UNIVERSITY OF BELGRADE

FACULTY OF BIOLOGY

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# DEMOGRAPHIC ANALYSIS OF ACARICIDE EFFECTS ON THE TWO-SPOTTED SPIDER MITE, *TETRANYCHUS URTICAE* KOCH (ACARI: TETRANYCHIDAE)

Doctoral Dissertation

Belgrade, 2023

UNIVERZITET U BEOGRADU

BIOLOŠKI FAKULTET

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# DEMOGRAFSKA ANALIZA EFEKATA AKARICIDA NA OBIČNOG PAUČINARA, *TETRANYCHUS URTICAE* KOCH (ACARI: TETRANYCHIDAE)

Doktorska disertacija

Beograd, 2023

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## ACKNOWLEDGEMENT

This research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project TR31043; Grants No. 451-03-68/2022-14/200214, 451-03-47/2023-01/ 200214).

Experimental part of this doctoral dissertation was carried out in Laboratory of Applied Entomology of the Institute of Pesticides and Environmental Protection, Belgrade – Zemun.

I would like to thank the following people, without whom I would not have been able to complete this research:

- supervisor Dr. Dejan Marčić, Principal Research Fellow of the Institute of Pesticides and Environmental Protection, whose insight and knowledge into the subject matter steered me throughout this research,

- supervisor Dr. Željko Tomanović, Professor of the Faculty of Biology, University of Belgrade, for his encouragement and guidance throughout the duartion of research,

- Dr. Irena Međo, Research Associate of the Institute of Pesticides and Environmental Protection, who planned and organized experimental work, analyzed data, and supported me throughout this research,

- all members of the Laboratory of Applied Entomology, for their help and support

- I would like to express my profound gratitude to my husband for his support.

**Demographic analysis of acaricide effects on the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae)**

Abstract

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a polyphagous and cosmopolitan species that attacks a number of agricultural crops, especially in greenhouses. The effects of acaricides hexythiazox and spirodiclofen on life history traits and population parameters of the $F_0$ and $F_1$ generationss of *T. urticae*, following the treatment of $F_0$ eggs and pre-ovipositing females, were evaluated in laboratory bioassays using the age-stage two-sex life table (in fecundity- and fertility-based variants). Hexythiazox caused a short-lived transovarial toxic effect on the eggs laid by treated females (i.e. reduced their fertility), and this effect was decisive for significant reduction in the population growth rates in both generations. Spirodiclofen reduced the population growth rates 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. Its transovarial toxicity was not sufficient to cause a significant reduction in the population growth of the $F_0$ generation, but contributed to population growth reduction in the $F_1$ generation. Ovicidal action of the acaricides, following the egg treatment, had a decisive contribution to reduction in the population growth rates of the $F_0$ generation. In the $F_1$ generation, shortening of development time compensated fecundity and longevity reductions. Considering the range of concentrations of hexathiazox and spirodiclofen causing significant reduction in the population growth rates of *T. urticae*, it can be concluded that the impact of the former is stronger than that of the latter. These results are discussed in the context of population biology of *T. urticae* as well as its management.

**Key words**: *T. urticae*, acaricides, sublethal effects, life tables, spirodiclofen, hexythiazox

**Scientific field**: biology

**Scientific subfield**: entomology, acarology, plant protection

**Demografska analiza efekata akaricida na običnog paučinara, *Tetranychus urticae* Koch (Acari: Tetranychidae)**

Sažetak

Obični paučinar, *Tetranychus urticae* Koch (Acari: Tetranychidae), polifagna je i kosmopolitska vrsta grinje koja napada veliki broj poljoprivrednih kultura, posebno u staklenicima i plastenicima. Efekti akaricida heksitiazoksa i spirodiklofena na osobine životne istorije i populacione parametre $F_0$ i $F_1$ generacija *T. urticae*, nakon tretiranja $F_0$ jaja i pre-ovipozicionih ženki, ocenjivani su u laboratorijskim biotestovima primenom starosno-razvojnih tabela života oba pola (u varijantama zasnovanim na fekunditetu i fertilitetu). Heksitiazoks je uzrokovao kratkotrajni transovarijalni toksični efekat na jaja koja su polagale tretirane ženke (tj. redukovao je njihov fertilitet), i ovaj efekat je bio odlučujući za značajnu redukciju stopa populacionog rasta obe generacije. Spirodiclofen je redukovao stope populacionog rasta uglavnom zahvaljujući sterilizaciji tretiranih ženki i njihovoj visokoj smrtnosti u početnom periodu ovipozicije, u kombinaciji sa redukcijom fekunditeta i dužine života reproduktivnih ženki. Njegova transovarijalna toksičnost nije bila dovoljna za značajnu redukciju stopa populacionog rasta $F_0$ generacije, ali je doprinela redukciji populacionog rasta $F_1$ generation. Ovicidno delovanje oba akaricida, nakon direktnog tretiranja jaja, bilo je odlučujuće za redukciju populacionog rasta $F_0$ generation. Skraćenje vremena razvića u $F_1$ generaciji kompenzovalo je redukciju fekunditeta i dužine života. Uzimajući u obzir opseg koncentracija oba akaricida koje uzrokuju značajnu redukciju stopa populacionog rasta *T. urticae*, može se zaključiti da je učinak heksitiazoksa jači od učinka spirodiklofena. Dobijeni rezultati razmotreni su u kontekstu populacione biologije *T. urticae* kao i njenog suzbijanja.

**Ključne reči:** *T. urticae*, acaricidi subletalni efekti, tabele života, spirodiklofen, heksitiazoks

**Naučna oblast**: biologija

**Uža naučna oblast**: entomologija, akarologija, zaštita bilja

## Contents

# 1. INTRODUCTION

## 1.1. Spider mites as plant pests

Spider mites, classified into the family Tetranychidae, are members of the subclass Acari (mites and ticks), which belongs to the class Arachnida, the subphylum Chelicerata and the phylum Arthropoda (Tab. 1.1). After insects, Acari represent the second largest group of terrestrial animals. They are small animals: most mites are less than 1 mm in length, while some engorged adult female ticks may reach a length of 20 – 30 mm. Among over 60,000 named species of Acari there are a number of species of agricultural, veterinary and medical importance (Walter and Proctor 2013; Stork 2018).

The family Tetranychidae represent the largest group of plant-feeding mites, with 1345 valid species and more than 4000 host plant records (Migeon and Dorkeld 2023). In a recent survey carried out in Serbia a total of 45 spider mite species were recorded (Marić et al. 2018, 2021). It is the second highest number of spider mite species, after Greece with 56 known species, recorded in South-Eastern Europe.

**Table 1.1.** Classification of mites (Lindquist et al. 2009)

| Phylum | Arthropoda |
|---|---|
| Subphylum | Chelicerata |
| Classis | Arachnida |
| **Subclassis** | **Acari** |
| Superorder | Acariformes |
| Order | Trombidiformes |
| Suborder | Prostigmata |
| Superfamily | Tetranychoidea |
| Family | Tetranychidae |

Until the 1950s, spider mites were minor pests of agricultural crops, presumably due to biological regulation by their mite and insect predators. Advances in agricultural production after World War II (e.g. extensive use of synthetic pesticides and fertilizers, irrigation and other cultural practices) induced increase in spider mite populations far above economic thresholds. Grown under favorable conditions, host plants became high quality food sources for the mites, which gave rise to outbreaks of their populations. Moreover, populations of spider mite predators were destroyed by overuse of organochlorine, organophosphorous and carbamate insecticides, neuroactive compounds intended for the control of a wide ramge of insect pests. These compounds were also toxic to insect predators, as well as to predatory and pest mites. Besides destroying predators, heavy selection pressure by the insecticides caused emergence of spider mite populations resistant to these compounds (Van de Vrie et al. 1972; Hoy 2011).

Spider mites are obligate plant feeders, and their main feeding activity usually occurs on the leaves. They puncture the cells of the palisade tissue by cheliceral stylets and sucks up their content, which causes leaves to become necrotic and dry. Heavily infested plants may be covered with silk webbing produced by mites. Spider mite outbreaks may result in significant yield losses in a range of greenhouse and field crops. Spider mites are economically the most important mite plant pests. Among the members of the family Tetranychidae more than a hundred species are considered as pests of agricultural crops, ornamentals and other economic plants (Zhang 2003; Vacante 2016).

## 1.2. The twospotted spider mite as the most important mite pest

Among spider mites, the two-spotted spider mite, *Tetranychus urticae* Koch (Fig. 1.1 – 1.4), is of greatest economic importance. This spider mite is a highly polyphagous and cosmopolitan species that attacks more than 1100 host plant species, including a number of agricultural crops and ornamentals, especially in greenhouses where this mite is a major pest (Zhang 2003; Migeon and Dorkeld, 2023).

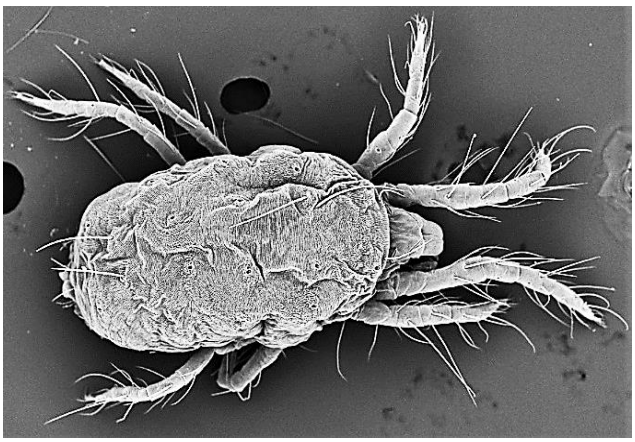

**Fig. 1.1.** SEM photo of *T. urticae* female (E. Palevsky)

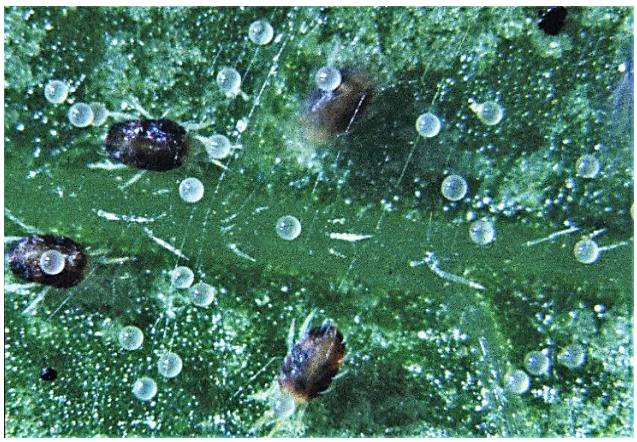

**Fig.1.2.**. *T. urticae* eggs and females (H. Tsolakis)

**Fig. 1.3.** *T. urticae* female (A. Migeon)

**Fig. 1.4**.. *T. urticae* male (A. Migeon)

The life cycle of *T. urticae* is short (developmental time is 7-10 days under optimal conditions) and it develops from the egg stage through the following four motile stages: larva, protonymph, deutonymph, and adult, with the quiescent stages between the motile stages called proto-, deuto- and teleiochrysalis. Two-spotted spider mites reproduce by arrhenotoky: females develop from fertilised eggs, whereas males develop from unfertilised eggs. Dispersal behavior is an important element in population biology of *T. urticae* as a colonizing species. The adaptive strategy of this species is based on high reproduction of young fertilized females as the main dispersers, so that natural populations are often close to a stable age distribution in which eggs, immatures, and adults account for around 66%, 26%, and 8%, respectively (Carey 1982; Sabelis 1985; Vaccante 2016).

## 1.3. Acaricides in spider mite control

Application of acaricides, a class of pesticide products toxic to plant-feeding mites, has been a predominating method of spider mite control. On the other hand, spider mites possess an exceptional intrinsic potential for rapid evolution of resistance, which has often been accelerated by misuse and overuse of acaricides. Therefore, the resistance of *T. urticae* and other spider mites to acaricides has become a global phenomenon (Van Leeuwen et al. 2009; Marčić 2022). The Arthropod Pesticide Resistance Database (APRD), a globally important source of data on the phenomenon of arthropod (insect and mite) resistance to pesticides, currently has as many as 983 resistance reports for 19 tetranychid species, of which 602 reports concerned *T. urticae*. On the list of top 10 resistant arthropod pest species *T. urticae* is ranked sixth (Mota-Sanchez and Wise 2023)

**Table 1.2.** Classification of acaricides on the basis of their modes of action (*NA* = Neuroactive compounds; *IRE* = Inhibitors of respiration; *IGD* = inhibitors of growth and development; *UN* = acaricides with unknown or uncertain modes of action)

| **Group names** (according to IRAC 2022) | **Chemical classes** | **Compounds** |
|---|---|---|
| ***NA*** | | |
| AChE inhibitors | Organophosphates | Chlorpyrifos* |
| | Carbamates | Formetanate* |
| GABA-Cl blocators | Cyclodienes | Endosulfan* |
| GABA-Cl allosteric modulators | Izoxazolines | Fluxametamide, isocycloseram |
| | Meta-diamides | Broflanilide |
| Glu-Cl allosteric modulators | Avermectins | Abamectin |
| | Milbemycins | Milbemectin |
| VG-Na modulators | Pyrethroids | Acrinathrin* |
| KCa2 modulators | Trifluoromethylpyridines | Acynonapyr |
| OAR agonists | Formaidines | Amitraz |
| ***IRE*** | | |
| MC I electron transport inhibitors | Quinazolines | Fenazaquin |
| | Pyrazole-oximes | Fenpyroximate |
| | Pyrazole-carboxamides | Tebufenpyrad |
| | Pyridazinones | Pyridaben |
| | Pyrimidin-amines | Pyrimidifen |
| MC II electron transport inhibitors | Beta-ketonitriles | Cyenopyrafen, cyflumetofen |
| | Carboxanilides | Pyflubumide |
| MC III electron transport inhibitors ($Q_0$ site) | Hydroxynaphthoquinones | Acequinocyl |
| | Carbazates | Bifenazate |
| | Methoxyacrylates | Fluacrypyrim |
| MC III electron transport inhibitors ($Q_i$ site) | Quinolines | Flometoquin |
| ATP inhibitors | Thioureas | Diafenthiuron |
| | Organotins | Cyhexatin, fenbutatin oxide |
| | Sulfite esters | Propargite |
| | Diphenyl sulfones | Tetradifon |
| Uncouplers of oxidative phosphorylation | Pyroles | Chlorfenapyr |
| ***IGD*** | | |
| Mite growth inhibitors affecting CHS1** | Thiazolidinones | Hexythiazox |
| | Tetrazines | Clofentezine, diflovidazine |
| | Oxazolines | Etoxazole |
| Inhibitors of chitin biosynthesis affecting CHS1** | Benzoylureas | Flucycloxuron* |
| Inhibitors of chitin biosynthesis (type 1)** | Thiadiazinones | Buprofezin |
| ACC inhibitors | Tetronic acid derivatives | Spirodiclofen, spiromesifen |
| | Tetramic acid derivatives | Spirotetramat |

* representatives of the chemical class ** CHS1 inhibitors (Douris et al. 2016)

**Primary targets**: **AChE** = acetylcholin esterase; **GABA-Cl** = GABA(γ-aminobutyric acid)-gated chlorid channels; **Glu-Cl** = glutamate-gated chlorid channels; **VG-Na** = voltage-gated sodium channels; **KCa2** = calcium-activated potassium channels; **OAR** = oktopamine receptors; **MC I** = mitochondrial complex I; **MC II** = mitochondrial complex II; **MC III** = mitochondrial complex III; **ATP** = mitochondrial ATP synthase; **CHS1** = chitin synthase 1; **ACC** = acetyl-coenzyme A carboxylase

Since the initial reports on resistant populations during the 1950s, chemical industry has sought to solve the resistance problems by developing and introducing acaricides with novel modes of toxic action against spider mites. In arthropod toxicology, mode of action is defined as specific binding of an acaricide to its primary target (enzyme, receptor, ion channel etc.) within the mite body. Several generations of structurally diverse acaricides, directed against various biochemical and physiological targets, have been launched on global market (Van Leeuwen et al. 2015; Marčić 2022).

Nowadays, acaricides are divided into 15 groups of compounds with known modes of action, which fall into three categories: neuroactive compounds, inhibitors of respiration, and inhibitors of growth and development (Tab. 1.2). An additional category comprises acaricides with unknown, uncertain and non-specific modes of action such as triterpenoid azadirachtin, essential oils and their components (monoterpenes), fatty acid monoesters, alcaloids matrine and oxymatrine, various bacterial and fungal agents. As a category comprising the highest number of compounds (and most of them are insecto-acaricides i.e. intended for the control of insects and mites) neuroactive acaricides hold more than 70% of global acaricide market. On the other hand, the most widely sold acaricidal compound in the world is spirodiclofen, a tetronic acid derivative and a growth and development inhibitor (Sparks et al. 2020).

Besides the continued development of new acaricides with novel modes of action, there is also a need for optimization of their use in order to delay the evolution of resistance and prolong their life span. An effective acaricide resistance management program should follow general resistance management principles endorsed by the expert organization IRAC (Sparks and Nauen 2015). The key recommendation is reduction of the selection pressure for resistance by alternations. sequences, rotations and mixtures of compounds with different modes of action. This approach is compatible with integration of chemical, biological, cultural and other control tactics in IPM (Integrated Pest Management) programs in which acaricides are considered as the last resort i.e. if other measures are not sufficient in keeping the pest population below the economic threshold (Hoy 1998; Marčić 2012; Adesanya et al. 2021).

