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Sterilization makes a difference: demographic analysis of spirodiclofen effects on *Tetranychus urticae* (Acari: Tetranychidae)

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Original research

ABSTRACT

Spirodiclofen is characterized by a relatively slow acaricidal action against adult females of the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), with reduction of their fecundity (eggs laid/female) and fertility (eggs hatched/female). Exposure of pre-ovipositing *T. urticae* females to the acaricide may affect population growth, considering that a high reproduction of young females is crucial in the population biology of this colonizing species. Effects of spirodiclofen on life-history traits and population parameters of *T. urticae* were evaluated in demographic bioassay using the age-stage two-sex life table, constructed in fecundity-based and fertility-based variants. The acaricide was applied against pre-ovipositing females in a series of nine concentrations, starting from the recommended field rate (96 mg/l). The treatments with concentrations ranging from 12 - 96 mg/l significantly reduced fecundity and longevity, while 27 - 40% of females didn't lay eggs. Exposure was lethal to 2 - 21% of the females, of whom a large majority didn't lay eggs. A considerable part of surviving females also failed to lay eggs within the first four post-treatment days (when around 50% of all eggs in the control were laid) i.e., they were sterilized by the acaricide. At the same time, the percentage of dead females rose to 17 - 55%, mainly due to the mortality of sterilized females. The three highest concentrations (24 – 96 mg/l) significantly reduced the net reproductive rate (R_0), intrinsic rate of increase (r) and finite rate of increase (λ), by 49-72%, 20-34%, and 4-6%, respectively, compared to the control. This reduction was mainly the result of sterilization and high mortality of treated females, in combination with reduced fecundity and longevity of reproductive ones. Application of the fertility-based life table showed significant decrease of r and λ (by 23-40%, and 4-7%, respectively, compared to the control), in the treatments with the three highest concentrations. The short-lived transovarial toxic effect observed in the fertility-based life table was not sufficient to cause a significant reduction in population parameters, compared to those acquired by the fecundity-based life table.

Keywords *Tetranychus urticae*; spirodiclofen; toxicity; sublethal effects; life tables

Introduction

Demographic toxicological studies, based on the incorporation of life tables in the context of toxicology, have become an essential element in the laboratory evaluation of pesticide effects on insects and mites. This approach accounts both for the lethal and sublethal effects of pesticides/acaricides and integrates their impact at an ecologically more relevant level into population growth rate and other demographic parameters (Stark and Banks 2003; Guedes *et*

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al. 2016). The age-stage two-sex life table (Chi *et al.* 2020) has become a frequent tool lately in various subject areas of entomological and acarological research, including the evaluation of acaricide effects on plant-inhabiting mites (e.g. Musa *et al.* 2017, 2022; Bozhgani *et al.* 2019; Havasi *et al.* 2021; Rimy *et al.* 2021; Rajaee *et al.* 2022).

Acaricides are an important component of the integrated management of spider mites (Acari: Tetranychidae) and other phytophagous mite pests (Marčić 2012; Van Leeuwen *et al.* 2015). It is, therefore, necessary to evaluate their effects on mites thoroughly, putting an emphasis on population-level response. Spirodiclofen, a tetronic acid derivative, is probably the most widely sold acaricide in the world (Van Leeuwen *et al.* 2015; Sparks *et al.* 2020). As an inhibitor of acetyl coenzym A carboxylase, spirodiclofen is active against all developmental stages of spider mites, and it strongly affects the fecundity and fertility of female adults. The symptomatology of poisoning observed in *T. urticae* females is unique: their bodies grow to unusually large size due to inability to lay eggs which accumulate in the genital tract (a part of females is being sterilized i.e. they don't lay eggs), and it take most of them several days to die (Nauen 2005; Van Pottelberge *et al.* 2009). Despite its success on the global market, there have been few demographic studies in which the effects of spirodiclofen on plant-inhabiting mite species were assessed using the age-stage two-sex life table (Alinejad *et al.* 2016; Sarbaz *et al.* 2017; Saber *et al.* 2018; Sani *et al.* 2019; Ahmed and Abdelwines 2021). All of these studies examined spirodiclofen effects on the F_1 generation i.e., on the offspring of F_0 females that survived treatments mostly with 1-3 concentrations ranging from LC_{10} to LC_{50}.

The aim of this study was to expand the knowledge of demographic toxicology of spirodiclofen action on spider mites. The objective was to assess whether and how spirodiclofen as a relatively slow-acting acaricide, causing unique symptoms of poisoning, affects population growth of the F_0 generation after treatment of pre-ovipositing *T. urticae* females with a wide range of concentrations. As a colonizing species, *T. urticae* is characterized by a high fecundity of young adult females (Sabelis 1985; Li and Margolies 1993). As spirodiclofen can lower significantly not only the female fecundity but fertility too, the age-stage two-sex life table was constructed in two variants: the fecundity-based life table, using the number of laid eggs, and the fertility-based life table, using the number of viable (hatched) eggs. Spirodiclofen effects were tested in a wide range of concentrations (i.e., a wide range of acaricide deposits on treated surface), from concentrations lethal to a part of treated females (and sublethal to the others) to concentrations sublethal to all treated females. The amount of an acaricide recommended for control of phytophagous mites is determined having in mind the goal that even the most tolerant individual in a population should absorb a sufficient amount of acaricide to cause its death i.e., to eliminate the entire population. In practice, however, acaricide distribution on treated plants is heterogeneous in space and time (Martini *et al.* 2012) which enables some individuals to survive as a result of absorbing smaller (sublethal) amounts of acaricides. In other words, spatial and temporal heterogeneity of spray coverage and exposure (variability of acaricide deposit on treated surface) in the field enables population recovery on poorly covered or uncovered plant surfaces, which depends on the reproductive potential of females that survived exposure.

Material and methods

Mite colony

A population of *T. urticae*, set up from samples collected on ruderal weeds in the vicinity of Belgrade, has been reared on bean plants in a climate-controlled room (25-30 ºC, 16/8 h light/dark photoperiod) in the Laboratory of Applied Entomology of the Institute of Pesticides and Environmental Protection, Belgrade, Serbia, since March 2004.

