study ranged from 1.26 to 1.20 day^{-1}, which was similar to that determined by Ibrahim and Knowles (1986) for the same species treated with amitraz (1.28 to 1.04 day^{-1}). The mean generation time (*T*) for the spider mites treated by the highest concentration of fenazaquin (LC_{30}) was remarkably lower than the control. In comparison, our results are in contrast with those obtained by Sáenz-de-Cabezón et al. (2006), who found that the mean generation time of the treated mites with different concentrations of triflumuron did not differ much from the untreated ones at 24±1 ºC, 65±5% RH. This variation may be due to the mode of action of different acaricides. Furthermore, the doubling time (*DT*) in treated females with the highest concentration was significantly extended, which means that it took more time for mites treated with the highest sublethal level of fenazaquin to compensate lost individuals. The similar patterns of sublethal activity of spirodiclofen and clofentezine on spider mites have been reported by Marcic (2007, 2003).

The results showed that the biological parameters of offspring of treated *T. urticae* were affected by sublethal concentrations of fenazaquin, which points to the efficiency of this acaricide in controlling the subsequent generation of the two-spotted spider mites. Additionally, reduced pesticide use is an essential element of any resistance management (Hoy 1998) and in conservation of biological control agents in the ecosystem. Consequently, we suggest that applying fenazaquin at lower rates with the release of the appropriate predatory mites, could lead to efficient control of *T. urticae*. On the other hand, when choosing recommended concentrations of acaricides, one should estimate the sublethal effects on different life stages of the target pest. Further research should be focused on the sublethal effects of fenazaquin on the biological control agents, in order to improve the management of *T. urticae* populations.

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