## 1.4. Acaricide effects and their evaluation

Knowing the biological profile of any pesticide (acaricide), which is based on laboratory bioassays, is a crucial precondition for its long-term sustainable use in multitactic IPM programs. Laboratory acute toxicity (concentration-mortality) bioassays are designed to assess lethal effects of pesticides on arthropods. The response of test organisms that either die or survive acute exposure to a pesticide applied in a series of doses/concentrations is known as the binary response, while the median lethal dose/concentration ($LD/LC_{50}$ i.e. dose/concentration expected to cause 50% mortality) is a parameter most frequently used for quantifying it (Finney 1971; Robertson et al. 2017). This parameter, however, offers no reliable estimate of the totality of effects of any pesticide in practice because pesticides may cause a variety of effects on surviving individuals (Robertson and Worner 1990; Stark and Banks 2003).

The amount of a pesticide (concentration) recommended for control of phytophagous insects and mites is determined having in mind the goal that even the most tolerant individual in a population should absorb a sufficient amount of pesticide (dose) to cause its death (lethal dose) i.e. to eliminate the entire population. In practice, however, pesticide 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 (i.e. sublethal) doses of pesticides. In other words, spatial and temporal heterogeneity of spray coverage in the field enables population recovery on poorly covered or uncovered plant surfaces, which depends on the reproductive potentials of females that survived or avoided exposure.

Considering the population level, pesticide concentrations range from those causing total elimination of a population, across a range of concentrations enabling the survival of a part of population (concentrations sublethal to some individuals), to concentrations causing no mortality (concentrations that are sublethal to all individuals in a population). Exposure to concentrations that are sublethal to a part of population or entire population is defined as sublethal exposure. Sublethal exposure results in ***sublethal effects*** that are described as changes in life-history traits of the surviving individuals i.e. their developmental rate, longevity, fecundity, etc. Those effects are manifested at population level as well, depending on the changing life-history traits, as well as in the next generations i.e. as transgenerational effects (Guedes et al. 2016).

To assess the sublethal effects of pesticides, bioassays are conducted to quantify changes in life-history traits and their integration at an ecologically more relevant population level (Guedes et al. 2016). One of widely accepted methods for assessing the response of arthropod (insect and mite) populations to various factors, including exposure to pesticides, is construction of ***life tables***.

## 1.5. Life tables: theory and application in acarology and acaricide toxicology

Life tables describe life history of populations. The life table approach was introduced in entomology by Birch (1948) who used the rice weevil, *Calandra oryzae* L. (Coleoptera: Curculionidae) as an example. Since then, life tables have been discussed in a number of books (e.g. Carey 1993), and a lot of scientific papers regarding insect and mite life tables have been published.

According to Birch (1948) and Carey (1993) demographic parameters are calculated from ***the female age-specific life table***, using two age-specific functions: ***age-specific survival,*** **($l_x$)**, the proportion of living females in the age interval *x*, and ***age-specific fertility*** **($m_x$)**, the number of female offspring per female in the age interval *x* (*x* is the age of females in days).

***The gross reproductive rate*** (*GRR*), which represents the mean number of female offspring that a female can produce during its life span, is calculated as:

$$GRR = \sum_{x=0}^{\infty} m_x \qquad (1)$$

***The net reproductive rate ($R_0$)***, which is the mean net number of female offspring that a female can produce during its life span i.e. a multiplication factor of a population in one generation or generation growth rate, is calculated as:

$$R_0 = \sum_{x=0}^{\infty} l_x m_x \qquad (2)$$

***The intrinsic rate of increase*** **(*r*)**, a basic demographic parameter for a population, is defined as the rate of natural increase in a closed population with constant age-specific survival and fertility schedules and a stable age distribution (the unit for *r* is usually day$^{-1}$). This growth rate indicates the potential of a population to use existing environmental conditions to its advantage, and it is primarily determined by the juvenile developmental rate and offspring production. It is estimated using the iterative bisection method from the Euler-Lotka equation:

$$\sum_{x=0}^{\infty} e^{-r\,(x+1)}\, l_x m_x = 1 \qquad (3)$$

with age indexed from day 0 (Goodman 1982).

***The finite rate of increase (λ)***, which is a multiplication factor of a population at each time unit i.e. daily growth rate (the unit for $\lambda$ is the same as for $r$), is calculated by using equation:

$$\lambda = e^{r} \tag{4}$$

***The mean generation time (T)***, defined as the length of time (in days) that is required for a population to increase $R_0$-fold of its size, is calculated as:

$$T = \frac{\ln R_0}{r} \tag{5}$$

Application of the female age-specific life table was criticized by Chi (1988), primarily for disregarding natural variation in juvenile developmental times among females in cohorts, but also for excluding males from the statistics, which result in errors in data analysis and interpretation.

A new approach was proposed (Chi 1988; Huang and Chi 2012; Chi et al. 2020) based on constructing of ***the age-stage two-sex life table*** from data for both sexes (females and males) and on including the variability of juvenile development into a theoretical model These data include the duration of the egg stage, immature stages, adult (female and male) stage in days, and the number of eggs laid daily.

Using these data, ***the age-specific survival rate ($l_x$),*** defined as the probability that a newly laid egg survives from birth to age $x$ and stage $j$, is calculated as:

$$l_{\mathrm{x}} = \sum_{j=1}^{k} s_{xj} \tag{6}$$

where $x$ = age, $j$ = stage, $k$ = the number of stages, and $s_{xj}$ = the age-stage specific survival rate i.e. the probability that a newborn egg will survive to age $x$ and stage $j$.

***The age-specific fecundity ($m_x$)***, which is the mean fecundity of all individuals of age $x$, is calculated as:

$$m_x = \frac{\sum_{j=1}^{k} s_{xj} f_{xj}}{\sum_{j=1}^{k} s_{xj}} \tag{7}$$

where $x$ = age, $j$ = stage, $k$ = the number of stages, $s_{xj}$ = the age-stage specific survival rate, and $f_{xj}$ = the age-stage-specific fecundity (the mean fecundity of individuals of age $x$ and stage $j$ i.e. the female age-stage-specific fecundity).

Weighting fecundity by survivorship [$l_x \times m_x$] gives ***the age-specific net maternity***. Using these age-specific functions (Fig. 1.5a,b) the above mentioned and defined demographic parameters (1 - 5) are calculated.

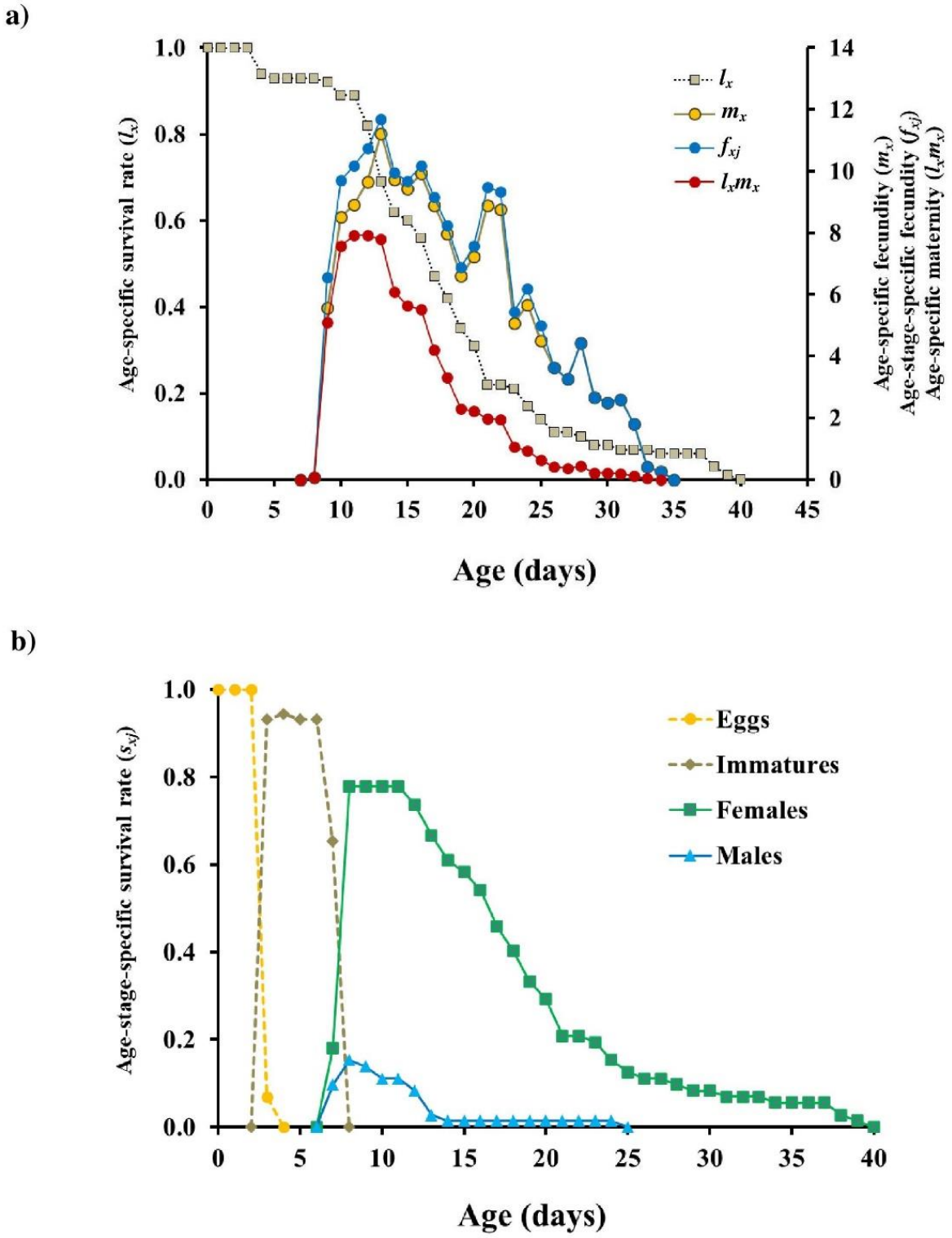


**Fig. 1.5.** Age-specific functions of a *T. urticae* population (adapted from: Musa et al. 2017): a) age-specific survival rate ($l_x$), age-specific fecundity ($m_x$), age-stage-specific (female) fecundity ($f_{xj}$), age-specific maternity ($l_xm_x$); b) age-stage-specifc survival rate ($s_{xj}$)

In addition, this type of demographic analyses usually include two more parameters (6, 7). ***The age-stage life expectancy ($e_{xj}$)*** is defined as expected survival time (in days) of an individual of age *x* and stage *j*:

$$e_{xj} = \sum_{i=x}^{\infty} \sum_{y=j}^{k} s'_{iy} \tag{6}$$

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

***The reproductive value ($v_{xj}$)*** is the contribution of an individual of age *x* and stage *j* to future population:

$$v_{xj} = \frac{e^{r(x+1)}}{s_{xj}} \sum_{i=x}^{\infty} e^{-r(i+1)} \sum_{y=j}^{k} s'_{iy} f_{iy} \tag{7}$$

where where *x* = age, *j* = stage, *k* = the number of stages, $s_{xj}$ = the age-stage specific survival rate, $s'_{iy}$ = the probability that an individual of age *x* and stage *j* will survive to age *i* and stage *y*, and $f_{iy}$ = the probability that an individual of age *x* and stage *j* will reproduce to age *i* and stage *y*. The unit for $v_{xj}$ is $day^{-1}$.

Besides, the age-stage two-sex life table theory (Chi 1988; Chi et al. 2020) provides several important life history traits, such as stage developmental time (in days), preadult survival rate (number of adults/initial number of eggs), stable age-stage distribution (proportion of individuals in age *x* and stage *j*), adult preovipositional period (APOP, the time period from the emergence of the adult female to its initial oviposition; in days), total preovipositional period (TPOP, the total period from the female's birth to its initial oviposition; in days), adult longevity (in days), oviposition days (the number of days in which females laid eggs), fecundity (the mean number of eggs laid per female). The relationship among the mean fecundity (F) and $R_0$ is always $R_0 = F \times N_f/N$, when $N_f$ is the mean number of female adults, and N is the total number of individuals.

The age-stage two-sex life table has received a growing acceptance in demographic analysis of effects of various factors on population growth of spider mites, such as host plant (Yin et al. 2013; Maleknia et al. 2016; Savi et al. 2019) and temperature (Bayu et al. 2017; Ullah et al. 2020). This type of life tables has gained preference over the application of the female age-specific life table in demographic analysis of acaricide effects on *T. urticae* (Tab. 1.3) and other spider mite species (Zanardi et al. 2018; Ahmed and Abdelwines 2021; Rimy et al. 2021).

Demographic analysis using the age-stage two-sex life table of *T. urticae* has usually been conducted in bioassays in which effects of 1-3 acaricide concentrations (mostly < $LC_{50}$) were evaluated on offspring of treated females ($F_1$ generation). However, this type of experimental design doesn't give a whole picture of acaricide effects. Testing a wide range of concentrations (including those sublethal to all individuals in a population) is needed because of possible *hormetic effect* (*hormesis*). It is defined as the concentration-response relationship in which low concentrations cause stimulation (e.g. increased fecundity, higher population growth rates), while high concentrations act inhibitory (Guedes and Cutler 2014).

In the context of the stable age distribution of *T. urticae*, it is very important to evaluate the effects occurring after treatment of eggs as the dominant stage. In addition to the egg stage, preovipositing female adults should be included in the analysis, considering that high reproduction of young, fertilized females is crucial in population biology of *T. urticae* as a colonizing species (Sabelis 1985; Li and Margolies 1993).

**Table 1.3.** Examples of demographic analysis of acaricide effects on *T. urticae* based on the age-stage two-sex life table (*NA* = Neuroactive compounds; *IRE* = Inhibitors of respiration; *IGD* = inhibitors of growth and development; *UN* = acaricides with unknown or uncertain modes of action

| Acaricides | Modes of action | Bioassays | References |
|---|---|---|---|
| ***NA*** | | | |
| Bifenthrin | VG-Na modulator | E - $LC_{10, 25}$ - $F_0$ | 11 |
| Abamectin | Glu-Cl allosteric modulator | Fe - $LC_{25}$ - $F_1$ | 10 |
| ***IRE*** | | | |
| Fenazaquin | MC I electron transport inhibitor | Fe - $LC_{5, 10, 20}$ - $F_1$ | 1 |
| Pyridaben | MC I electron transport inhibitor | Fe - $LC_{25}$ - $F_1$ | 10 |
| Bifenazate | MC III electron transport inhibitor ($Q_0$ site) | Fe - $LC_{10, 20}$ - $F_1$ | 6 |
| Chlorfenapyr | Uncoupler of oxidative phosphorylation | Fe - $LC_{10, 20, 30}$ - $F_1$ | 2 |
| ***IGD*** | | | |
| Diflovidazin | Mite growth inhibitor affecting CHS1 | Fe - $LC_{10, 20, 30}$ - $F_1$ | 4 |
| Hexythiazox | Mite growth inhibitor affecting CHS1 | Fe - $LC_{10, 20, 30}$ - $F_1$ | 5 |
| Spirodiclofen | ACC inhibitor | Fe - $LC_{25}$ - $F_1$ | 10 |
| | | Fe - $LC_{5, 15, 35}$ - $F_1$ | 9 |
| Spiromesifen | ACC inhibitor | Fe - $LC_{20, 25, 35}$ - $F_1$ | 3 |
| | | Fe - $LC_{20}$ - $F_1$ | 8 |
| ***UN*** | | | |
| Monoterpenes (α-terpinene, *p*-cymene, limonene) | - | E - $LC_{50}$ - $F_0$ | 7 |

1 = Alinejad et al. (2015); 2 - 3 = Bozhgani et al. (2018; 2019); 4 -5 = Havasi et al. (2018; 2021); 6 = Li et al. (2017); 7 = Musa et al. (2017); 8 = Rajaee et al. (2022); 9 = Sani et al. (2019); 10 = Saber et al. (2018); 11 = Wang et al. (2014)

VG-Na = voltage-gated sodium channels; Glu-Cl = glutamate-gated chlorid channels; MC I = mitochondrial complex I; MC III = mitochondrial complex III; CHS1 = chitin synthase 1; ACC = acetyl-coenzyme A carboxylase

E = egg treatment; Fe = female treatment; $F_0$ = assessment on $F_0$ generation; $F_1$ = assessment on $F_1$ generation

On the other hand, mode of action of some acaricides should be taken into account when using the age-stage two-sex life table. Mite growth inhibitors (MGI) affecting CHS1 (clofentezine, diflovidazin, etoxazole, hexythiazox) have excellent toxic activity on eggs and immatures of spider mites but they are considered non-toxic to adults. It was found, however, that females of *T. urticae* and other spider mites treated with these acaricides laid eggs that did not hatch i.e. their fertility was reduced. This toxic effect, assumed to be caused by transovarial transfer of the uptaken acaricide from the maternal body to eggs, was most evident on eggs laid over the initial days of oviposition (Chapman and Marris 1986; Yamada et al. 1987; Pap et al. 1996; Sáenz-de-Cabezón Irigaray and Zalom 2012; Adesanya et al. 2018). Spirodiclofen and spiromesifen, ACC inhibitors, are also highly toxic to the egg and immature stages, while the symptomology of poisoning observed in adult females is unique. Female bodies grow to unusual size due to egg accumulation in their genital tract, and it take most of females several days to die (Nauen 2005; Van Pottelberge et al. 2009). Their fertility is also affected due to a relatively short-lived transovarial toxic effect (Marčić 2007; Van Pottelberge et al. 2009; Marčić et al. 2010). Although short-lived, however, transovarial toxic effect may have a significant impact taking into account the demographic rule that initial reproduction primarily determines the population growth rate of *r*-selected species (Birch 1948; Snell 1978). Considering these facts, it is obvious that application of the age-stage two-sex life table, based on fecundity data, may underestimate population-level effects of acaricides acting against growth and development targets.