Acaricide

The commercial product Envidor (manufactured by Bayer AG, a suspension concentrate containing 240 g/l spirodiclofen) was purchased from the company representative in Serbia.

Life table bioassay

Primary kidney bean leaf discs (3 cm diameter) positioned upon moistened cotton wads in Petri dishes were used in a life table bioassay. The Petri dishes were kept in a Binder KBWF 720 plant growth chamber at 26 ± 1 ºC, under 60 ± 5% RH and 16/8 h light/dark photoperiod. The leaf discs were sprayed with the acaricide suspended in distilled water using an air-pressure sprayer (1 ml of liquid, 100 kPa air pressure, aqueous deposit 2.2 ± 0.2 mg/cm^2).

To assess the effects of spirodiclofen on life-history traits and demographic parameters of *T. urticae*, life tables were constructed for 10 cohorts of mites. The cohorts were formed by placing a single fertilized female (2–3 days old) on each leaf disc and removing it after 24 h, as well as all eggs laid, except one. The development and survival of immature mites that hatched from those eggs were checked daily. Newly-emerged females were transferred individually to fresh leaf discs and paired with adult males. As new females normally outnumber males, the missing number of males was transferred from the mite colony. Nine cohorts of paired mites were sprayed with spirodiclofen, diluted in distilled water, in a series of concentrations (mg/l): 96 (the recommended field rate), 48, 24, 12, 6, 3, 1.5, 0.75 and 0.375. A control cohort was sprayed with distilled water only. The initial number of eggs per cohort was 70–420, depending on expected lethal and sublethal effect on females. After 24-h exposure to the acaricide, the surviving mites were transferred to untreated leaf discs. The mites were continuously transferred to fresh leaf discs on a daily basis, and their survival (both sexes) and the number of eggs laid (females) were recorded until all individuals in the cohort were dead. Leaf discs containing the eggs were kept for another 7-8 days under the same conditions, and then unhatched eggs were counted. Two variants of the age-stage two-sex life table were constructed: a fecundity-based life table, using the number of eggs laid, and a fertility-based life table, using the number of viable (hatched) eggs.

The following life-history traits were calculated: adult longevity (in days), fecundity (total number of eggs laid per female), oviposition days (number of days in which females laid eggs), and fertility (total number of viable eggs per female). Fecundity, fertility and longevity were calculated in two ways: based on the total number of females in cohort, Nf (fecundity-I, fertility–I, longevity-I), and based on the number of reproductive females, Nfr (fecundity-II, fertility-II, longevity-II). The proportion of reproductive females (Nfr/Nf), was calculated with respect to the entire duration of adult life, including the 24-h exposure to acaricide, and the total number of days after treatment that individual mites lived on untreated surface. Females that laid eggs (in fecundity-based life tables) and females that laid viable eggs (in fertility-based life tables) were considered as reproductive. Females that did not lay eggs after treatment were considered as sterilized.

Data analysis

Raw life table data were analyzed according to the age-stage two-sex life table theory (Chi and Liu 1985; Chi 1988; Chi *et al.* 2020), using the software TWOSEX-MSChart (Chi 2021; Chi *et al.* 2022). Males obtained from the colony as well as escaped or drowned individuals were excluded from the analysis. The life table data included the number of eggs laid (fecundity-based life table) or the number of viable eggs (fertility-based life table). The demographic parameters are detailed in Table 1. Standard errors of the life-history traits and demographic parameters were estimated by the bootstrap technique with 50,000 bootstrap replicates. Differences between control and treatment were compared by the paired bootstrap test (50,000 replicates) based on the confidence interval of difference (Efron and Tibshirani 1993; Chi *et al.* 2020, 2022).

Table 1 The parameters used in demographic analysis (Chi and Liu 1985; Tuan *et al.* 2016; Taghizadeh and Chi 2022).

Parameter	Equations and definitions	
Age-specific survival rate (l_x)	$l_x = \sum_{j=1}^{k} s_{xj}$	the probability that a newly laid egg survives to age x
Age-stage-specific survival rate (s_{xj})		the probability that a newly laid egg survives to age x and stage j
Age-specific fecundity (m_x)	$m_x = \frac{\sum_{j=1}^{k} s_{xj} f_{xj}}{\sum_{j=1}^{k} s_{xj}}$	the mean fecundity of all individuals of age x
Female age-stage-specific fecundity (f_{xj})		the mean fecundity of individual of age x and stage j (female, j =3)
Age-specific net maternity ($l_x m_x$)		age-specific fecundity weighted by survival rate
The gross reproductive rate (GRR)	$GRR = \sum_{x=0}^{\infty} m_x$	the mean number of offspring that an individual can produce during its life span
The net reproductive rate (R_0)	$R_0 = \sum_{x=0}^{\infty} l_x m_x$	the mean net number of offspring that an individual can produce during its life span (a multiplication factor in one generation)
The intrinsic rate of increase (r)*	$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1$	the rate of natural increase in a closed population with constant age-specific mortality and reproduction schedules and a stable age distribution
The finite rate of increase (λ)	$\lambda = e^r$	a multiplication factor of a population at each time unit (daily growth rate)
The mean generation time (T)	$T = \frac{\ln R_0}{r}$	the lenght of time that is required by a population to increase R_0-fold
Age-stage-specific reproductive value (v_{xj})	$v_{xj} = \frac{e^{r(x+1)}}{s_{xj}} \sum_{i=x}^{\infty} e^{-r(i+1)} \sum_{y=j}^{k} s'_{iy} f_{iy}$	the contribution of an individual of age x and stage j to future population

* estimated using the iterative bisection method from the Euler-Lotka equation, with the age indexed from 0 (Goodman 1980)

k = number of stages (egg, immature, female/male)

s'_{iy} = the probability that an individual of age x and stage j will survive to age i and stage y, calculated by assuming $s'_{iy} = 1$

Results

Exposure to spirodiclofen affected the longevity and reproduction of *T. urticae* females (Table 2). In treatments with the four highest acaricide concentrations (12 – 96 mg/l) the proportion of reproductive females (Nfr/Nf) was significantly lowered than in the control. It means that a considerable part of females (27 – 40%) failed to lay eggs. These four treatments also caused statistically significant reductions in fecundity-I and longevity-I (by 29 - 73% and 2.9 - 6.3 days, respectively, compared to the control). Considering reproductive females only, the three highest concentrations (24 – 96 mg/l) significantly reduced fecundity-II, longevity-II and oviposition duration in days (by 34 - 59%, 2.9 - 5.5 days, and 3.6 - 6.5 days, respectively, compared to the control). It is interesting that the highest values of fecundity-II were found after treatment with 1.5 mg/l concentration, but the difference from control values was not statistically significant. The two highest concentrations (48 - 96 mg/l) reduced significantly the adult longevity of males (Table 2).

Figure 1 shows the proportion of reproductive females over the entire adult life, including the 24-h exposure to acaricide on treated leaf discs. Depending on acaricide concentration, exposure was found to be lethal to 2 - 21% treated females, most of which failed to lay a single egg during that time period. Regarding females that survived the exposure, a considerable part of them laid no eggs at all after treatment with concentrations 12 – 96 mg/l over the first four post-treatment days (when around 50% of all eggs in the control were laid). During that time period, the total percentage of dead females rose to 17 – 55%, mostly as a result of the deaths of those females that were sterilized by the toxic action of spirodiclofen (Figure 1).

The age-specific survival and reproduction of *T. urticae* are presented in Figure 2. Spirodiclofen treatment caused a concentration-dependent decrease in age-specific survival rates (l_x), compared to the control. This effect is primarily due to a decrease in age-stage specific survival rates of females (Figure 2a-c). Regarding reproduction, both female age-specific fecundity (f_{xj}) and age-specific fecundity (m_x) in treatments were lower than control data over most of the oviposition period. The only exception was treatment with 1.5 mg/l concentration, where f_{xj} and m_x values were mostly above control data (Figure 2d-e). Resultingly, age-specific maternity ($l_x m_x$) in all treatments was lower than it was in the control over the entire oviposition period. Treatment with 1.5 mg/l concentration remained an exception but only with several

Table 2 Life history traits (mean ± SE) of *Tetranychus urticae* treated with spirodiclofen (mg/l) at the beginning of the females' pre-oviposition period (fecundity-based life tables)

	Females								Males	
mg/l	Nf	Fecundity-I	Long-I	Nfr	Fecundity-II	Ovd	Long-II	Nfr/Nf	Nm	Long
96	234	15.28 a	6.59 a	140	25.54 a	5.19 a	8.31 a	0.60 a	170	5.21 a
		(± 1.61)	(± 0.31)		(± 2.34)	(± 0.37)	(± 0.40)	(± 0.03)		(± 0.21)
48	215	22.14 b	7.48 b	145	32.83 b	6.46 b	9.52 b	0.67 ab	169	5.24 a
		(± 1.91)	(± 0.33)		(± 2.37)	(± 0.34)	(± 0.37)	(± 0.03)		(± 0.19)
24	127	29.20 b	8.65 c	89	41.66 c	8.06 c	10.89 c	0.70 b	108	5.63 ab
		(± 3.11)	(± 0.48)		(± 3.72)	(± 0.53)	(± 0.52)	(± 0.04)		(± 0.28)
12	126	40.79 c	9.98 d	92	55.86 d	9.93 d	12.36 d	0.73 b	111	6.29 b
		(± 3.51)	(± 0.50)		(± 3.74)	(± 0.49)	(± 0.47)	(± 0.04)		(± 0.28)
6	76	50.46 d	11.59 e	70	54.79 d	9.60 d	12.33 cd	0.92 d	66	6.26 b
		(± 4.34)	(± 0.63)		(± 4.34)	(± 0.57)	(± 0.61)	(± 0.03)		(± 0.36)
3	75	49.95 d	11.37 e	64	58.53 de	10.78 de	12.88 d	0.85 cd	65	5.97 ab
		(± 4.06)	(± 0.62)		(± 3.86)	(± 0.51)	(± 0.54)	(± 0.04)		(± 0.35)
1.5	47	56.40 d	11.36 e	36	73.64 e	12.06 e	14.00 d	0.77 bc	40	6.65 b
		(± 7.08)	(± 1.01)		(± 7.00)	(± 0.93)	(± 0.93)	(± 0.06)		(± 0.52)
0.75	45	49.22 d	11.40 e	40	55.38 d	10.42 de	12.40 cd	0.89 cd	40	6.22 b
		(± 4.91)	(± 0.80)		(± 4.67)	(± 0.73)	(± 0.76)	(± 0.05)		(± 0.40)
0.375	48	47.96 d	11.48 e	41	56.15 d	10.73 de	12.71 cd	0.85 cd	38	5.71 ab
		(± 5.53)	(± 0.84)		(± 5.54)	(± 0.76)	(± 0.79)	(± 0.05)		(± 0.42)
0	35	57.51 d	12.86 e	32	62.91 de	11.66 de	13.81 d	0.91 cd	29	6.52 b
		(± 6.72)	(± 1.01)		(± 6.56)	(± 0.94)	(± 0.93)	(± 0.05)		(± 0.48)

Means in rows followed by different letters are significantly different (the paired bootstrap test at 5% significance level, B = 50,000)

Nf = total number of females; Nfr = number of reproductive females; Nfr/Nf = proportion of reproductive females;

Nm = number of males; Fecundity-I = eggs laid/Nf; Fecundity-II = eggs laid/Nfr; Long = adult longevity (days);

Ovd = number of oviposition days; Long-I = adult longevity of all females; Long-II = adult longevity of reproductive females

$l_x m_x$ values exceeding control data (Figure 2f). The age-stage-specific reproductive value (v_{xj}) was affected by spirodiclofen in a similar manner as fecundity, i.e., v_{xj} values of treated females were predominantly lower than control data over most of the oviposition period, the only exception being the concentration 1.5 mg/l, where the data exceeded the control (Figure 3).