# 2. RESEARCH AIMS

Taking into account that acaricides are and will be an important element of management of *T. urticae* populations, as well as that knowledge of population-level effects of acaricide exposure is essential for any assessment of its overall impact in practice, the program of this study will include a series of laboratory bioassays focusing on the following aims:

1) to assess the effects of sublethal exposure of *T. urticae* to acaricides acting against growth and development (hexythiazox, spirodiclofen) on life-history traits and population parameters after the treatment of the selected stages (eggs, preovipositional females) using the age-stage two-sex life table.

2) to assess the transgenerational effects of sublethal exposure of *T. urticae* to the acaricides using the age-stage two-sex life table.

3) to evaluate application of the age-stage two-sex life table in demographic analysis of the acaricide effects on *T. urticae* with the emphasis on transovarial toxicity.

4) to evaluate results obtained in demographic analysis in the context of pest management, taking into account population biology of *T. urticae*.

# 3. MATERIALS AND METHODS

## 3.1. 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 at 25-30 ºC and 16/8 h light/dark photoperiod (Fig. 3.1) in the Laboratory of Applied Entomology of the Institute of Pesticides and Environmental Protection, Belgrade, Serbia, since March 2004.

## 3.2. Bioassays

All bioassays were carried out on primary bean leaves or leaf discs (30 mm diameter) positioned upon moistened cotton wads in Petri dishes with the abaxial surface upward (Fig. 3.2). The leaves/leaf discs were sprayed with the acaricide diluted in distilled water using an air pressure sprayer (1 ml of liquid, 100 kPa air pressure, aqueous deposit 2.2 ± 0.2 mg/cm$^2$) (Fig. 3.3). Controls were sprayed with distilled water only. The Petri dishes were kept in a Binder KBWF 720 and KBWF 240 plant growth chambers at 26 ± 1 ºC, under 60 ± 5% RH and 16/8 h light/dark photoperiod (Fig.3.4).

**Fig. 3.1.** Laboratory population of *T. urticae* reared on bean plants

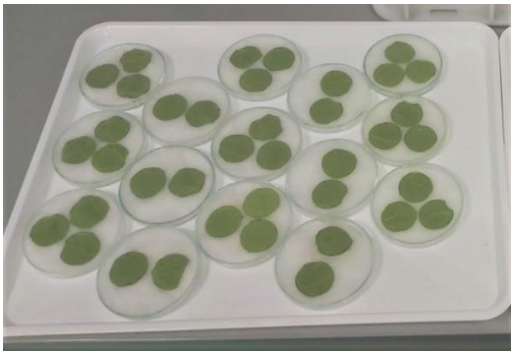

**Fig. 3.2.** Petri dishes with bean leaf discs


**Fig. 3.3.** Air pressure sprayer

**Fig. 3.4.** Petri dishes in the Binder plant growth chamber

### 3.2.1. Acaricides

The following commercial acaricide products were purchased from the companies' representatives in Serbia: a) "Hexythiazox 250 SC" (manufactured by Nisso Chemical Europe GmbH, Germany), a suspension concentrate containing 250 g/l a.i. hexythiazox, a thiazolidinone derivative (Fig. 3.5); b) "Envidor" (manufactured by Bayer AG, Germany), a suspension concentrate containing 240 g/l spirodiclofen, a tetronic acid derivative (Fig. 3.6).

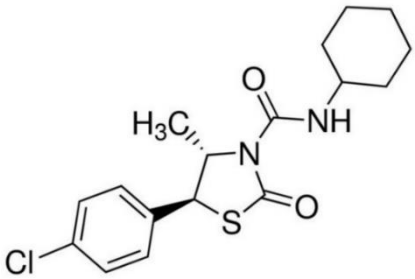


**Fig. 3.5.** Structural formula of hexythiazox

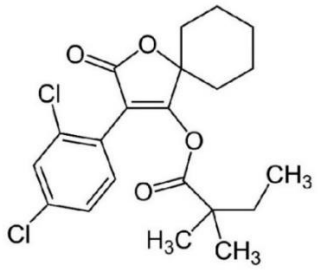


**Fig. 3.6.** Structural formula of spirodiclofen

### 3.2.2. Acute toxicity bioassays

The acute toxicity to eggs of *T. urticae* (ovicidal activity) was assessed in acute toxicity bioassays carried out in five replications by spraying serially diluted concentrations of the acaricides covering a range of 10-90% mortality. The concentrations were chosen after preliminary tests. The bioassays consisted of spraying primary bean leaves that carried 30-50 eggs (24 h old) per leaf. Mortality was assessed based on the number of mites that hatched from the treated eggs and reached the adult stage.

### 3.2.3. Life table bioassays

To assess the effects of acaricides on life history traits and demographic parameters of *T. urticae*, life tables were constructed for 9-10 cohorts (depending on the number of acaricide concecntrations). The cohorts were formed by placing a single adult female (2-3 days old) on each leaf disc and removing it after 24 h along with all eggs laid, except one. The initial number of eggs in cohorts was 60-1000, depending on expected survivorship and reproduction. Development and survival of mites that hatched from those eggs were observed daily. Newly emerged preovipositional adult females were transferred individually to new leaf discs and paired with adult males. Because more females than males emerged, the missing number of males was transferred from the mass-rearing population. The pairs were kept together and their survival (both sexes) and the number of eggs laid were recorded daily until all individuals in a cohort were dead.

***Egg treatment bioassays.*** The eggs (0-24 h old) were treated with a series of concentrations of the acaricide. The three highest concentrations were within 95% confidence limits of the $LC_{10}$, $LC_{50}$ and $LC_{90}$ estimates from the acute toxicity bioassays, while the others were sublethal. The mites that hatched from those eggs completed their juvenile development on the same treated leaf surfaces.

***Female treatment bioassays***. The acaricides were applied in a series of concentrations, starting with a field-relevant rate. In the bioassay with hexythiazox, nine cohorts of paired mites were sprayed when females were in the preovipositional period or in the first day of oviposition. The acaricide was applied in a series of eight concentrations, starting with 50 mg/l. In the bioassay with spirodiclofen, 10 cohorts of paired mites were sprayed when females were in the preovipositional period. The acaricide was applied in a series of nine concentrations, starting with 96 mg/l.

After 24-h exposure, surviving mites were transferred to untreated leaf discs. The discs with laid eggs were kept for another seven days, 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/female, and a fertility-based life table, using the number of viable (hatched) eggs/female. Fecundity and fertility were calculated in two ways: related to the total number of females in cohort (fecundity-I, fertility–I), and related to the number of females alive 24 h after treatment or the number of reproductive females (fecundity-II, fertility-II). Egg viability (%) was calculated as (fertility-II/fecundity-II) × 100, from the total number of eggs laid after the treatment, as well as from the number of eggs laid over the first four days after the treatment (when around 50% of all eggs were laid). 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.

***The effects on $F_1$ generation*** were assessed on offspring of $F_0$ generation, treated at the egg stage or at the adult stage (1- 2 days old females and males). The offspring eggs were collected from the first or the second day of oviposition. As in the $F_0$ generation bioassay, the development and survival of hatched individuals were observed daily until adulthood, preovipositional adult females were paired with adult males, and their survival and the number of eggs laid were recorded until the death of all individuals.

### 3.2.4. Data analysis

***Acute toxicity bioassays.*** Concentration-mortality data were subjected to probit analysis that relies on linear regression following probit transformation of percent mortalities and logatithmic transformation of concentration data. This type of analysis provides the $LC_{50}$ and its 95% confindence limits (CLs), the slope of regression line and its standard error (b ± SE), and $\chi^2$ value for testing the discrepancies between observed and expected numbers (Finney 1971). The probit analysis was conducted using the POLO Plus software (LeOra Sotfware, Berkeley, CA). A pairwise comparison of LCs of the life stages was performed using the lethal dose ratio test: when 95% confidence limits for LC ratios included 1 the LCs were not significantly different (Robertson et al. 2017).

***Life table bioassays.*** Life history traits and population parameters used in demographic analysis are given in Table 2.1. Raw life table data were analyzed according to the age-stage two-sex life table theory (Chi 1988; Chi et al. 2020), using the software TWOSEX-MSChart (Chi 2021; Chi et al. 2022). Examples of raw table data and life table output data are presented in Table 2.2. and Table 2.3. Males obtained from the colony as well as escaped or drowned individuals were excluded from the analysis. Standard errors of the life-history traits and population parameters were estimated by the bootstrap technique with 50,000 bootstrap replicates. Differences between control and treatment were compared by the paired bootstrap test (Efron and Tibshirani 1993; Chi et al. 2020, 2022).

**Table 2.1**. The life history traits and population parameters used in demographic analysis (definitions and equations are presented in chapter 1.5)

| **Life history traits** | | **Population parameters** | |
|---|---|---|---|
| Preadult developmental time* | *Pa* | Age-specific survival rate | $l_x$ |
| Preadult survival rate | *Sa* | Age-stage-specific survival rate | $s_{xj}$ |
| Total preovipositional period | *TPOP* | Age-specific fecundity | $m_x$ |
| Adult longevity | - | Age-specific net maternity | $l_x m_x$ |
| Fecundity | - | The net reproductive rate | $R_0$ |
| Oviposition days | - | The intrinsic rate of increase | $r$ |
| Fertility | - | The finite rate of increase | $\lambda$ |
| Reproduction days** | - | The mean generation time | $T$ |

* developmental times of the egg and all immature stages ** number of days in which females laid viable eggs

**Table 2.2.** Example of the age-stage two-sex life table data format for the TWOSEX-MSChart

***Project***: Spirodiclofen: Females F1
***User***: Asma
***Treatment code***: Control
***Initial number of eggs***: 75

***Number of types and stages***: 3, 3
F, Egg, Immature, Female
M, Egg, Immature, Male
N, Egg, Immature, Unknown

*Descriptive data and definitions*

1,F,4,6,16,0,3,5,7,8,8,7,8,5,3,3,2,1,0,0,-1
2,M,4,6,2
3,F,4,6,14,0,2,4,7,5,7,6,6,4,3,3,2,0,0,-1
4,M,4,6,7
5,F,4,6,13,0,1,6,9,9,8,8,7,10,7,5,3,0,-1
6,M,4,6,8
7,F,4,6,12,0,0,1,3,7,8,7,7,6,5,3,1,-1
8,M,4,6,6
9,F,4,6,8,0,2,3,6,5,4,3,1,-1
10,M,4,6,5
11,F,4,6,18,0,0,4,8,8,8,7,7,6,6,6,5,2,3,4,3,1,0,-1
12,M,4,6,5
13,F,4,6,12,0,1,4,8,7,6,6,5,4,3,2,0,-1
14,M,4,6,4
15,F,4,6,17,0,0,0,3,5,6,6,5,4,5,5,4,4,4,3,2,0,-1
16,M,4,6,6
17,F,4,6,13,0,0,0,3,4,4,5,4,6,5,3,1,0,-1
18,M,4,6,8
19,F,4,6,19,0,0,4,9,10,9,8,9,8,7,7,6,5,5,3,2,2,1,0,-1
20,M,4,6,5
21,F,4,6,16,0,1,3,5,9,8,7,7,8,9,7,5,4,2,1,0,-1
22,M,4,6,5
23,F,4,6,6,0,0,0,1,2,0,-1
24,M,4,6,2
25,F,4,6,9,0,0,1,3,7,6,5,4,0,-1
26,M,4,6,4
27,F,4,6,15,0,5,6,10,12,8,7,6,5,6,6,5,3,1,0,-1
28,M,4,6,6
29,F,4,6,17,0,3,5,6,5,6,6,5,6,7,6,5,4,4,3,1,0,-1
30,M,4,6,8
31,F,4,6,14,0,0,1,4,6,6,7,7,6,7,7,3,1,0,-1
32,M,4,6,8
33,F,4,6,15,0,4,4,8,9,7,6,6,5,5,5,3,3,1,0,-1
34,M,4,6,9
35,F,4,6,16,0,0,1,4,4,5,7,8,7,10,11,7,6,5,3,0,-1
36,M,4,6,5
37,F,4,6,11,0,1,5,5,10,8,7,6,4,3,0,-1
38,M,4,6,4
39,F,4,6,19,0,0,5,7,10,8,8,6,6,7,8,7,5,4,3,1,1,0,0,-1
40,M,4,6,6
41,F,4,6,12,0,0,5,8,9,7,6,4,5,3,1,0,-1
42,M,4,6,8
43,F,4,6,9,0,2,4,7,7,6,6,4,0,-1
44,M,4,6,7
45,F,4,6,16,0,2,5,5,9,8,7,6,7,6,5,4,3,3,1,0,-1
46,M,4,6,6
47,F,4,6,11,0,5,7,8,10,8,7,5,3,1,0,-1
48,M,4,6,3
49,F,4,6,10,0,1,5,5,7,6,5,5,2,0,-1
50,M,4,6,5
51,F,4,6,11,0,3,3,6,5,7,7,7,4,3,0,-1
52,M,4,6,6
53,F,4,6,9,0,1,4,7,6,5,5,4,1,-1
54,M,4,6,7
55,F,4,6,8,0,0,6,6,6,5,3,1,-1
56,M,4,6,7
57,F,4,6,8,0,0,1,9,6,4,2,0,-1
58,M,4,6,6
59,F,4,6,17,0,4,6,5,7,6,6,9,8,7,5,4,4,5,2,0,-1
60,M,4,6,5
61,F,4,6,9,0,3,4,6,6,4,2,1,0,-1
62,M,4,6,6
63,F,4,6,7,0,0,4,7,4,1,0,-1
64,M,4,6,3
65,F,4,6,6,0,0,2,5,3,1,-1
66,F,5,6,9,0,3,6,7,6,5,4,2,0,-1
67,F,5,6,9,0,6,8,7,7,5,3,0,0,-1
68,F,5,6,13,0,2,7,4,5,6,5,3,5,3,1,0,0,-1
69,F,5,6,14,0,3,7,6,7,8,9,7,5,5,3,3,1,0,-1
70,F,5,6,21,0,1,7,5,5,7,8,8,8,7,8,8,8,5,7,6,7,5,4,2,0,-1
71,F,5,6,11,0,2,8,7,10,7,6,4,3,1,0,-1
72,M,5,6,4
73,N,-5
74,N,-5
75,N,-5

duration of egg, immature*, adult stages (days)
* all immature stages (from larva to teleiochrisalys)
the number of eggs laid daily
the end of fecundity data

*Developmental time for each individual and fecundity for each female*

**Table 2.3.(a-g).** Example of the age-stage two-sex life table output data obtained from the TWOSEX-MSChart

**a) age-specific survival (actual number)**

| Age | Egg | Immature | Female | Male |
|---|---|---|---|---|
| 0 | 75 | | | |
| 1 | 75 | | | |
| 2 | 75 | | | |
| 3 | 75 | | | |
| 4 | 10 | 65 | | |
| 5 | | 72 | | |
| 6 | | 72 | | |
| 7 | | 72 | | |
| 8 | | 72 | | |
| 9 | | 72 | | |
| 10 | | 7 | 33 | 32 |
| 11 | | | 39 | 33 |
| 12 | | | 39 | 31 |
| 13 | | | 39 | 29 |
| 14 | | | 39 | 26 |
| 15 | | | 39 | 18 |
| 16 | | | 37 | 10 |
| 17 | | | 36 | 6 |
| 18 | | | 33 | 1 |
| 19 | | | 29 | |
| 20 | | | 26 | |
| 21 | | | 23 | |
| 22 | | | 19 | |
| 23 | | | 17 | |
| 24 | | | 14 | |
| 25 | | | 11 | |
| 26 | | | 7 | |
| 27 | | | 4 | |
| 28 | | | 3 | |
| 29 | | | 1 | |
| 30 | | | 1 | |
| 31 | | | 1 | |

**b) age – stage – specific total fecundity**

| Age | Egg | Immature | Female | Male |
|---|---|---|---|---|
| 0 | 0 | | | |
| 1 | 0 | | | |
| 2 | 0 | | | |
| 3 | 0 | | | |
| 4 | 0 | 0 | | |
| 5 | | 0 | | |
| 6 | | 0 | | |
| 7 | | 0 | | |
| 8 | | 0 | | |
| 9 | | 0 | | |
| 10 | | 0 | 0 | 0 |
| 11 | | | 44 | 0 |
| 12 | | | 135 | 0 |
| 13 | | | 243 | 0 |
| 14 | | | 263 | 0 |
| 15 | | | 238 | 0 |
| 16 | | | 217 | 0 |
| 17 | | | 195 | 0 |
| 18 | | | 154 | 0 |
| 19 | | | 139 | |
| 20 | | | 114 | |
| 21 | | | 79 | |
| 22 | | | 56 | |
| 23 | | | 46 | |
| 24 | | | 28 | |
| 25 | | | 16 | |
| 26 | | | 10 | |
| 27 | | | 8 | |
| 28 | | | 5 | |
| 29 | | | 4 | |
| 30 | | | 2 | |
| 31 | | | 0 | |
| | | | 1996 | |

**c) survival rate to each age-stage interval ($s_{xj}$)**

| Age | Egg | Immature | Female | Male |
|---|---|---|---|---|
| 0 | 1 | | | |
| 1 | 1 | | | |
| 2 | 1 | | | |
| 3 | 1 | | | |
| 4 | 0.1333 | 0.8667 | | |
| 5 | | 0.96 | | |
| 6 | | 0.96 | | |
| 7 | | 0.96 | | |
| 8 | | 0.96 | | |
| 9 | | 0.96 | | |
| 10 | | 0.0933 | 0.44 | 0.4267 |
| 11 | | | 0.52 | 0.44 |
| 12 | | | 0.52 | 0.4133 |
| 13 | | | 0.52 | 0.3867 |
| 14 | | | 0.52 | 0.3467 |
| 15 | | | 0.52 | 0.24 |
| 16 | | | 0.4933 | 0.1333 |
| 17 | | | 0.48 | 0.08 |
| 18 | | | 0.44 | 0.0133 |
| 19 | | | 0.3867 | |
| 20 | | | 0.3467 | |
| 21 | | | 0.3067 | |
| 22 | | | 0.2533 | |
| 23 | | | 0.2267 | |
| 24 | | | 0.1867 | |
| 25 | | | 0.1467 | |
| 26 | | | 0.0933 | |
| 27 | | | 0.0533 | |
| 28 | | | 0.04 | |
| 29 | | | 0.0133 | |
| 30 | | | 0.0133 | |
| 31 | | | 0.0133 | |

**d) age – stage – specific fecundity ($f_{xj}$)**

| Age | Egg | Immature | Female | Male |
|---|---|---|---|---|
| 0 | 0 | | | |
| 1 | 0 | | | |
| 2 | 0 | | | |
| 3 | 0 | | | |
| 4 | 0 | 0 | | |
| 5 | | 0 | | |
| 6 | | 0 | | |
| 7 | | 0 | | |
| 8 | | 0 | | |
| 9 | | 0 | | |
| 10 | | 0 | 0 | 0 |
| 11 | | | 1.1282 | 0 |
| 12 | | | 3.4615 | 0 |
| 13 | | | 6.2308 | 0 |
| 14 | | | 6.7436 | 0 |
| 15 | | | 6.1026 | 0 |
| 16 | | | 5.8649 | 0 |
| 17 | | | 5.4167 | 0 |
| 18 | | | 4.6667 | 0 |
| 19 | | | 4.7931 | |
| 20 | | | 4.3846 | |
| 21 | | | 3.4348 | |
| 22 | | | 2.9474 | |
| 23 | | | 2.7059 | |
| 24 | | | 2 | |
| 25 | | | 1.4545 | |
| 26 | | | 1.4286 | |
| 27 | | | 2 | |
| 28 | | | 1.6667 | |
| 29 | | | 4 | |
| 30 | | | 2 | |
| 31 | | | 0 | |

**e) age-specific life table**

| Age | $l_x$ | $m_x$ | $l_xm_x$ | $e^{-r(x+1)}l_xm_x$ | SAD (%) | $e_{xj}$ | $v_{xj}$ |
|---|---|---|---|---|---|---|---|
| 0 | 1 | 0 | 0 | 0 | | | |
| 1 | 1 | 0 | 0 | 0 | | | |
| 2 | 1 | 0 | 0 | 0 | | | |
| 3 | 1 | 0 | 0 | 0 | | | |
| 4 | 1 | 0 | 0 | 0 | | | |
| 5 | 0.96 | 0 | 0 | 0 | | | |
| 6 | 0.96 | 0 | 0 | 0 | | | |
| 7 | 0.96 | 0 | 0 | 0 | | | |
| 8 | 0.96 | 0 | 0 | 0 | | | |
| 9 | 0.96 | 0 | 0 | 0 | | | |
| 10 | 0.96 | 0 | 0 | 0 | | | |
| 11 | 0.96 | 0.61 | 0.59 | 0.0537 | 2.02 | 8.49 | 11.38 |
| 12 | 0.933 | 1.93 | 1.8 | 0.135 | 1.61 | 7.7 | 13.52 |
| 13 | 0.907 | 3.57 | 3.24 | 0.199 | 1.28 | 6.9 | 14.5 |
| 14 | 0.867 | 4.05 | 3.51 | 0.1765 | 1 | 6.17 | 14.04 |
| 15 | 0.76 | 4.18 | 3.17 | 0.1309 | 0.72 | 5.89 | 13.9 |
| 16 | 0.627 | 4.62 | 2.89 | 0.0978 | 0.49 | 5.94 | 14.4 |
| 17 | 0.56 | 4.64 | 2.6 | 0.072 | 0.36 | 5.52 | 13.37 |
| 18 | 0.453 | 4.53 | 2.05 | 0.0466 | 0.24 | 5.59 | 13.15 |
| 19 | 0.387 | 4.79 | 1.85 | 0.0344 | 0.17 | 5.38 | 12.34 |
| 20 | 0.347 | 4.38 | 1.52 | 0.0231 | 0.12 | 4.88 | 10.2 |
| 21 | 0.307 | 3.43 | 1.05 | 0.0131 | 0.09 | 4.39 | 8.12 |
| 22 | 0.253 | 2.95 | 0.75 | 0.0076 | 0.06 | 4.11 | 6.93 |
| 23 | 0.227 | 2.71 | 0.61 | 0.0051 | 0.04 | 3.47 | 5.43 |
| 24 | 0.187 | 2 | 0.37 | 0.0026 | 0.03 | 3 | 4.04 |
| 25 | 0.147 | 1.45 | 0.21 | 0.0012 | 0.02 | 2.55 | 3.17 |
| 26 | 0.093 | 1.43 | 0.13 | 0.0006 | 0.01 | 2.43 | 3.29 |
| 27 | 0.053 | 2 | 0.11 | 0.0004 | 0 | 2.5 | 3.97 |
| 28 | 0.04 | 1.67 | 0.07 | 0.0002 | 0 | 2 | 3.21 |
| 29 | 0.013 | 4 | 0.05 | 0.0001 | 0 | 3 | 5.64 |
| 30 | 0.013 | 2 | 0.03 | 0.0001 | 0 | 2 | 2 |
| 31 | 0.013 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 60.94 | 26.61 | | | | |

**f) Basic statistics of life history data**

| Stage | Sex | N | Mean | S.D. | S.E. | Variance |
|---|---|---|---|---|---|---|
| Egg | All | 72 | 4.1 | 0.3 | 0.04 | 0.09 |
| Immature | All | 72 | 6 | 0 | 0 | 0 |
| Adult | All | 72 | 9.39 | 4.66 | 0.55 | 21.71 |
| Egg | F | 39 | 4.15 | 0.37 | 0.06 | 0.13 |
| Immature | F | 39 | 6 | 0 | 0 | 0 |
| Adult | F | 39 | 12.56 | 3.91 | 0.63 | 15.3 |
| Egg | M | 33 | 4.03 | 0.17 | 0.03 | 0.03 |
| Immature | M | 33 | 6 | 0 | 0 | 0 |
| Adult | M | 33 | 5.64 | 1.78 | 0.31 | 3.18 |
| Egg - Immature | All | 72 | 10.1 | 0.3 | 0.04 | 0.09 |
| Egg - Adult | All | 72 | 19.49 | 4.71 | 0.56 | 22.23 |
| Egg - Immature | F | 39 | 10.15 | 0.37 | 0.06 | 0.13 |
| Egg - Adult | F | 39 | 22.72 | 3.94 | 0.63 | 15.52 |
| Egg - Immature | M | 33 | 10.03 | 0.17 | 0.03 | 0.03 |
| Egg - Adult | M | 33 | 15.67 | 1.76 | 0.31 | 3.1 |
| Fecundity | F | 39 | 51.18 | 24.7 | 3.96 | 610.2 |
| TPOP | F | 39 | 11.62 | 0.63 | 0.1 | 0.4 |
| APOP | F | 39 | 1.46 | 0.64 | 0.1 | 0.41 |
| Oviposition days | F | 39 | 10.05 | 3.79 | 0.61 | 14.37 |

The variances are calculated by using routine statistics (the variance of all individuals)

**g) Bootstrapping results of population parameters**

| | *GRR* | $R_0$ | *r* | $\lambda$ | *T* | *F* |
|---|---|---|---|---|---|---|
| ORIGINAL | 60.94 | 26.61 | 0.199 | 1.221 | 16.47 | 51.18 |
| N | 75 | 75 | 75 | 75 | 75 | 39 |
| B | 50000 | 50000 | 50000 | 50000 | 50000 | 50000 |
| Boot. max | 80.99 | 42.10667 | 0.226328 | 1.253987 | 17.421 | 69.2 |
| Boot. mean | 58.62 | 26.62039 | 0.198809 | 1.219988 | 16.46 | 51.15 |
| Boot. min | 32.5 | 11.41333 | 0.148729 | 1.160359 | 15.509 | 33.41 |
| Variance | 71 | 12.98703 | 0.000065 | 0.000096 | 0.047 | 15.52 |
| S.E. | 8.426 | 3.60375 | 0.00804 | 0.009794 | 0.217 | 3.94 |

Bootstrap sample size: N =75

Total number of effective bootstrap: B = 50000

The variance is calculated by using all bootstrap sample means.

# 4. RESULTS

## 4.1. Acute toxicity of the acaricides to the eggs

Concentration-response curves of hexythiazox and spirodiclofen acute toxicity to *T. urticae* eggs (ovicidal activity) are shown in Fig. 4.1. and Fig. 4.2. Parameters of acute toxicity of the acaricides, obtained by probit analysis, are presented in Table 4.1.

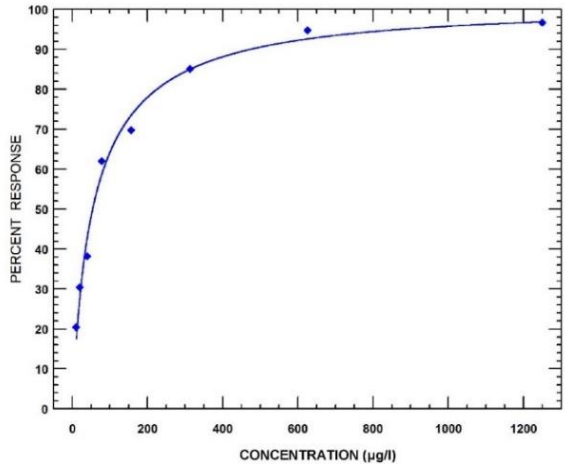


**Fig. 4.1.** Concentration (µg/l) – response (egg mortality, %) curve of hexythiazox activity of against *T. urticae* eggs

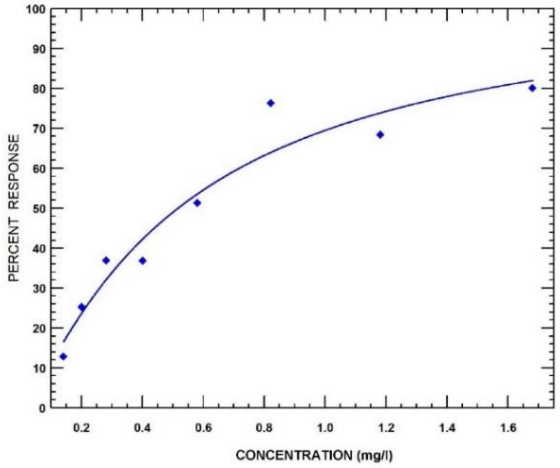


**Fig. 4.2.** Concentration (mg/l) – response (egg mortality, %) curve of spirodiclofen activity of against *T. urticae* eggs

**Table 4.1.** Acute toxicity of acaricides to *T. urticae* eggs

| Acaricides | n | $LC_{10}$ (mg/l) (95% CLs) | $LC_{50}$ (mg/l) (95% CLs) | $LC_{90}$ (mg/l) (95% CLs) | b (± SE) | $\chi^2$ | df |
|---|---|---|---|---|---|---|---|
| Hexythiazox | 1998 | 0.007 (0.004 – 0.010) | 0.06 (0.05 – 0.07) | 0.49 (0.38 – 0.67) | 1.37 (± 0.07) | 7.34 | 6 |
| Spirodiclofen | 1205 | 0.10 (0.05 – 0.16) | 0.54 (0.42 – 0.68) | 2.75 (1.82 – 5.45) | 1.80 (± 0.13) | 14.92 | 6 |

n = number of treated eggs; CLs = confidence limits; b = slope of the regression line; df = degress of freedom

Both acaricides showed strong ovicidal activity, but hexythiazox was more toxic to the eggs than spirodiclofen: the $LC_{50}$ of the latter was 9 times as high as the $LC_{50}$ of the former. At the $LC_{90}$ level this ratio was lowered, due to lower slope of the hexythiazox regression line. A pairwise comparison of LCs between the acaricides, using the lathal dose ratio test, confirmed that 95% CLs were significantly different.

## 4.2. The effects of hexythiazox on life history traits and population growth

### 4.2.1. Female treatment

#### *4.2.1.1. $F_0$ generation bioassay*

Exposure to hexythiazox affected the adult longevity, oviposition duration in days and fecundity of *T. urticae* females (Table 4.2). The three highest acaricide concentrations reduced significantly the longevity of female adults, whereas 50 and 12.5 mg/l significantly reduced their oviposition duration (by 2 – 2,5 days, and 1.5 - 2 days, respectively, compared to the control). Treatments with the three highest concentrations and 0.195 mg/l caused statistically significant reduction in fecundity-I (by 26–37%, compared to the control).

**Table 4.2.** Life history traits (mean ± SE) of *T. urticae* after the treatment of females with hexythiazox (mg/l) (fecundity-based life tables)

| mg/l | Females | | | | | | | Males | |
|---|---|---|---|---|---|---|---|---|---|
| | Nf | Fecundity-I* | Long | Ovd | Nfs | Fecundity-II** | Long | Nm | Long |
| 50 | 66 | 36.48 bc (± 4.34) | 7.98 c (± 0.53) | 6.44 bc (± 0.51) | 51 | 45.18 ab (± 4.57) | 9.57 a (± 0.50) | 20 | 2.55 e (± 0.17) |
| 12.5 | 73 | 31.04 c (± 3.33) | 7.82 c (± 0.46) | 5.98 c (± 0.44) | 58 | 35.55 bc (± 3.45) | 9.17 b (± 0.42) | 12 | 3.83 d (± 0.24) |
| 3.125 | 49 | 32.61 c (± 4.69) | 8.33 bc (± 0.66) | 6.54 a (± 0.66) | 40 | 36.72 abc (± 4.96) | 9.62 a (± 0.65) | 15 | 6.07 b (± 0.47) |
| 0.781 | 42 | 41.14 ab (± 5.00) | 9.43 ab (± 0.71) | 7.18 a (± 0.74) | 40 | 40.15 abc (± 4.86) | 9.80 a (± 0.69) | 9 | 5.89 b (± 1.15) |
| 0.390 | 46 | 50.93 a (± 5.19) | 10.20 a (± 0.60) | 7.50 ab (± 0.57) | 44 | 49.07 a (± 4.90) | 10.54 a (± 0.57) | 11 | 6.09 b (± 1.16) |
| 0.195 | 49 | 32.37 b (± 5.38) | 9.57 ab (± 0.66) | 6.72 a (± 0.68) | 49 | 30.20 c (± 5.18) | 9.57 a (± 0.66) | 8 | 4.75 c (± 0.31) |
| 0.049 | 45 | 46.13 ab (± 5.00) | 10.16 a (± 0.59) | 7.86 ab (± 0.58) | 43 | 45.58 ab (± 4.98) | 10.51 a (± 0.56) | 12 | 7.42 ab (± 1.00) |
| 0.012 | 44 | 40.86 ab (± 5.32) | 9.95 ab (± 0.65) | 7.52 a (± 0.66) | 42 | 39.98 abc (± 5.21) | 10.29 a (± 0.63) | 12 | 9.58 a (± 1.65) |
| 0.0 | 56 | 49.41 a (± 4.64) | 10.36 a (± 0.59) | 7.98 a (± 0.58) | 54 | 47.78 a (± 4.63) | 10.63 a (± 0.58) | 19 | 5.32 bc (± 0.30) |

Means in rows followed by different letters are significantly different (the paired bootstrap test)
Nf = total number of females; Nfs = number of females alive 24 h after the treatment; Nm = total number of males; Long = adult longevity (days); Ovd = oviposition days; *eggs laid/Nf **eggs laid/Nfs

Such effects mostly resulted from female mortality during 24-h exposure, which was 22.7% (50 mg/l), 20.6% (12.5 mg/l), and 18.4% (3.125 mg/l), whereas female mortality stayed below 5% in all other treatments and the control. Fecundity-II was not significantly different from the control in treatments with 50 and 3.125 mg/l, whereas adult female longevity was significantly reduced only by 12.5 mg/l. The two highest concentrations reduced significantly the adult longevity of males, whereas the lowest concentration extended male longevity significantly (Table 4.2).