Demographic parameters are detailed in Table 3. The net reproductive rate (R_0), intrinsic rate of increase (r) and finite rate of increase (λ) were significantly reduced by the three highest concentrations (by 49-72%, 20-34%, and 4-6%, respectively), compared to the control. In treatments with 96 mg/l concentration, these three demographic parameters were significantly lower also in comparison with treatments with concentrations of 48 mg/l and 24 mg/l. The gross reproductive rate (*GRR*) was significantly reduced by treatments with concentrations of 96 mg/l and 48 mg/l (by 39%, and 49%, respectively) compared to the control. As in the life history traits, the highest *GRR* value was found in treatment with 1.5 mg/l, but it was not significantly different from the control (Table 3).

The fertility-based life table gave a similar picture of spirodiclofen impact on the life-history traits and demographic parameters of *T. urticae* (Table 4, Table 5). The four highest concentrations caused statistically significant reduction in fertility-I (by 30 - 76%, compared to control values). These concentrations also significantly lowered the proportion of reproductive females (i.e., females that laid viable eggs), compared to the control. In these treatments, 30 - 62% of females produced no offspring at all as they were either sterilized or laid non-viable eggs. Regarding reproductive females only, fertility-II was significantly reduced by treatments with the three highest concentrations (by 26 - 41%, compared to the control). The highest value

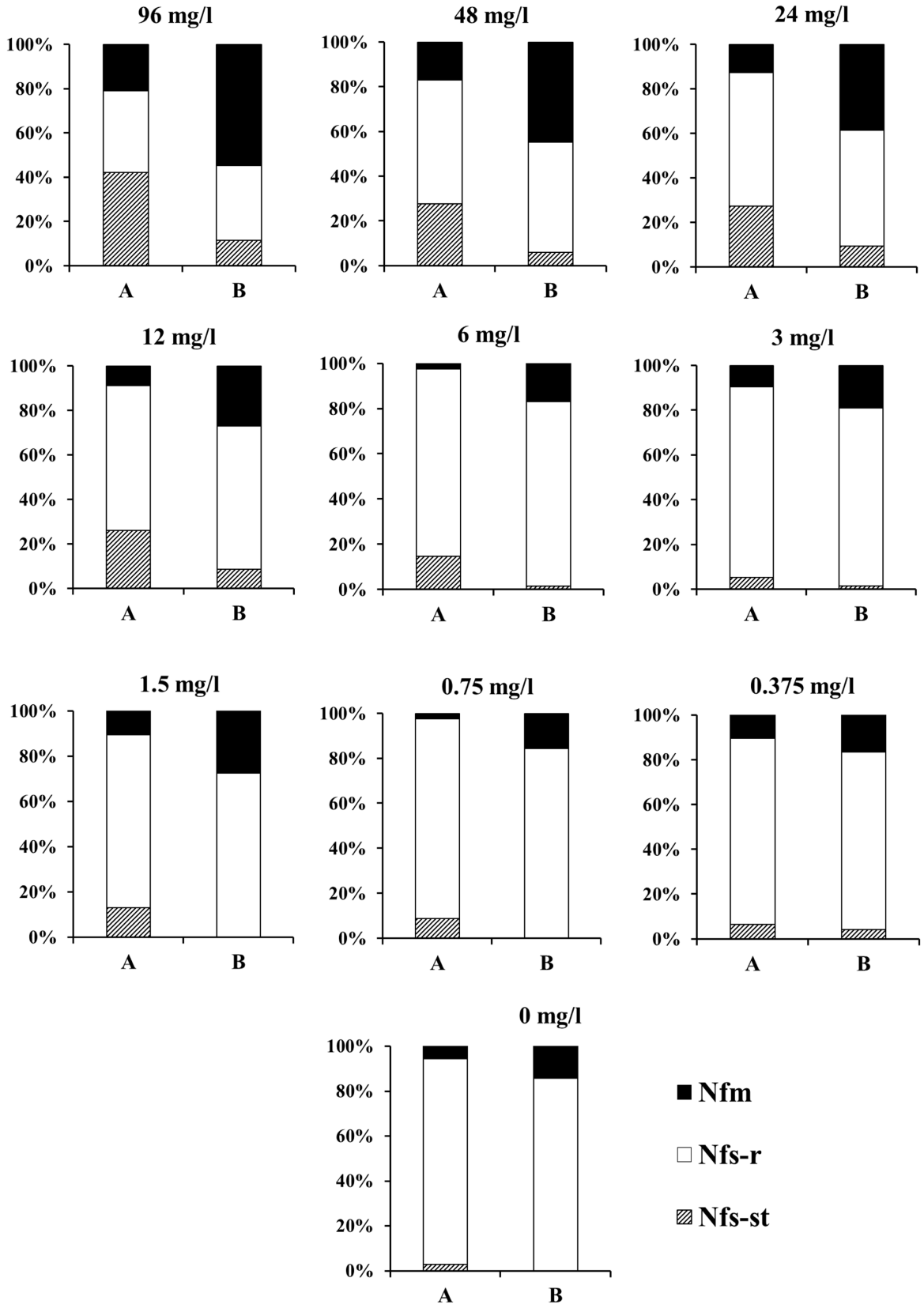

Figure 1 Survival and reproduction of *Tetranychus urticae* treated with spirodiclofen (mg/l) at the beginning of the females' pre-oviposition period; **Nfm** = dead females after 24-h exposure (A) and total number of dead females 96 h after treatment (B), **Nfs-r** = females that survived 24-h exposure and laid eggs on untreated surface at least 24 h after treatment (A) or 96 h after treatment (B) - reproductive females; **Nfs-st** = females that survived 24-h exposure and failed to lay eggs on untreated surface 24 h after treatment **(A)** or 96 h after treatment **(B)** - sterilized females.