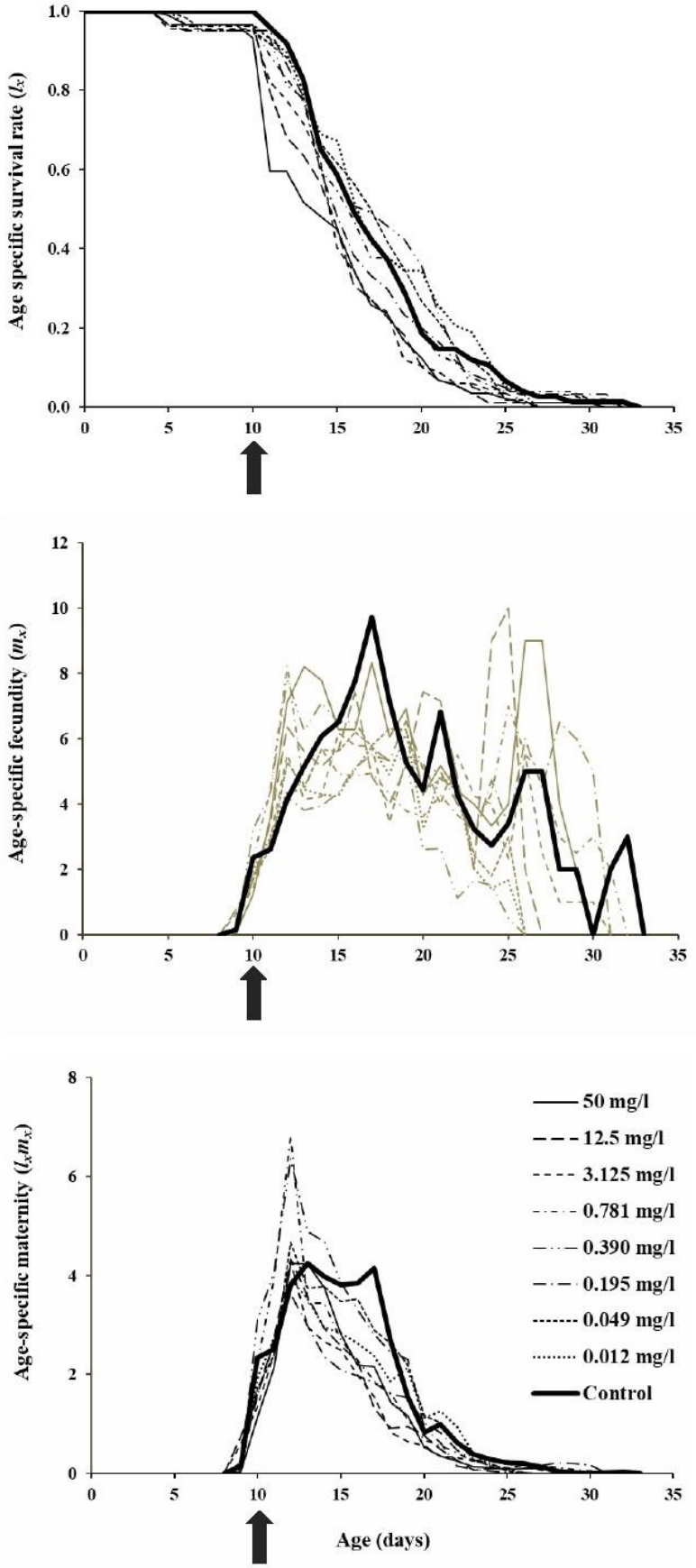


**Fig. 4.3.** Age-specific survival rate ($l_x$), age-specific fecundity ($m_x$) and age-specific maternity ($l_xm_x$) of *T. urticae* after the treatment of females with hexythiazox (mg/l); arrow indicate the treatment

Relatively high female mortality over the 24-h treatment with the three highest concentrations caused a decrease in age-specific survival rates ($l_x$) which remained lower in those treatments, compared to the control (Figure 4.3). Over the initial 4 days after treatment, $l_x$ values were lower for all treatments compared to the control. By the end of the trial, $l_x$ values in treatments with lower concentrations mostly exceeded control values. On the other hand, age-specific fecundity ($m_x$) was higher in all treatments, compared to the control, for at least two of the initial four post-treatment days, and during most days of the second half of the oviposition period. Reductions in $l_x$ after treatment with the three highest concentrations were the decisive contribution to age-specific maternity ($l_xm_x$) reduction as the parameter was below the control value toward the end of oviposition (except only the second day after treatment). Treatments with all other concentrations resulted in higher $l_xm_x$ values than the control during 5–8 days of oviposition, mostly in the latter half of the oviposition period.

Population parameters are detailed in Table 4.3. Net reproductive rate ($R_0$) was significantly reduced by the three highest concentrations (27–35%, compared to the control) which was expected considering the described reduction in age-specific maternity in those treatments. However, such reduction was insufficient to decrease significantly the intrinsic rate of increase ($r$) or consequently the finite rate of increase ($\lambda$). On the other hand, the highest $r$ and $\lambda$ values were recorded in treatments with 0.390 mg/l. These values significantly exceeded those recorded for treatments with the three highest concentrations, as well as with two lower ones, but not the control values. The treatments with concentrations 12.5, 0.781, and 0.390 mg/l. significantly shortened the eneration time.

**Table 4.3.** Population parameters (mean ± SE) of *T. urticae* after the treatment of females with hexythiazox (mg/l) (fecundity-based life tables)

| mg/l | N | $R_0$ (eggs laid/mite) | $r$ (days$^{-1}$) | $\lambda$ (days$^{-1}$) | $T$ (days) |
|---|---|---|---|---|---|
| 50 | 89 | 27.06 bc (± 3.64) | 0.224 b (± 0.008) | 1.251 b (± 0.011) | 14.72 ab (± 0.23) |
| 12.5 | 88 | 25.75 c (± 3.02) | 0.229 b (± 0.007) | 1.258 b (± 0.009) | 14.17 b (± 0.23) |
| 3.125 | 67 | 23.85 c (± 3.85) | 0.222 b (± 0.010) | 1.248 b (± 0.012) | 14.31 ab (± 0.32) |
| 0.781 | 53 | 32.60 ab (± 4.58) | 0.244 a (± 0.008) | 1.277 a (± 0.011) | 14.26 b (± 0.26) |
| 0.390 | 59 | 39.71 a (± 4 87) | 0.257 a (± 0.008) | 1.293 a (± 0.011) | 14.32 b (± 0.17) |
| 0.195 | 60 | 26.43 ab (± 4.63) | 0.220 b (± 0.009) | 1.246 b (± 0.012) | 14.91 ab (± 0.34) |
| 0.049 | 60 | 34.60 ab (± 4.56) | 0.236 ab (± 0.008) | 1.267 ab (± 0.010) | 14.99 a (± 0.23) |
| 0.012 | 58 | 31.00 ab (± 4.63) | 0.230 b (± 0.009) | 1.259 b (± 0.011) | 14.92 a (± 0.24) |
| 0.0 | 75 | 36.89 a (± 4.25) | 0.240 ab (± 0.006) | 1.272 ab (± 0.008) | 15.01 a (± 0.23) |

Means in rows followed by different letters are significantly different (the paired bootstrap test)
N = sample size (initial number of eggs in cohort); $R_0$ = net reproductive rate;
$r$ = intrinsic rate of increase; $\lambda$ = finite rate of increase; $T$ = generation time

The fertility-based life table gave a different picture of hexythiazox impact. Fertility was significantly reduced, compared to control values, by treatments with 50, 12.5, 3.125, 0.781 and 0.195 mg/l, including both fertility-I (38–58%) and fertility-II (32–48%). Besides, the three highest concentrations significantly reduced the number of reproduction days (Table 4.4).

**Table 4.4.** Life history traits (mean ± SE) of *T. urticae* after the treatment of females with hexythiazox (mg/l) (fertility-based life tables)

| mg/l | Nf | Fertility-I* | Rpd | Nfs | Fertility-II** |
|---|---|---|---|---|---|
| 50 | 66 | 24.03 c (± 3.32) | 5.16 bc (± 0.50) | 51 | 31.04 bc (± 3.78) |
| 12.5 | 73 | 19.73 c (± 2.54) | 4.55 c (± 0.42) | 58 | 23.76 c (± 2.87) |
| 3.125 | 49 | 24.18 c (± 3.65) | 5.80 bc (± 0.66) | 40 | 28.68 bc (± 3.98) |
| 0.781 | 42 | 29.31 b (± 4.08) | 6.85 ab (± 0.75) | 40 | 29.48 bc (± 4.17) |
| 0.390 | 46 | 40.74 a (± 4.20) | 7.50 a (± 0.57) | 44 | 39.43 ab (± 4.06) |
| 0.195 | 49 | 27.41 bc (± 4.68) | 6.64 ab (± 0.68) | 49 | 25.78 c (± 4.54) |
| 0.049 | 45 | 39.96 ab (± 4.37) | 7.86 a (± 0.58) | 43 | 39.93 ab (± 4.35) |
| 0.012 | 44 | 36.23 ab (± 4.81) | 7.52 a (± 0.66) | 42 | 35.90 ab (± 4.73) |
| 0.0 | 56 | 47.18 a (± 4.38) | 7.98 a (± 0.58) | 54 | 45.67 a (± 4.44) |

Means in rows followed by different letters are significantly different (the paired bootstrap test)
Nf = total number of females; Nfs = number of females alive 24 h after the treatment
Rpd = number of reproduction days; *viable (hatched) eggs/Nf **viable (hatched) eggs/Nfs

The ultimate outcome resulted from the transovarial toxic effects of hexythiazox, i.e. reduction in egg viability of treated females. Transovarial toxicity of hexythiazox was at its highest in eggs laid over the initial 24 h following treatment, as their hatching was 15–81%, compared to 95% in the control. By the end of the trial, egg viability in treated females gradually increased, approaching the control values (Figure 4.4.a). Total egg viability was concentration-dependent and ranged in treated females between 67% and 90%, whereas it was 96% in control females. The viability of eggs from treated females over the initial four post-treatment days ranged from 52 to 89%, whereas the control percentage was 95%. A statistically significant difference between fecundity and fertility values (i.e., significant reductions in egg viability) in the first four post-treatment days was observed for treatments with concentrations from 50 to 0.390 mg/l, whereas only the two highest concentrations significantly reduced total egg viability (Figure 4.4.b).

The reduction in egg viability also affected the age-specific functions. In contrast to the age-specific fecundity (Figure 4.3), the age-specific fertility of treated females was mostly below the control values in initial days of oviposition. Excepting some lower concentrations, the $l_x m_x$ values of treated females were lower than the control toward the end of the bioassay (Figure 4.5).

Hexythiazox concentrations that significantly reduced fertility-I and fertility-II also significantly decreased population parameters (Table 4.5): compared to the control, $R_0$ was reduced by 34–54%, $r$ by 12–24%, and $\lambda$ by 3–5%. The values of $r$ and $\lambda$ in the treatments with the four highest concentrations were significantly lowered, compared to those obtained from the fecundity-based life table (the paired bootstrap test)

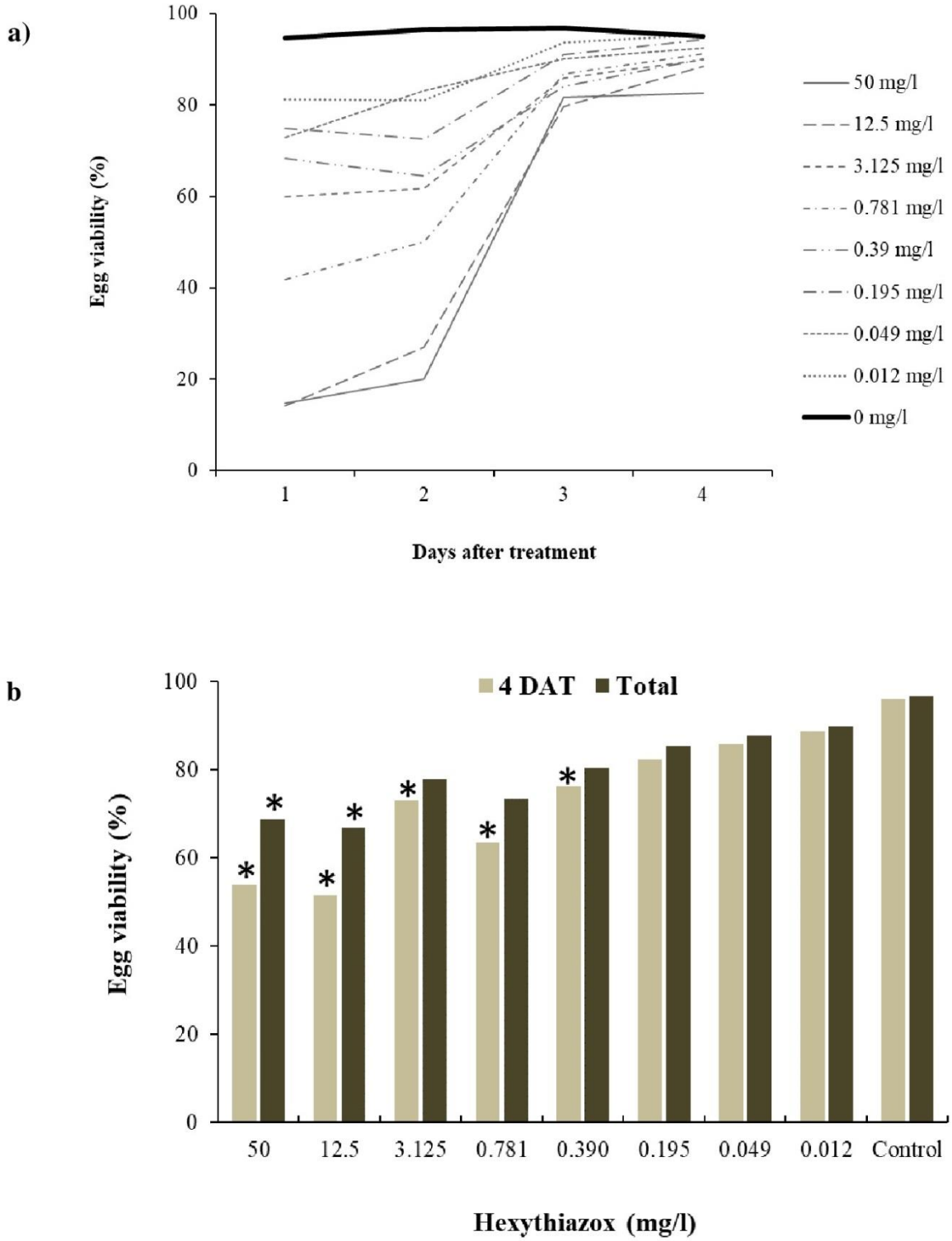


**Fig. 4.4.** Viability (%) of the eggs laid by *T. urticae* females treated with hexythiazox (mg/l), calculated from: **a)** no. eggs laid over the first 4 days after the treatment, and **b)** the total no. eggs laid after the treatment (Total) and the total no. eggs laid over the first 4 days after the treatment (4 DAT); asterisk indicate statistically signficant differences between fecundity (no. eggs laid/female) and fertility (no. viable eggs/female)

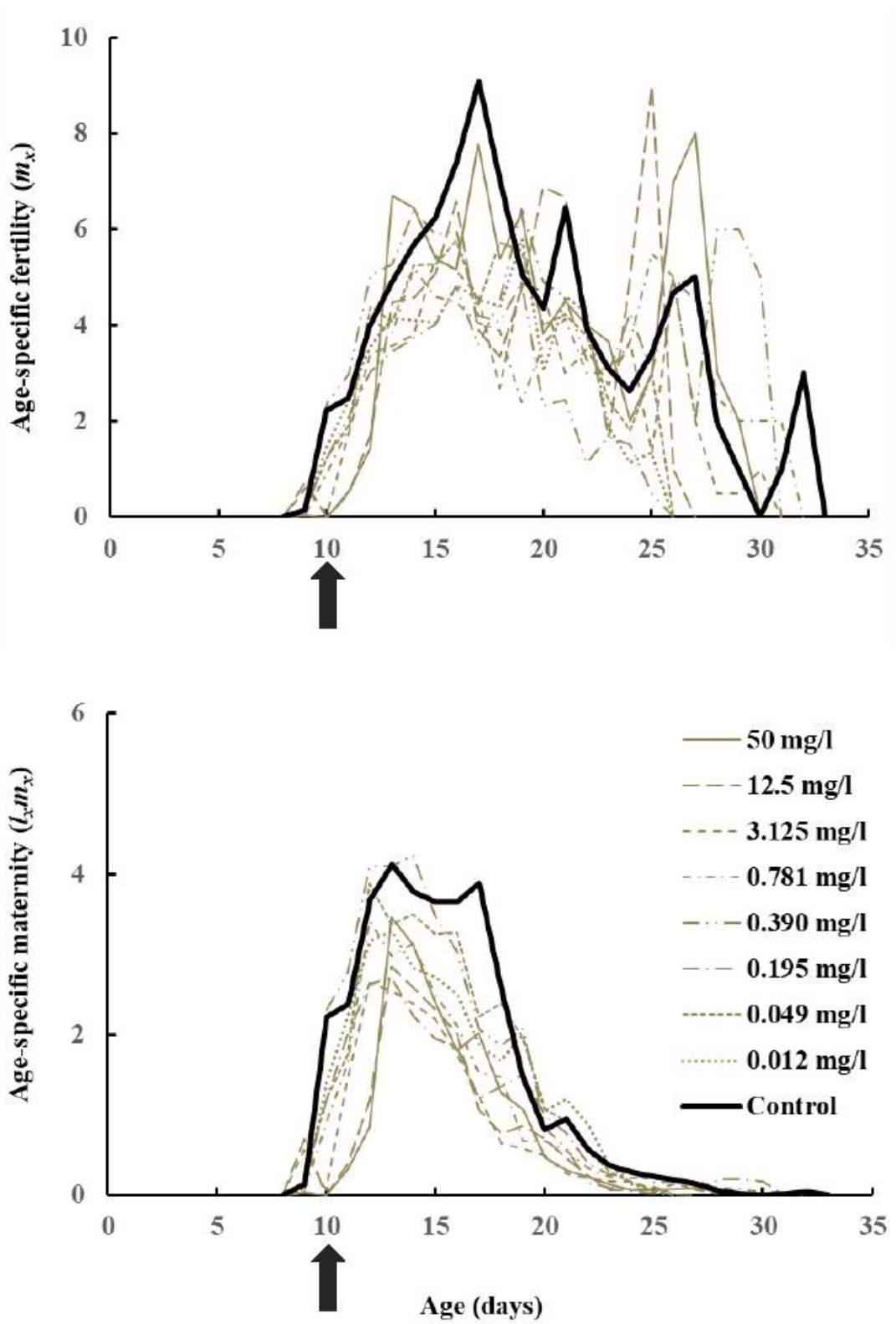


**Fig. 4.5.** Age-specific fertility ($m_x$) and age-specific maternity ($l_x m_x$) of *T. urticae* after the treatment of females with hexythiazox (mg/l); arrow indicate the treatment

**Table 4.5.** Population parameters (mean ± SE) of *T. urticae* after the treatment of females with hexythiazox (mg/l) (fertility-based life tables)

| mg/l | N | $R_0$ (viable eggs/mite) | $r$ (days$^{-1}$) | $\lambda$ (days$^{-1}$) | $T$ (days) |
|---|---|---|---|---|---|
| 50 | 89 | 17.82 c (± 2.70) | 0.180 c ↓ (± 0.008) | 1.198 c ↓ (± 0.010) | 15.97 a (± 0.27) |
| 12.5 | 88 | 16.36 c (± 2.24) | 0.182 c ↓ (± 0.007) | 1.199 c ↓ (± 0.009) | 15.39 ab (± 0.27) |
| 3.125 | 67 | 17.69 c (± 2.97) | 0.193 c ↓ (± 0.010) | 1.213 c ↓ (± 0.012) | 14.87 bc (± 0.36) |
| 0.781 | 53 | 23.23 bc (± 3.62) | 0.209 bc ↓ (± 0.008) | 1.232 bc ↓ (± 0.010) | 15.07 bc (± 0.29) |
| 0.390 | 59 | 31.76 ab (± 3.94) | 0.237 a (± 0.008) | 1.267 a (± 0.010) | 14.59 c (± 0.18) |
| 0.195 | 60 | 22.38 bc (± 4.05) | 0.205 bc (± 0.009) | 1.227 bc (± 0.012) | 15.17 bc (± 0.36) |
| 0.049 | 60 | 29.97 ab (± 3.96) | 0.227 ab (± 0.007) | 1.251 ab (± 0.009) | 15.19 ab (± 0.24) |
| 0.012 | 58 | 27.48 ab (± 4.18) | 0.219 ab (± 0.008) | 1.245 ab (± 0.010) | 15.14 bc (± 0.25) |
| 0.0 | 75 | 35.23 a (± 4.02) | 0.237 a (± 0.006) | 1.267 a (± 0.008) | 15.03 bc (± 0.23) |

Means in rows followed by different letters are significantly different (the paired bootstrap test)
N = sample size (initial number of eggs in cohort); $R_0$ = net reproductive rate;
$r$ = intrinsic rate of increase; $\lambda$ = finite rate of increase; $T$ = generation time
↓ significantly lower compared to the fecundity-based life table

The bioassay results for *T. urticae* $F_0$ generation revealed that negative hexythiazox effects were much more evident when assessed by the fertility-based life table than fecundity-based life table, whereas fertility reduction during the initial days of oviposition was decisive for reductions in population growth. Hexythiazox concentrations ranging from the field-relevant rate of 50 mg/l down to a 256× lower concentration of 0.195 mg/l caused significant reductions. On the other hand, testing a wide range of concentrations revealed that the concentration-response relationship was not linear, i.e., maximum values of fecundity, fertility, $R_0$, and rates of increase were recorded in treatments with 0.390 mg/l, which is a mid-test-range value. These values significantly exceeded those recorded for some treatments with higher and lower concentrations, but not the control values.

#### *4.2.1.2. $F_1$ generation bioassay*

Considering the transovarial effects of hexythiazox on eggs laid over the initial 24 h after treatment, the $F_1$ generation bioassay expectedly revealed reductions in the age-stage specific survival rate ($s_{xj}$) at the immature and adult stages, and preadult survival rate (*Sa*), which were concentration-dependent (Figure 4.6; Table 4.6). The *Sa* of the offspring of treated females was significantly lower in all treatments than in the control. Besides, concentrations ranged 50–0.390 mg/l slightly but significantly extended offspring preadult developmental time (*Pa*) and the total preovipositional period (TPOP) of $F_1$ females. The four highest concentrations, as well as the two lowest concentrations, significantly reduced the fecundity of $F_1$ females (by 26–34% compared to the control). The highest fecundity (not different from the control) was recorded in the treatment with 0.195 mg/l. Females' longevity was significantly reduced by the four highest concentrations, as well as the lowest one, while the number of oviposition days was significantly reduced by treatments with 50, 12.5, 0.781, 0.049 and 0.012 mg/l (by 1.4-2.1 days, and 1.4-1.9 days, respectively, compared to the control.

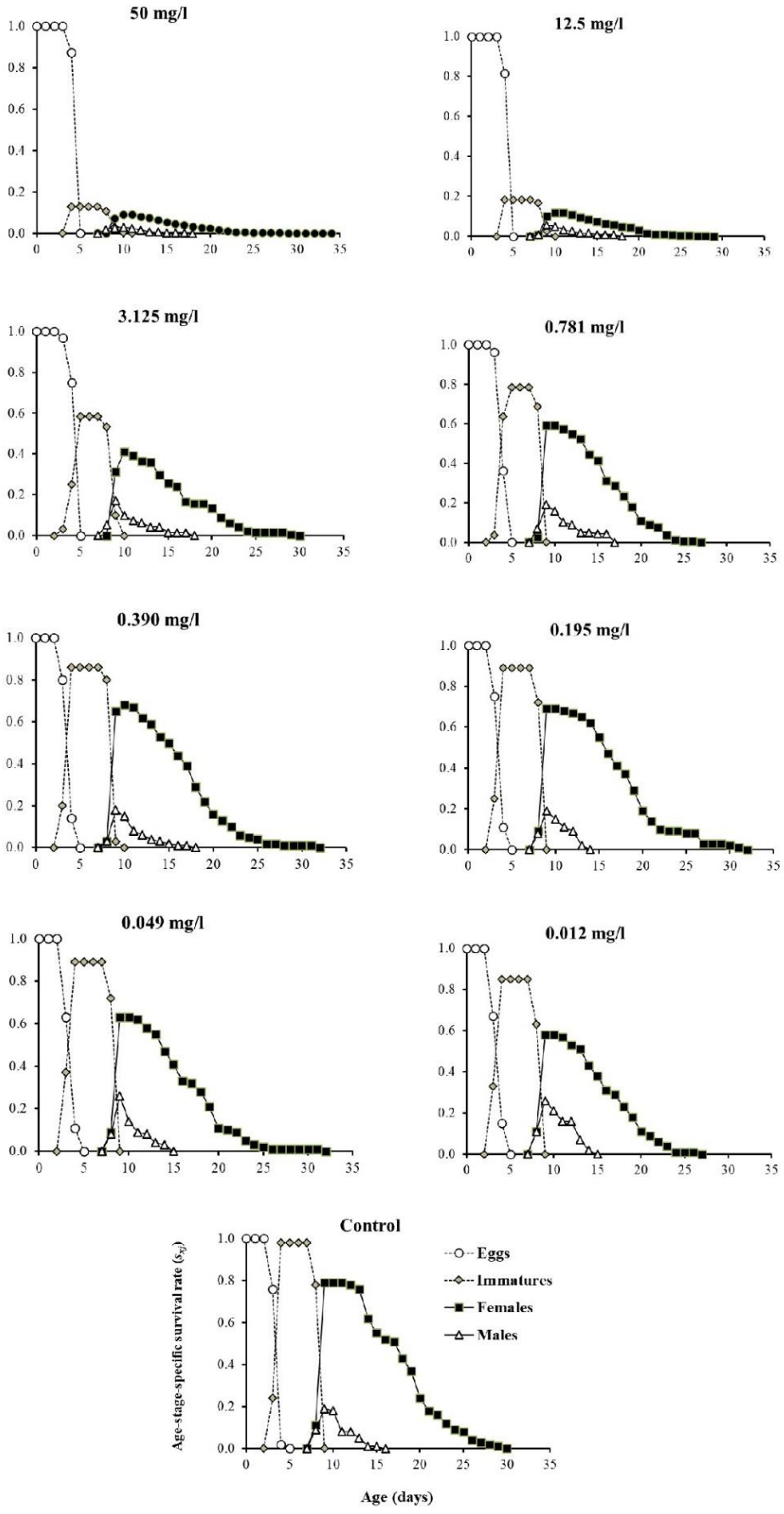


**Fig. 4.6.** Age-stage-specific survival rate ($s_{xj}$) of the offspring of *T. urticae* after the treatment of $F_0$ females with hexythiazox (mg/l)

**Table 4.6.** Life history traits (mean ± SE) of the offspring of *T. urticae* after the treatment of $F_0$ females with hexythiazox (mg/l)

| mg/l | N | *Sa* | Females | | | | | | Males | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Nf | *Pa* | TPOP | Fecundity* | Ovd | Long | Nm | *Pa* | Long |
| 50 | 1020 | 0.13 f (± 0.01) | 97 | 9.27 a (± 0.05) | 10.34 b (± 0.06) | 37.38 cd (± 3.28) | 6.38 c (± 0.41) | 7.99 b (± 0.43) | 33 | 8.64 bc (±0.15) | 4.24 a (± 0.39) |
| 12.5 | 743 | 0.18 e (± 0.01) | 89 | 9.09 b (± 0.05) | 10.17 cd (± 0.06) | 42.24 c (± 3.51) | 6.64 bc (± 0.39) | 8.40 b (± 0.42) | 48 | 8.98 a (±0.07) | 3.67 ab (± 0.38) |
| 3.125 | 192 | 0.58 d (± 0.04) | 79 | 9.24 a (± 0.05) | 10.57 a (± 0.07) | 39,30 cd (± 4.00) | 7.54 abc (± 0.48) | 8.57 b (± 0.49) | 33 | 8.70 bc (±0.08) | 3.42 ab (± 0.47) |
| 0.781 | 157 | 0.78 c (± 0.03) | 93 | 8.96 cd (± 0.02) | 10.20 bc (± 0.05) | 38.61 cd (± 3.19) | 6.87 bc (± 0.35) | 8.55 b (± 0.37) | 30 | 8.63 bc (±0.09) | 4.20 a (± 0.49) |
| 0.390 | 100 | 0.86 bc (± 0.03) | 68 | 9.00 bc (± 0.04) | 10.11 cd (± 0.06) | 52.97 ab (± 4.43) | 7.62 ab (± 0.45) | 9.16 ab (± 0.50) | 18 | 8.83 ab (±0.09) | 3.39 ab (± 0.50) |
| 0.195 | 100 | 0.89 b (± 0.03) | 69 | 8.87 de (± 0.04) | 10.03 de (± 0.06) | 60.61 a (± 4.77) | 8,32 a (± 0.46) | 10.25 a (± 0.53) | 20 | 8.60 bc (±0.11) | 3.20 ab (± 0.37) |
| 0.049 | 100 | 0.89 b (± 0.03) | 63 | 8.86 e (± 0.04) | 10.11 cd (± 0.08) | 46.81 bc (± 4.65) | 6.90 bc (± 0.48) | 8.86 ab (± 0.51) | 26 | 8.69 bc (±0.09) | 2.77 b (± 0.37) |
| 0.012 | 100 | 0.85 bc (± 0.04) | 58 | 8.81 e (± 0.05) | 10.00 de (± 0.08) | 41.78 c (± 3.64) | 6.93 bc (± 0.43) | 8.67 b (± 0.48) | 27 | 8.59 bc (±0.10) | 3.67 ab (± 0.34) |
| 0.0 | 100 | 0.98 a (± 0.01) | 79 | 8.86 e (± 0.04) | 9.97 e (± 0.06) | 56.84 ab (± 3.92) | 8.29 a (± 0.44) | 10.11 a (± 0.48) | 19 | 8.53 c (±0.12) | 3.63 ab (± 0.43) |

Means in rows followed by different letters are significantly different (the paired bootstrap test)
N = sample size (initial number of eggs in cohort); Nf = number of females; Nm = number of males;
*Sa* = preadult survival rate; *Pa* = preadult developmental time (days); TPOP = total preoviposition period;
Ovd = oviposition days; Long = adult longevity (days); *eggs laid/**N**f

Due to reduction in $s_{xj}$ at the egg stage, the $l_x$ of the offspring of treated females was lower than in the control (Figure 4.7). The age-specific fecundity of offspring produced by females treated with the four highest acaricide concentrations, as well as the two lowest, was mostly lower than it was among control offspring in the initial half of the oviposition period. On the other hand, $m_x$ values in treatments with 0.390 and 0.195 mg/l were higher than the control values during four of the six initial days of oviposition. Resultingly, $l_x m_x$ values in all treatments, except with 0.390 and 0.195 mg/l, were lower than the control values over the entire oviposition period (excluding a few days at the very end of the period).

Consistent with the reduction in the age-specific maternity, all treatments (except with concentrations 0.390 and 0.195 mg/l) significantly reduced population parameters: $R_0$ was reduced by 34–92%, $r$ by 10–68%, and $\lambda$ by 3–17% (Table 4.7).

The $F_1$ generation bioassay showed that the transovarial toxic effect of hexythiazox had a decisive contribution to reducing population growth of *T. urticae* offspring whereas its influence on life-history traits (TPOP, fecundity, longevity) was less important. Hexythiazox concentrations ranging from the field-relevant rate of 50 mg/l down to a 1020× lower concentration caused significant reductions. As in the $F_0$ generation bioassay, the concentration-response relationship was not linear.

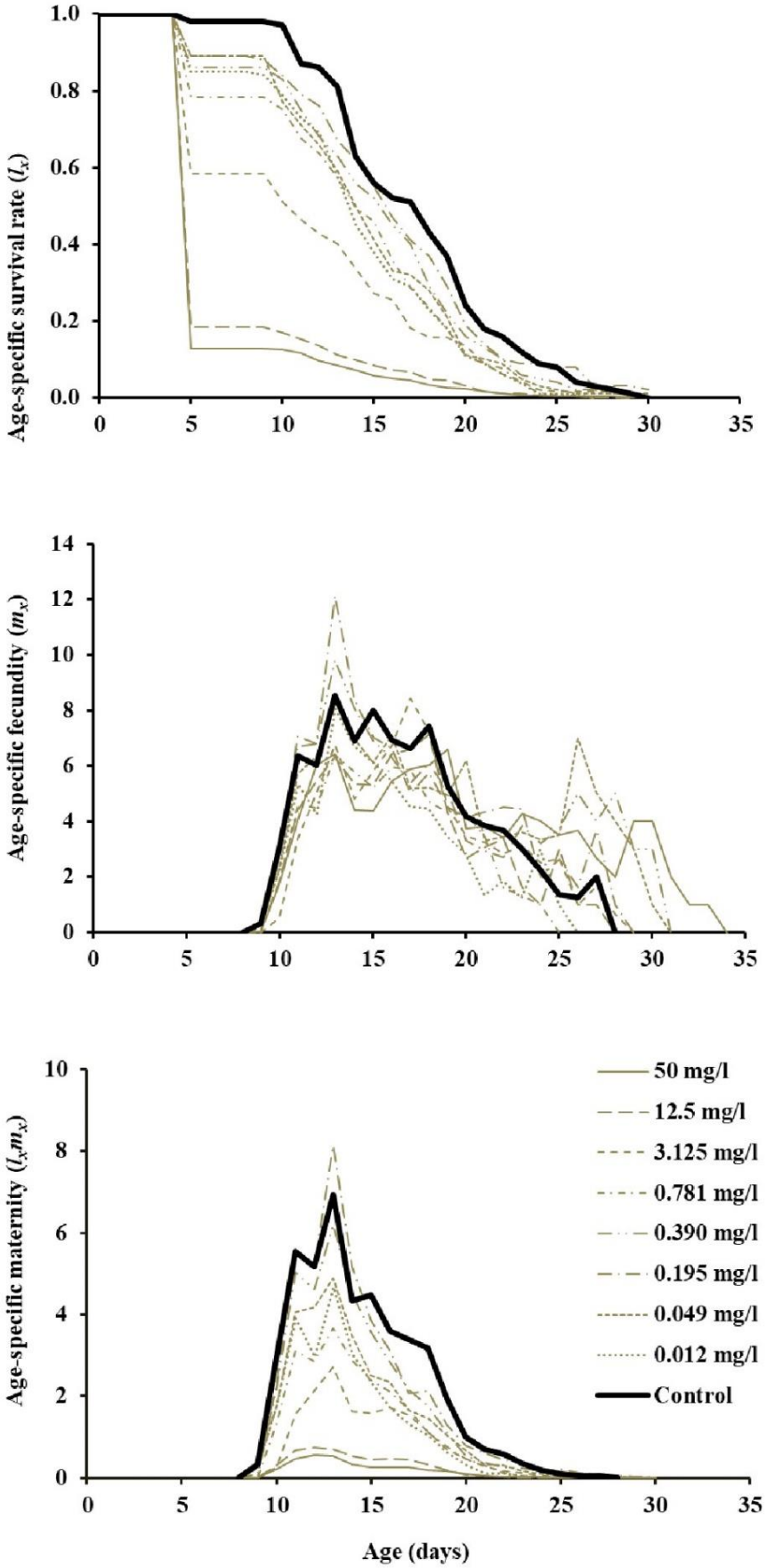


**Fig. 4.7.** Age-specific survival rate ($l_x$), age-specific fecundity ($m_x$) and age-specific maternity ($l_xm_x$) of the offspring of *T. urticae* after the treatment of $F_0$ females with hexythiazox (mg/l)