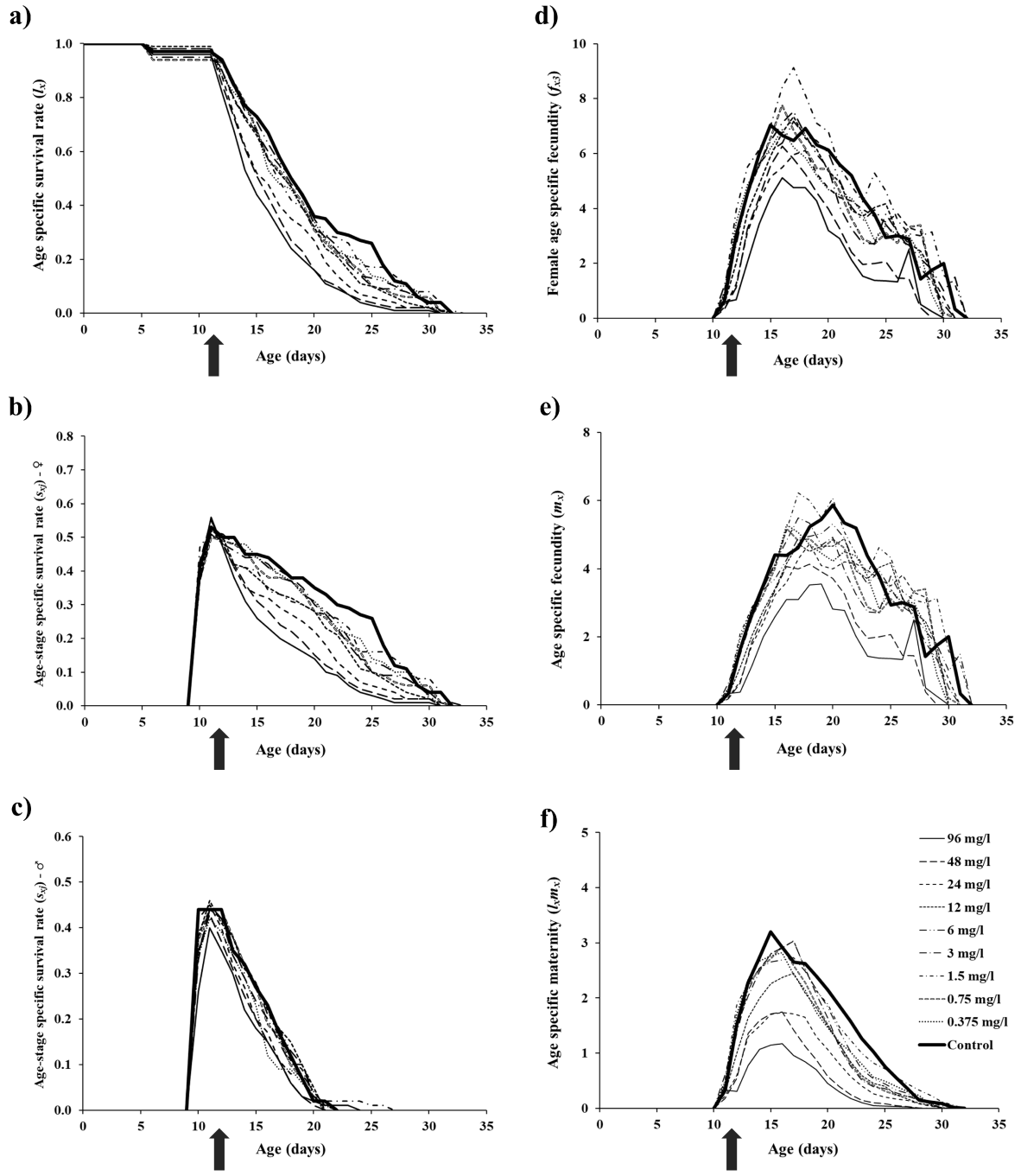

Figure 2 **a)** Age-specific survival rate (l_x), **b)** age-stage-specific survival rate (s_{xj}) - females, **c)** age-stage-specific survival rate (s_{xj}) – males, **d)** female age-specific fecundity (f_{xj}), **e)** age-specific fecundity (m_x) and **f)** age-specific maternity ($l_x m_x$) of *Tetranychus urticae* treated at the beginning of the females' pre-oviposition period with spirodiclofen (mg/l); arrow indicates the treatment (s_{xj} for egg and immature stages are not shown for better clarity).

of fertility-II was recorded in the treatment with 1.5 mg/l, but it was not significantly different from the control data (Table 4).

Compared to fecundity-I (Table 2), fertility-I was somewhat lower as maximum reduction was 11% in treatment with the highest concentration (Table 4). Fertility reduction was the

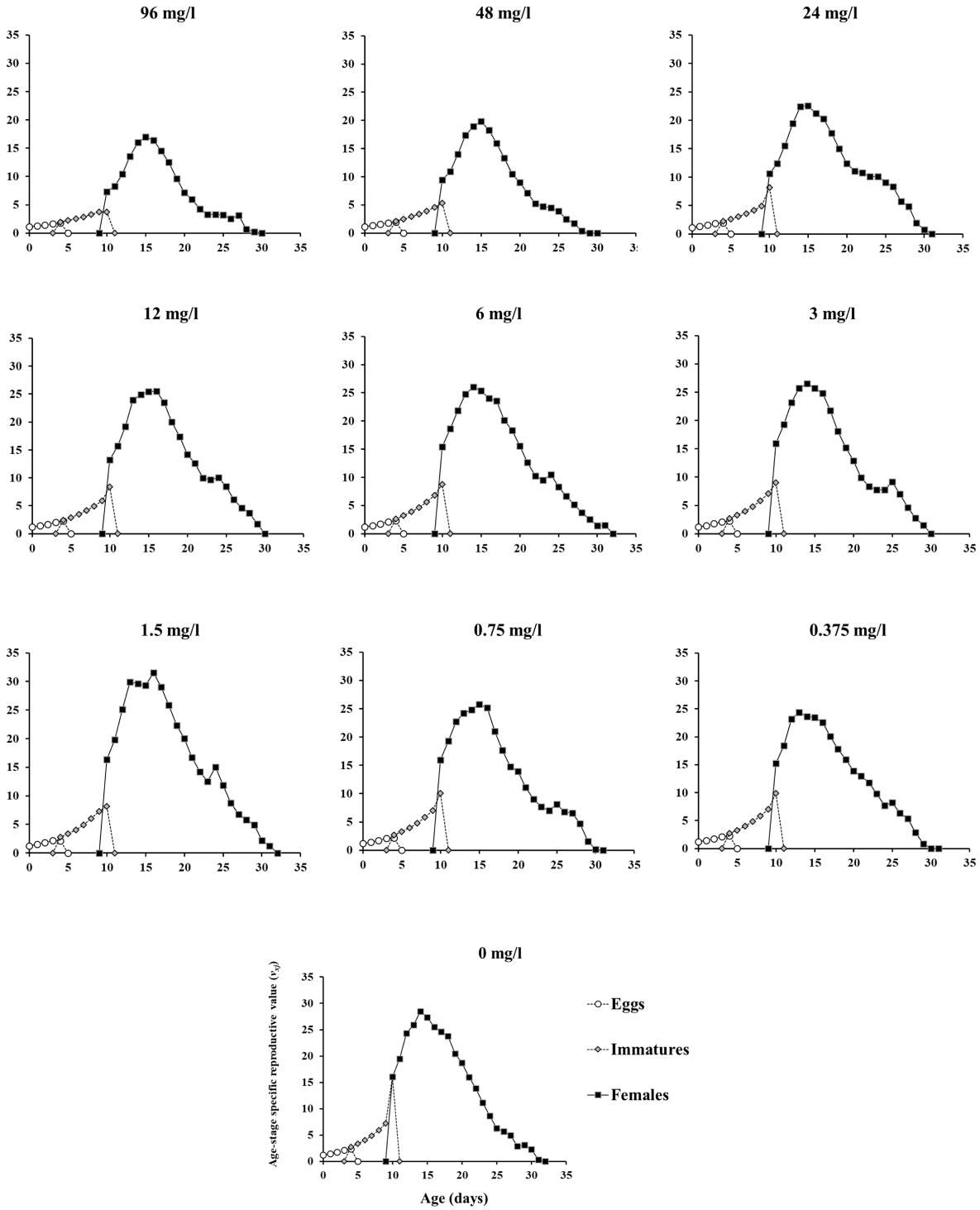

Figure 3 Age-stage-specific reproductive value (v_{xj}) of *Tetranychus urticae* treated at the beginning of the females' pre-oviposition period with spirodiclofen (mg/l).

consequence of two factors. The first factor was the residual toxic action of spirodiclofen on eggs laid on treated leaf discs during 24-h exposure. In treatments with concentrations of 3 - 96 mg/l, the percentage of unhatched eggs ranged between 17% and 98%, while it was less than

2% after treatments with concentrations of 0.375 – 1.5 mg/l and in the control. The second factor was transovarial toxic effect on eggs laid on untreated surface following the exposure, which was most evident in treatments with the three highest concentrations. However, this effect was short-lived: the percentage of unhatched eggs was 13- 32% on the first day, 8-20% on the second day, while it remained between 2% and 4% from the third day until the end of trial, which is similar to the other treatments and the control. Residual toxicity to eggs laid on treated discs was the primary contribution towards reduced proportion of reproductive females in treatments with concentrations of 12 – 96 mg/l. Resulting from the lower number of reproductive females (compared to fecundity-based life tables) fertility-II was higher than fecundity-II in those treatments.

Demographic parameters obtained from fertility-based life tables are detailed in Table 5. Spirodiclofen concentration that significantly reduced fertility-II (24 – 96 mg/l) also decreased r and λ significantly (by 23-40%, and 4-7%, respectively) compared to the control. The concentrations 96 mg/l and 48 mg/l significantly reduced *GRR* (by 50% and 40%, respectively) and R_0 (by 75% and 61%, respectively), compared to the control. The highest GRR value was recorded after treatment with 1.5 mg/l, but this value was not significantly different from the control (Table 5). All these parameters were lower than those acquired from fecundity-based life tables but the differences had no statistical significance (the paired bootstrap test at 5% significance level, B = 50,000).

Discussion

Our demographic study analyzed spirodiclofen effects on the survival, reproduction and population growth of the F_0 generation, considering that high reproduction of young fertilized

Table 3 Demographic parameters (mean ± SE) of *Tetranychus urticae* treated with spirodiclofen (mg/l) at the beginning of the females' pre-oviposition period (fecundity-based life tables).

mg/l	N	*GRR* (eggs laid)	R_0 (eggs laid/mite)	r ($days^{-1}$)	λ ($days^{-1}$)	T (days)
96	420	36.11 a	8.51 a	0.128 a	1.136 a	16.80 ab
		(± 5.99)	(± 0.97)	(± 0.007)	(± 0.007)	(± 0.17)
48	390	43.47 a	12.21 b	0.150 b	1.162 b	16.71 a
		(± 3.78)	(± 1.20)	(± 0.006)	(± 0.006)	(± 0.13)
24	240	61.06 b	15.45 b	0.157 b	1.170 b	17.44 c
		(± 6.36)	(± 1.89)	(±0.007)	(±0.008)	(± 0.19)
12	240	63.10 b	21.41 c	0.176 c	1.192 c	17.41 c
		(± 4.25)	(± 2.25)	(± 0.006)	(± 0.007)	(± 0.15)
6	147	67.89 bc	26.09 c	0.189 c	1.208 c	17.27 bc
		(± 7.35)	(± 3.07)	(± 0.006)	(± 0.008)	(± 0.19)
3	147	63.50 bc	25.40 c	0.191 c	1.210 c	16.99 ab
		(± 4.53)	(± 2.92)	(± 0.007)	(± 0.009)	(± 0.17)
1.5	90	79.67 c	29.46 c	0.195 c	1.215 c	17.36 bc
		(± 7.27)	(± 4.72)	(± 0.009)	(± 0.011)	(± 0.23)
0.75	90	64.31 bc	24.61 c	0.189 c	1.208 c	16.97 ab
		(± 5.32)	(± 3.56)	(±0.008)	(±0.010)	(± 0.21)
0.375	90	64.64 bc	25.58 c	0.191 c	1.210 c	17.01 abc
		(± 6.35)	(± 3.87)	(± 0.009)	(± 0.010)	(± 0.2)
0	66	71.01 bc	30.50 c	0.195 c	1.215 c	17.56 c
		(± 6.02)	(± 5.01)	(± 0.009)	(± 0.011)	(±0.20)