**Table 4.7.** Population parameters (mean ± SE) of the offspring of *T. urticae* after the treatment of $F_0$ females with hexythiazox (mg/l)

| mg/l | N | $R_0$ (eggs laid/mite) | $r$ (days$^{-1}$) | $\lambda$ (days$^{-1}$) | $T$ (days) |
|---|---|---|---|---|---|
| 50 | 1020 | 3.55 e (± 0.46) | 0.084 f (± 0.008) | 1.088 f (± 0.009) | 15.03 a (± 0.46) |
| 12.5 | 743 | 5.06 e (± 0.65) | 0.110 e (± 0.008) | 1.116 e (± 0.009) | 14.77 ab (± 0.46) |
| 3.125 | 192 | 16.17 d (± 2.16) | 0.184 d (± 0.008) | 1.202 d (± 0.010) | 15.14 a (± 0.46) |
| 0.781 | 157 | 22.88 c (± 2.42) | 0.218 c (± 0.007) | 1.243 c (± 0.008) | 14.38 bc (± 0.46) |
| 0.390 | 100 | 36.02 ab (± 3.89) | 0.252 ab (± 0.007) | 1.286 ab (± 0.009) | 14.24 c (± 0.46) |
| 0.195 | 100 | 41.82 a (± 4.30) | 0.262 a (± 0.007) | 1.299 a (± 0.009) | 14.26 c (± 0.46) |
| 0.049 | 100 | 29.49 bc (± 3.68) | 0.238 bc (± 0.007) | 1.269 bc (± 0.009) | 14.22 bc (± 0.46) |
| 0.012 | 100 | 24.23 c (± 2.93) | 0.229 c (± 0.008) | 1.257 c (± 0.010) | 13.93 bc (± 0.46) |
| 0.0 | 100 | 44.90 a (± 3.86) | 0.265 a (± 0.005) | 1.304 a (± 0.007) | 14.35 bc (± 0.46) |

Means in rows followed by different letters are significantly different (the paired bootstrap test)
N = sample size (initial number of eggs in cohort); $R_0$ = net reproductive rate;
$r$ = intrinsic rate of increase; $\lambda$ = finite rate of increase; $T$ = generation time

### 4.2.2. Egg treatment

#### *4.2.2.1. $F_0$ generation bioassay*

The three highest hexythiazox concentrations (500, 62.5, and 6.25 µg/l, that fall within the 95% CLs of $LC_{90}$, $LC_{50}$, and $LC_{10}$ estimates from the acute toxicity bioassay) significantly lowered $s_{xj}$ values at immature and adult stages after the treatment of *T. urticae* eggs (Fig. 4.8).

These treatments also significantly lowered *Sa*, fecundity (by 33-41%), female adult longevity (by 2.9-3.5 days) and the number of oviposition days (by 2-2.8 days), while *Pa* and TPOP of females were slightly but significantly extended, compared to the control (Tab. 4.8). The treatment with concentration 0.781 µg/l significanlty reduced *Sa*, fecundity and the number of oviposition days, and extended female *Pa*, while the treatment with concentration 0.391 µg/l significanltly reduced only fecundity. On the other hand, *Pa* of males was significantly extended in the treatments with concentrations ranged 0.781-500 µg/l.

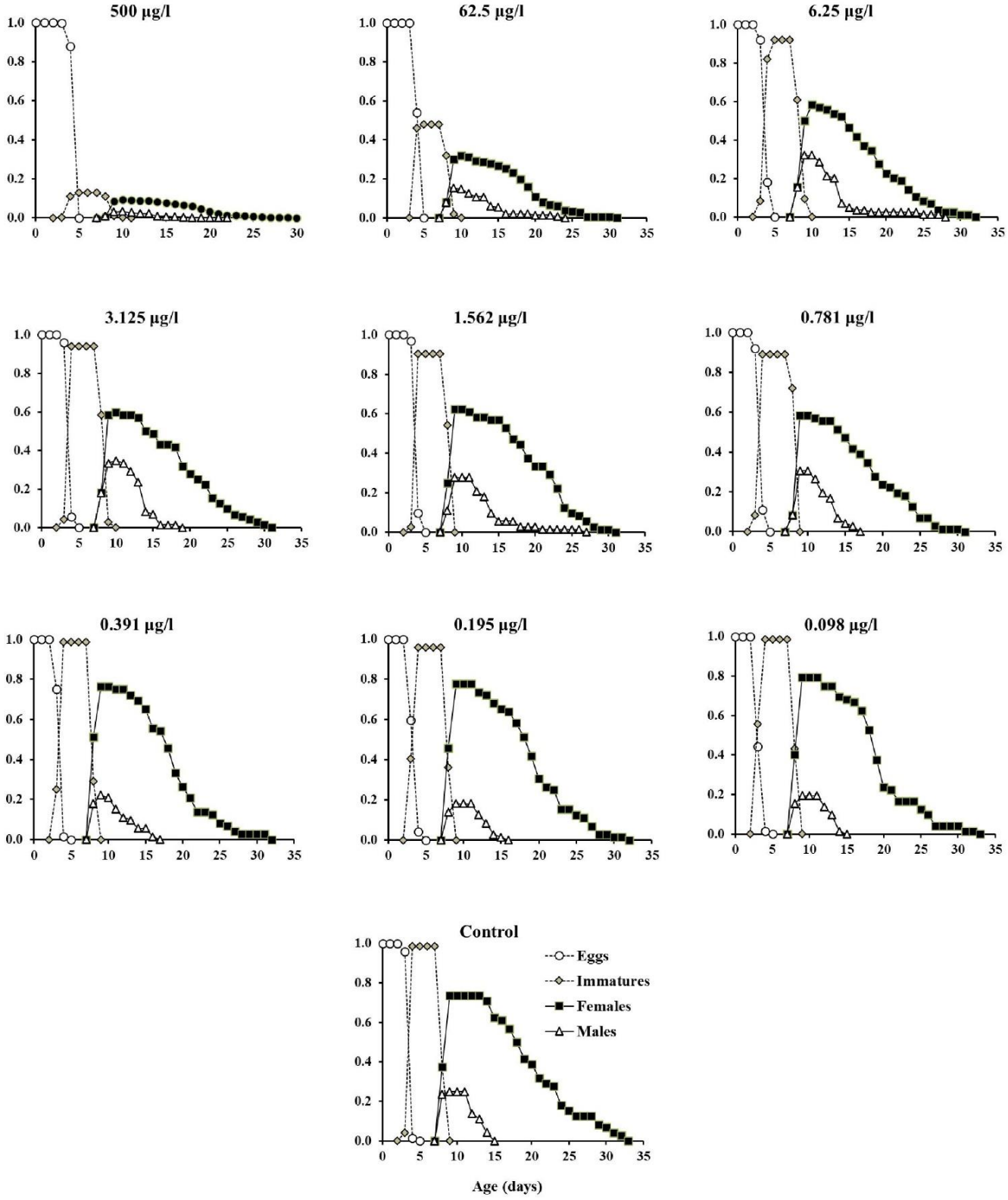


**Fig. 4.8.** Age-stage-specific survival rate ($s_{xj}$) of *T. urticae* after the treatment of eggs with hexythiazox (µg/l)

**Table 4.8.** Life history traits (mean ± SE) of *T. urticae* after the treatment of eggs with hexythiazox (μg/l)

| μg/l | N | *Sa* | Females | | | | | | Males | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Nf | *Pa* | TPOP | Fecundity* | Ovd | Long | Nm | *Pa* | Long |
| 500● | 780 | 0.13 d (± 0.01) | 73 | 9.03 e (± 0.05) | 10.24 a (± 0.07) | 46.68 cd (± 2.76) | 8.15 c (± 0.41) | 10.23 c (± 0.46) | 25 | 8.80 a (± 0.10) | 5.28 a (± 0.49) |
| 62.5● | 150 | 0.48 c (± 0.04) | 49 | 8.82 d (± 0.08) | 10.23 a (± 0.08) | 41.94 d (± 3.38) | 7.87 c (± 0.49) | 10.51 c (± 0.65) | 23 | 8.48 b (± 0.11) | 6.26 a (± 0.75) |
| 6.25● | 84 | 0.92 b (± 0.03) | 50 | 8.90 de (± 0.09) | 10.36 a (± 0.09) | 47.28 cd (± 3.83) | 8.66 bc (± 0.59) | 10.80 bc (± 0.68) | 27 | 8.52 b (± 0.10) | 5.89 a (± 0.74) |
| 3.125 | 72 | 0.94 ab (± 0.03) | 43 | 8.72 cd (± 0.08) | 10.12 ab (± 0.07) | 54.00 bc (± 4.80) | 9.63 ab (± 0.65) | 11.74 abc (± 0.75) | 25 | 8.52 b (± 0.12) | 5.52 a (± 0.36) |
| 1.562 | 72 | 0.90 b (± 0.03) | 45 | 8.60 bc (± 0.07) | 9.93 cd (± 0.06) | 61.80 ab (± 4.62) | 10.28 ab (± 0.64) | 12.53 ab (± 0.70) | 20 | 8.60 ab (± 0.11) | 6.35 a (± 0.84) |
| 0.781 | 72 | 0.89 b (± 0.04) | 42 | 8.86 d (± 0.05) | 10.02 bc (± 0.04) | 52.31 bc (± 4.26) | 8.54 bc (± 0.53) | 11.17 abc (± 0.75) | 22 | 8.73 ab (± 0.10) | 4.77 a (± 0.40) |
| 0.391 | 72 | 0.99 a (± 0.01) | 55 | 8.33 a (± 0.06) | 9.83 d (± 0.07) | 53.51 bc (± 3.57) | 9.04 abc (± 0.53) | 11.36 abc (±0.61) | 16 | 8.19 c (± 0.10) | 4.94 a (± 0.53) |
| 0.195 | 72 | 0.96 ab (± 0.02) | 56 | 8.41 ab (± 0.07) | 9.80 d (± 0.06) | 54.62 bc (± 3.31) | 9.09 abc (± 0.51) | 11.89 abc (± 0.62) | 13 | 8.23 bc (± 0.12) | 5.15 a (± 0.34) |
| 0.098 | 72 | 0.99 a (± 0.01) | 57 | 8.49 ab (± 0.07) | 9.93 cd (± 0.06) | 60.02 ab (± 4.10) | 9.62 ab (± 0.59) | 11.65 abc (± 0.62) | 14 | 8.21 c (± 0.11) | 5.07 a (± 0.32) |
| 0.0 | 72 | 0.99 a (± 0.01) | 53 | 8.49 ab (± 0.06) | 9.92 cd (± 0.07) | 70.72 a (± 4.98) | 10.66 a (± 0.69) | 13.17 a (± 0.71) | 18 | 8.06 c (± 0.06) | 5.11 a (± 0.28) |

Means in rows followed by different letters are signficantly different (the paired bootstrap test)
N = sample size (initial number of eggs in cohort); Nf = total number of females; Nm = total number of males;
*Sa* = preadult survival rate; *Pa* = preadult developmental time (days), TPOP = total preoviposition period;
Ovd = Oviposition days; Long = adult longevity (days); *eggs laid/**N**f
● concentrations within the 95% CLs of $LC_{90}$, $LC_{50}$ and $LC_{10}$ estimates from the acute toxicity bioassay

As a consequence of the reduction of $s_{xj}$ values in the treatments with the three highest concentrations, corresponding $l_x$ values were also considerably lowered, compared to the control. In other treatments, the $l_x$ values were also lower than the control ones over the entire oviposition period, with few exception (Fig. 4.9). The $m_x$ and $l_x m_x$ values in the treatments were mostly lower than the control ones. Although several $m_x$ values at the end of oviposition period were higher than the control, their contribution to the population growth was negligible.

Population parameters are detailed in Table 4.9. The treatments with the concentrations ranged 0.781-500 μg/l significantly reduced $R_0$ (by 41-92%), $r$ (by 12-64%), and $\lambda$ (by 3-16%), compared to the control. These reductions were mainly due to the reduction of *Sa* (i.e. ovicidal action) as well as reduced fecundity and longevity of females. The treatments with concentrations 0.391 and 0.195 μg/l slightly but significantly shortened *T*, compared to the control.

The $F_0$ generation bioassay showed that ovicidal action of hexythiazox had a decisive contribution to reducing population growth, whereas the negative effects on life history traits were less important. Hexythiazox concentrations ranging from 500 mg/l down to a 6400× lower concentration caused significant reduction of population growth after the egg treatment.

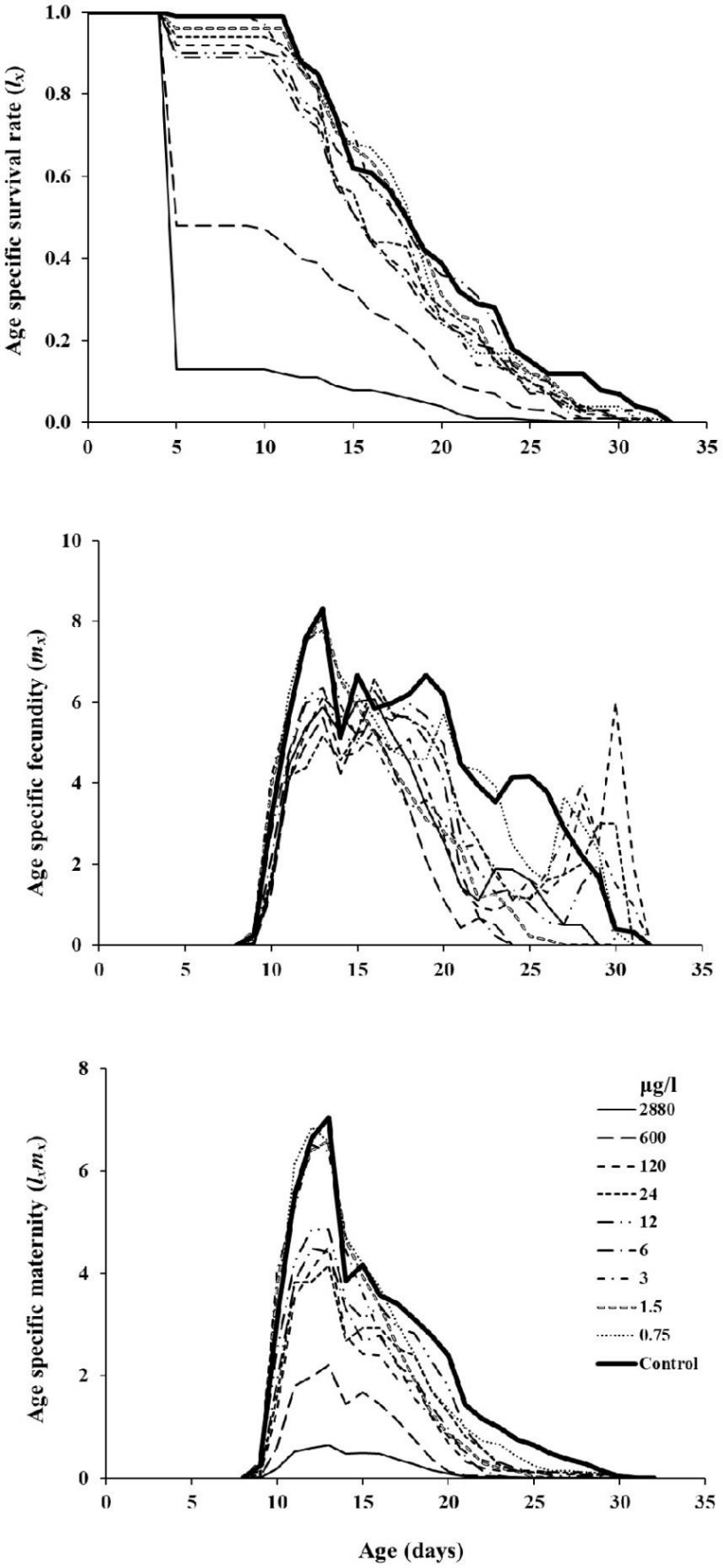


**Fig. 4.9.** Age-specific survival rate ($l_x$), age-specific fecundity ($m_x$) and age-specific maternity ($l_x m_x$) of *T. urticae* after the treatment of eggs with hexythiazox (µg/l)

**Table 4.9.** Population parameters (mean ± SE) of *T. urticae* after the treatment of egg with hexythiazox (μg/l)

| μg/l | N | $R_0$ (eggs laid/mite) | $r$ (days$^{-1}$) | $\lambda$ (days$^{-1}$) | $T$ (days) |
|---|---|---|---|---|---|
| 500● | 780 | 4.37 e (± 0.55) | 0.098 e (± 0.008) | 1.103 e (± 0.009) | 15.01 a (± 0.16) |
| 62.5● | 150 | 13.70 d (± 1.94) | 0.180 d (± 0.010) | 1.198 d (± 0.012) | 14.51 b (± 0.12) |
| 6.25● | 84 | 28.14 c (± 3.41) | 0.229 c (± 0.008) | 1.258 c (± 0.010) | 14.55 ab (± 0.20) |
| 3.125 | 72 | 32.25 bc (± 4.23) | 0.234 bc (± 0.009) | 1.264 bc (± 0.011) | 14.83 ab (± 0.23) |
| 1.562 | 72 | 38.62 b (± 4.54) | 0.249 b (± 0.008) | 1.283 b (± 0.010) | 14.65 ab (± 0.16) |
| 0.781 | 72 | 30.51 bc (± 3.92) | 0.238 bc (± 0.009) | 1.268 bc (± 0.011) | 14.39 b (± 0.15) |
| 0.391 | 72 | 40.88 ab (± 3.82) | 0.267 a (± 0.006) | 1.306 a (± 0.008) | 13.92 c (± 0.15) |
| 0.195 | 72 | 42.49 ab (± 3.71) | 0.269 a (± 0.006) | 1.308 a (± 0.008) | 13.95 c (± 0.13) |
| 0.098 | 72 | 47.51 a (± 4.34) | 0.270 a (± 0.006) | 1.310 a (± 0.007) | 14.31 bc (± 0.16) |
| 0.0 | 72 | 52.06 a (± 5.19) | 0.269 a (± 0.006) | 1.308 a (± 0.008) | 14.70 ab (± 0.19) |

Means in rows followed by different letters are significantly different (the paired bootstrap test)
N = sample size (initial number of eggs in cohort); $R_0$ = net reproductive rate;
$r$ = intrinsic rate of increase; $\lambda$ = finite rate of increase; $T$ = generation time
● concentrations within the 95% CLs of $LC_{90}$, $LC_{50}$ and $LC_{10}$ estimates from the acute toxicity bioassay

#### *4.2.2.2. $F_1$ generation bioassay*

The treatment of $F_0$ eggs with hexythiazox significantly affected several life history traits of $F_1$ generation of *T. urticae* (Tab. 4.10). Fecundity was significantly reduced (by 20-30%, compared to the control) in the treatments with concentrations ranged 0.195-500 μg/l (with the exception of 6.25 μg/l), while only the highest concentration significantly reduced female longevity and the number of oviposition days. On the other hand, in the treatments with the same range of concentrations (with few exceptions), *Pa* of both sexes and TPOP were slightly but significantly shortened.