Means in rows followed by different letters are significantly different
(the paired bootstrap test at 5% significance level, B = 50,000)
N = sample size (number of eggs at the begining of the life table study); GRR = gross reproductive rate;
R_0 = net reproductive rate; r = intrinsic rate of increase; λ = finite rate of increase; T = generation time

females is crucial for the population biology of *T. urticae* as a colonizing species (Sabelis 1985; Li and Margolies 1993). The fecundity-based life table revealed that a significant reduction in population growth was mainly due to sterilization of females (i.e., their inability to lay eggs), followed by their high mortality, and additionally reduced fecundity and longevity of reproductive females. Sterilization was the most evident during the initial period of reproduction, which primarily determines the magnitude of *r* (Birch 1948; Snell 1978). Sterilization of *T. urticae* females by intoxication by spirodiclofen, accompanied by accumulation of eggs in the genital tract and body swelling, had been reported in previous studies (Nauen 2005; Marčić 2007; Van Pottelberge *et al.* 2009).

Demographic analyses of the effects of spirodiclofen on population growth of spider mites using the age-stage two-sex life table have been carried out in few studies so far. Regarding *T. urticae*, Saber *et al.* (2018) treated females at the LC_{25} level and observed significant reductions in R_0, r and λ of the offspring of treated females. Sani *et al.* (2019) found that treatement of females at the LC_{15} and LC_{35} levels reduced significantly the same parameters in their offspring. Considering that the effects were examined on the F_1 generation, these results cannot be compared to our study. Ahmed and Abdelwines (2021) also found significant reduction in population growth of the F_1 generation the citrus red mite, *Panonychus citri* McGregor, following of exposure of parental females to LC_{20}. Marčić (2007) analyzed the effects of spirodiclofen on population growth of *T. urticae* F_0 generation and found significant

Table 4 Life history traits (mean ± SE) of *Tetranychus urticae* females treated with spirodiclofen (mg/l) at the beginning of the females' pre-oviposition period (fertility-based life tables).

mg/l	Nf	Fertility-I	Nfr	Fertility-II	Nfr/Nf
96	234	13.62 a	88	36.22 a	0.38 a
		(± 1.51)		(± 2.62)	(± 0.03)
48	215	20.97 b	129	34.95 a	0.60 b
		(± 1.83)		(± 2.35)	(± 0.03)
24	127	27.31 b	76	45.63 b	0.60 b
		(± 3.00)		(± 3.74)	(± 0.04)
12	126	39.19 c	88	56.11 c	0.70 bc
		(± 3.39)		(± 3.59)	(± 0.04)
6	76	48.38 cd	70	52.53 bc	0.92 d
		(± 4.17)		(± 4.18)	(± 0.03)
3	75	48.43 cd	64	56.75 cd	0.85 cd
		(± 3.93)		(± 3.71)	(± 0.04)
1.5	47	54.87 d	36	71.64 d	0.77 cd
		(± 6.82)		(± 6.78)	(± 0.06)
0.75	45	48.38 cd	40	54.42 bc	0.89 cd
		(± 4.78)		(± 4.54)	(± 0.05)
0.375	48	46.67 cd	41	54.63 bc	0.85 cd
		(± 5.34)		(± 5.34)	(± 0.05)
0	35	56.29 d	32	61.56 cd	0.91 d
		(± 6.53)		(± 6.39)	(± 0.05)

Means in rows followed by different letters are significantly different
(the paired bootstrap test at 5% significance level, B = 50,000)
Nf = total number of females;
Nfr = number of reproductive females (i.e. females that laid viable eggs);
Nfr/Nf = proportion of reproductive females;
Fertility-I = eggs hatched/Nf ; Fertility-II = eggs hatched/Nfr

reduction in R_0, r and λ following treatment of pre-ovipositing females with 12 mg/l. However, a different type of life table - the female age-specific life table (Birch 1948; Carey 1993) - was used in the study. This type of life table ignores males and natural variations in juvenile developmental times among females (Chi *et al.* 2020).

Our analysis of spirodiclofen effects showed that concentrations that are as many as four times lower than the recommended field rate (i.e. leaving 4-fold thinner acaricide deposit on plant surface) may cause significant reduction in *T. urticae* population growth. This finding has practical value, considering that spray coverage in the field is spatially heterogeneous (Martini *et al.* 2012). On the other hand, the highest values of *GRR*, fecundity-II and fertility-II were noted after treatment with the concentration of 1.5 mg/l, which is 64-fold lower than the field rate. Such stimulated reproduction was caused by the concentration entering the hormetic zone, i.e. within a concentration range which is up to 10-fold lower than the highest concentration that has no significant effect (Guedes and Cutler 2014). However, *GRR,* fecundity-II and fertility-II in the treatment with 1.5 mg/l concentration were significantly higher, compared to the treatments with concentrations of 12 - 96 mg/l, but not compared to the control. For this reason, it is hard to regard it as hormesis, which is defined as a concentration-response relationship in which low concentrations cause stimulation, whereas high concentrations act inhibitory. Besides, the maximum magnitude of stimulation in hormetic response is mostly 30–60% greater than control magnitude (Guedes and Cutler 2014), while it was no more than 17% in this study. However, it is still possible that a bioassay with more concentrations within the hormetic zone would yield response that fit the hormetic model both in quantitative and qualitative terms.

The fertility-based life table did not change the general picture of spirodiclofen effects generated by the fecundity-based variant of life table. Short-lived and weak transovarial

Table 5 Demographic parameters (mean ± SE) of *Tetranychus urticae* treated with spirodiclofen (mg/l) at the beginning of the females' pre-oviposition period (fertility-based life tables).

mg/l	N	*GRR* (eggs hatched)	R_0 (eggs hatched/mite)	r ($days^{-1}$)	λ ($days^{-1}$)	T (days)
96	420	34.28 a	7.59 a	0.117 a	1.124 a	17.28 bcd
		(± 5.78)	(± 0.90)	(± 0.007)	(± 0.008)	(± 0.17)
48	390	41.76 a	11.56 b	0.145 b	1.156 b	16.86 a
		(± 3.61)	(± 1.14)	(± 0.006)	(± 0.006)	(± 0.13)
24	240	59.11 b	14.45 c	0.150 b	1.162 b	17.75 d
		(± 6.28)	(± 1.81)	(±0.007)	(±0.008)	(± 0.19)
12	240	61.29 b	20.58 c	0.173 c	1.189 c	17.50 cd
		(± 4.17)	(± 2.18)	(± 0.006)	(± 0.007)	(± 0.15)
6	147	65.53 bc	25.01 c	0.186 c	1.204 c	17.32 bcd
		(± 7.23)	(± 2.94)	(± 0.006)	(± 0.008)	(± 0.20)
3	147	62.12 bc	24.71 c	0.188 c	1.207 c	17.03 abc
		(± 4.42)	(± 2.83)	(± 0.007)	(± 0.008)	(± 0.17)
1.5	90	77.52 c	28.66 c	0.194 c	1.214 c	17.33 bcd
		(± 7.04)	(± 4.59)	(± 0.009)	(± 0.011)	(± 0.23)
0.75	90	63.28 bc	24.19 c	0.188 c	1.207 c	16.96 ab
		(± 5.21)	(± 3.51)	(±0.008)	(±0.010)	(± 0.21)
0.375	90	63.11 bc	24.89 c	0.189 c	1.208 c	17.01 abc
		(± 6.14)	(± 3.74)	(± 0.009)	(± 0.010)	(± 0.21)
0	66	69.30 bc	29.85 c	0.194 c	1.214 c	17.55 cd
		(± 5.85)	(± 4.89)	(± 0.009)	(± 0.011)	(±0.20)

Means in rows followed by different letters are significantly different
(the paired bootstrap test at 5% significance level, B = 50,000)
N = sample size (number of eggs at the begining of the life table study); GRR = gross reproductive rate;
R_0 = net reproductive rate; r = intrinsic rate of increase; λ = finite rate of increase; T = generation time

toxic effect (assumed to be caused by transovarial transfer of the uptaken acaricide from the maternal body to eggs) did not reduce significantly the fertility, nor consequently the population parameters, compared to the same parameters acquired in the fecundity-based life table. Short-lived reductions in *T. urticae* fertility had been also reveiled in other studies involving spirodiclofen (Marčić 2007; Van Pottelberge *et al.* 2009). Transovarial toxicity to eggs laid by treated *T. urticae* females was also noted for spiromesifen (Marčić *et al.* 2010), as well as acaricides that inhibit chitin biosynthesis, such as etoxazole (Sáenz-de-Cabezón Irigaray and Zalom 2012) and hexythiazox (Adesanya *et al.* 2018). In our previous study (Musa *et al.* 2022), hexythiazox was also applied when *T. urticae* females were at the pre-oviposition period. The fertility-based variant of the age-stage two-sex life table revealed a significant decrease in population parameters, mostly caused by the transovarial toxicity of hexythiazox to eggs laid by treated females. Fertility reduction during the initial few days of oviposition, although short-lived, was sufficient to cause a significant reduction in population growth. On the other hand, application of fecundity-based life tables underestimated the population-level effects of hexythiazox on *T. urticae* (Musa *et al.* 2022). Although more labour-intensive, application of the fertility-based variant of age-stage two-sex life table is necessary for evaluation of acaricides that may exhibit transovarial toxicity, such as those interfering with growth and development of mites.

Considering the biological profile of spirodiclofen toxicity to *T. urticae* life stages (Wachendorff *et al.* 2002; Nauen 2005), as well as our own data on its residual toxicity to eggs laid during exposure, concentrations that sterilized females in our trial or reduced their reproduction could be considered as highly toxic to eggs and immatures on treated surface. Therefore, the recovery potential of a population mainly depends on females reaching leaf surface free of or poorly covered by acaricide deposit. Although sterilization and reproductive reduction significantly weakened the recovery potential of these females, it still remained considerable. On the other hand, the fact that spirodiclofen concentrations below recommended rate reduce populations growth leaves a possibility for combining reduced rates of the acaricide with predatory mites within an integrated spider mite management program.

In conclusion, our demographic analysis showed that spirodiclofen significantly reduced population growth rates of *T. urticae* when it was applied against pre-ovipositing females in concentrations up to 4-fold lower than the recommended rate. Application of the fecundity-based variant of age-stage two-sex life table showed that this effect was mainly due to sterilization of treated females and their high mortality in the initial period of oviposition, in combination with reduced fecundity and longevity of reproductive females. The short-lived transovarial toxic effect observed in the fertility-based variant was not sufficient to cause a significant reduction in population parameters, compared to parameters acquired by the fecundity-based variant. Future research may focus on the evaluation of transgenerational population-level effects of spirodiclofen, applied in a wide range of concentrations.

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Author contribution

D.Marčić conceived the original idea, D.Marčić and I. Međo planned the experiments, A. Musa, I. Međo and I. Marić carried out the experiments, D. Marčić and I. Međo analysed the data, D. Marčić wrote the manuscript.

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