The age-specific survival and reproduction of $F_1$ generation are presented in Figure 4.10. The $l_x$ values in the treatments were mostly lower than the control values over the entire lifetime. but not considerably. With several exceptions, $m_x$ and $l_x m_x$ values in the treatments were higher than the control ones over the initial four days of oviposition; in the treatment with concentration 6.25 μg/l more than a half of $m_x$ values were higher than the control.

Population parameters are detailed in Tab. 4.11. Reduction of $R_0$ was observed in the treatments with concentrations 500, 62.5, and 3.125 μg/l, by 25 – 42%, compared to the control. However, this reduction was insufficient to cause significant decrease in population growth rates. On the other hand, the highest $R_0$, $r$ and $\lambda$ values were found in the treatment with concentration 6.25 μg/l; the values were significantly higher than the values in treatments with both higher and 1-3 lower concentrations, but not than the control. In the treatments with concentrations ranged 0.195-500 μg/l (with the exception of 62.5 μg/l), $T$ was significantly shortened, compared to the control.

**Table 4.10.** Life history traits (mean ± SE) of the offspring of *T. urticae* after the treatment of $F_0$ eggs with hexythiazox (µg/l)

| µg/l | N | Females | | | | | | Males | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Nf | *Pa* | TPOP | Fecundity* | Ovd | Long | Nm | *Pa* | Long |
| 500● | 75 | 46 | 8.50 bc (± 0.07) | 9.74 bc (± 0.10) | 47.04 c (± 2.99) | 8.48 b (± 0.40) | 10.50 b (± 0.43) | 26 | 8.42 b (± 0.10) | 5.12 a (± 0.32) |
| 62.5● | 75 | 46 | 8.67 ab (± 0.07) | 10.02 ab (± 0.12) | 48.70 bc (± 4.33) | 9.63 ab (± 0.66) | 11.52 ab (± 0.70) | 27 | 8.44 b (± 0.10) | 5.26 a (± 0.28) |
| 6.25● | 75 | 56 | 8.41 c (± 0.07) | 9.53 d (± 0.09) | 57.16 ab (± 3.87) | 10.02 a (± 0.58) | 11.86 ab (± 0.64) | 18 | 8.33 b (± 0.11) | 5.44 a (± 0.34) |
| 3.125 | 75 | 40 | 8.50 bc (± 0.08) | 9.85 abc (± 0.12) | 43.68 c (± 4.01) | 8.40 b (± 0.63) | 10.75 ab (± 0.68) | 32 | 8.44 b (± 0.09) | 5.34 a (± 0.31) |
| 1.562 | 75 | 49 | 8.53 bc (± 0.07) | 9.75 bc (± 0.10) | 49.65 bc (± 4.40) | 8.67 ab (± 0.59) | 10.88 ab (± 0.59) | 26 | 8.38 b (± 0.10) | 5.19 a (± 0.37) |
| 0.781 | 75 | 52 | 8.44 c (± 0.08) | 9.61 cd (± 0.09) | 48.44 bc (± 3.75) | 9.49 ab (± 0.56) | 11.29 ab (± 0.60) | 19 | 8.42 b (± 0.11) | 4.47 a (± 0.35) |
| 0.391 | 75 | 49 | 8.47 c (± 0.07) | 9.74 bc (± 0.11) | 49.14 bc (± 4.27) | 9.05 ab (± 0.64) | 11.59 ab (±0.68) | 23 | 8.43 b (± 0.10) | 4.96a (± 0.52) |
| 0.195 | 75 | 52 | 8.36 c (± 0.07) | 9.65 cd (± 0.11) | 49.64 bc (± 4.08) | 8.67 ab (± 0.57) | 10.87 ab (± 0.61) | 20 | 8.40 b (± 0.11) | 5.15 a (± 0.57) |
| 0.098 | 75 | 54 | 8.69 ab (± 0.06) | 9.93 ab (± 0.10) | 53.06 ab (± 3.77) | 9.19 ab (± 0.53) | 11.44 ab (± 0.57) | 18 | 8.56 ab (± 0.12) | 5.00 a (± 0.49) |
| 0.0 | 75 | 48 | 8.78 a (± 0.08) | 10.10 a (± 0.10) | 62.25 a (± 4.70) | 10.15 a (± 0.67) | 12.44 a (± 0.67) | 27 | 8.77 a (± 0.07) | 5.63 a (± 0.45) |

Means in rows followed by different letters are signficantly different (the paired bootstrap test)
N = sample size (initial number of eggs in cohort); Nf = total number of females; Nm = total number of males;
*Pa* = preadult developmental time (days), TPOP = total preoviposition period;
Ovd = oviposition days; Long = adult longevity (days); *eggs laid/**N**f
● concentrations within the 95% CLs of $LC_{90}$, $LC_{50}$ and $LC_{10}$ estimates from the acute toxicity bioassay

**Table 4.11.** Population parameters (mean ± SE) of the offspring of *T. urticae* after the treatment of $F_0$ eggs with hexythiazox (µg/l)

| µg/l | N | $R_0$ (eggs laid/mite) | $r$ ($days^{-1}$) | $\lambda$ ($days^{-1}$) | $T$ (days) |
|---|---|---|---|---|---|
| 500● | 75 | 28.85 c (± 3.22) | 0.244 bc (± 0.008) | 1.277 bc (± 0.008) | 13.76 c (± 0.14) |
| 62.5● | 75 | 29.87 bc (± 3.79) | 0.235 bc (± 0.009) | 1.265 bc (± 0.011) | 14.46 ab (± 0.23) |
| 6.25● | 75 | 42.68 a (± 4.06) | 0.270 a (± 0.007) | 1.309 a (± 0.008) | 13.93 bc (± 0.17) |
| 3.125 | 75 | 23.29 c (± 3.30) | 0.225 bc (± 0.010) | 1.253 bc (± 0.013) | 13.96 bc (± 0.17) |
| 1.562 | 75 | 32.44 abc (± 3.96) | 0.249 bc (± 0.008) | 1.283 bc (± 0.011) | 13.97 bc (± 0.17) |
| 0.781 | 75 | 33.59 ab (± 3.66) | 0.252 ab (± 0.008) | 1.287 ab (± 0.010) | 13.95 bc (± 0.14) |
| 0.391 | 75 | 32.11 abc (± 3.88) | 0.247 bc (± 0.009) | 1.280 bc (± 0.011) | 14.05 bc (± 0.16) |
| 0.195 | 75 | 34.41 ab (± 3.85) | 0.256 ab (± 0.008) | 1.291 ab (± 0.010) | 13.85 c (± 0.16) |
| 0.098 | 75 | 38.20 ab (± 3.87) | 0.254 ab (± 0.007) | 1.289 ab (± 0.009) | 14.33 ab (± 0.14) |
| 0.0 | 75 | 39.84 ab (± 4.54) | 0.251 ab (± 0.008) | 1.285 ab (± 0.010) | 14.71 a (± 0.19) |

Means in rows followed by different letters are significantly different (the paired bootstrap test)
N = sample size (initial number of eggs in cohort); $R_0$ = net reproductive rate;
$r$ = intrinsic rate of increase; $\lambda$ = finite rate of increase; $T$ = generation time
● concentrations within the 95% CLs of $LC_{90}$, $LC_{50}$ and $LC_{10}$ estimates from the acute toxicity bioassay

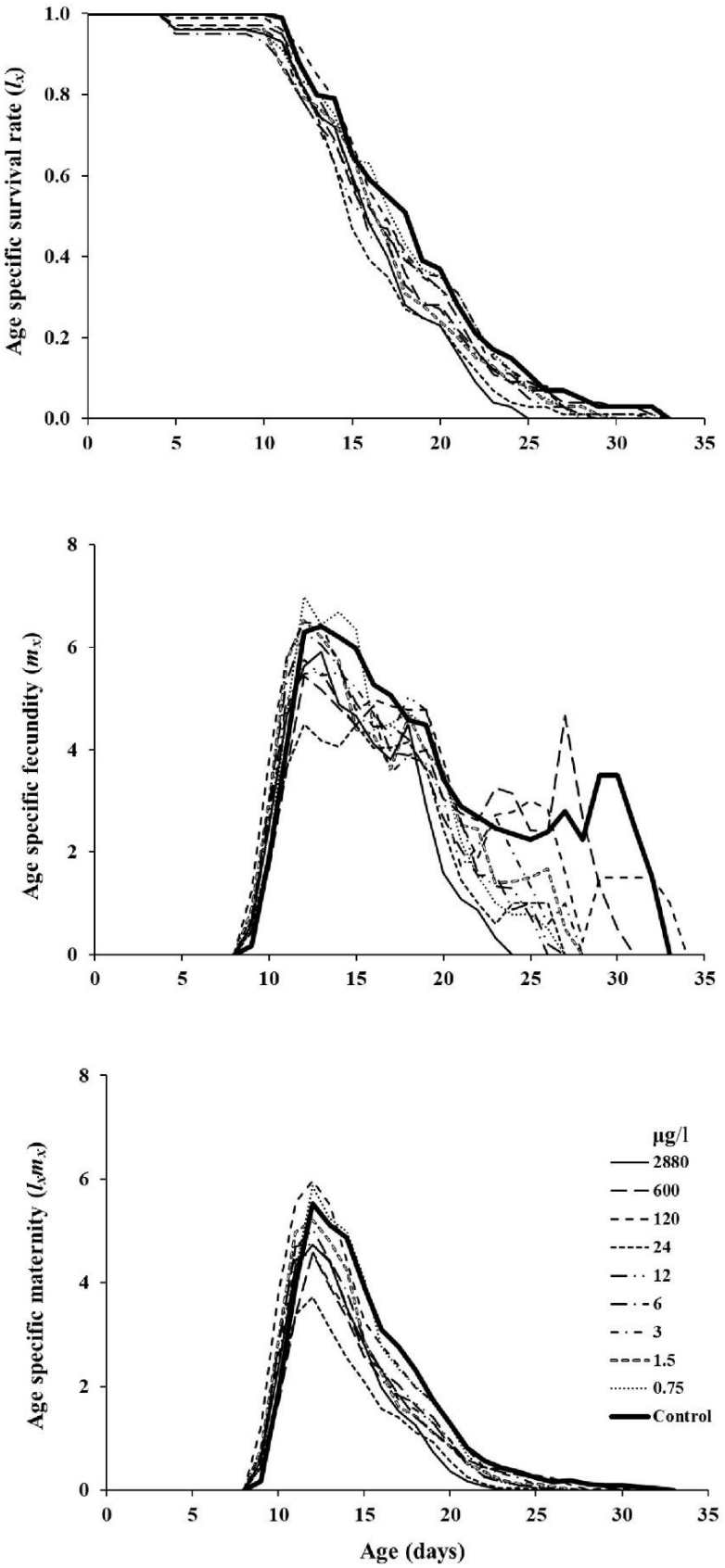


**Fig. 4.10.** Age-specific survival rate ($l_x$), age-specific fecundity ($m_x$) and age-specific maternity ($l_x m_x$) of the offspring of *T. urticae* after the treatment of $F_0$ eggs with hexythiazox (µg/l)

## 4.3. The effects of spirodiclofen on life history traits and population growth

### 4.3.1. Female treatment

#### *4.3.1.1. $F_0$ generation bioassay*

Exposure to spirodiclofen affected the longevity and reproduction of *T. urticae* females (Table 4.12). 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 was 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 4.12).

**Table 4.12.** Life history traits (mean ± SE) of *T. urticae* after the treatment of females with spirodiclofen (mg/l) (fecundity-based life tables)

| mg/l | Females | | | | | | | | Males | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Nf | Fecundity-I | Long-I | Nfr | Fecundity-II | Ovd | Long-II | Nfr/Nf | Nm | Long |
| 96 | 234 | 15.28 a (± 1.61) | 6.59 a (± 0.31) | 140 | 25.54 a (± 2.34) | 5.19 a (± 0.37) | 8.31 a (± 0.40) | 0.60 a (± 0.03) | 170 | 5.21 a (± 0.21) |
| 48 | 215 | 22.14 b (± 1.91) | 7.48 b (± 0.33) | 145 | 32.83 b (± 2.37) | 6.46 b (± 0.34) | 9.52 b (± 0.37) | 0.67 ab (± 0.03) | 169 | 5.24 a (± 0.19) |
| 24 | 127 | 29.20 b (± 3.11) | 8.65 c (± 0.48) | 89 | 41.66 c (± 3.72) | 8.06 c (± 0.53) | 10.89 c (± 0.52) | 0.70 b (± 0.04) | 108 | 5.63 ab (± 0.28) |
| 12 | 126 | 40.79 c (± 3.51) | 9.98 d (± 0.50) | 92 | 55.86 d (± 3.74) | 9.93 d (± 0.49) | 12.36 d (± 0.47) | 0.73 b (± 0.04) | 111 | 6.29 b (± 0.28) |
| 6 | 76 | 50.46 d (± 4.34) | 11.59 e (± 0.63) | 70 | 54.79 d (± 4.34) | 9.60 d (± 0.57) | 12.33 cd (± 0.61) | 0.92 d (± 0.03) | 66 | 6.26 b (± 0.36) |
| 3 | 75 | 49.95 d (± 4.06) | 11.37 e (± 0.62) | 64 | 58.53 de (± 3.86) | 10.78 de (± 0.51) | 12.88 d (± 0.54) | 0.85 cd (± 0.04) | 65 | 5.97 ab (± 0.35) |
| 1.5 | 47 | 56.40 d (± 7.08) | 11.36 e (± 1.01) | 36 | 73.64 e (± 7.00) | 12.06 e (± 0.93) | 14.00 d (± 0.93) | 0.77 bc (± 0.06) | 40 | 6.65 b (± 0.52) |
| 0.75 | 45 | 49.22 d (± 4.91) | 11.40 e (± 0.80) | 40 | 55.38 d (± 4.67) | 10.42 de (± 0.73) | 12.40 cd (± 0.76) | 0.89 cd (± 0.05) | 40 | 6.22 b (± 0.40) |
| 0.375 | 48 | 47.96 d (± 5.53) | 11.48 e (± 0.84) | 41 | 56.15 d (± 5.54) | 10.73 de (± 0.76) | 12.71 cd (± 0.79) | 0.85 cd (± 0.05) | 38 | 5.71 ab (± 0.42) |
| 0.0 | 35 | 57.51 d (± 6.72) | 12.86 e (± 1.01) | 32 | 62.91 de (± 6.56) | 11.66 de (± 0.94) | 13.81 d (± 0.93) | 0.91 cd (± 0.05) | 29 | 6.52 b (± 0.48) |

Means in rows followed by different letters are significantly different (the paired bootstrap test)
Nf = total number of females; Nfr = number of reproductive females; Nfr/Nf = proportion of reproductive females; Nm = total number of males; Fecundity-I = eggs laid/Nf; Fecundity-II = eggs laid/Nfr; Long = adult longevity (days); Ovd = oviposition days; Long-I = adult longevity of all females; Long-II = adult longevity of reproductive females

Figure 4.11 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 were laid). During that time period, the percentage of dead females rose to 17 – 55%, mostly as a result of the deaths of those females that failed to lay eggs, i.e. that were sterilized by the toxic action of spirodiclofen (Figure 4.11).

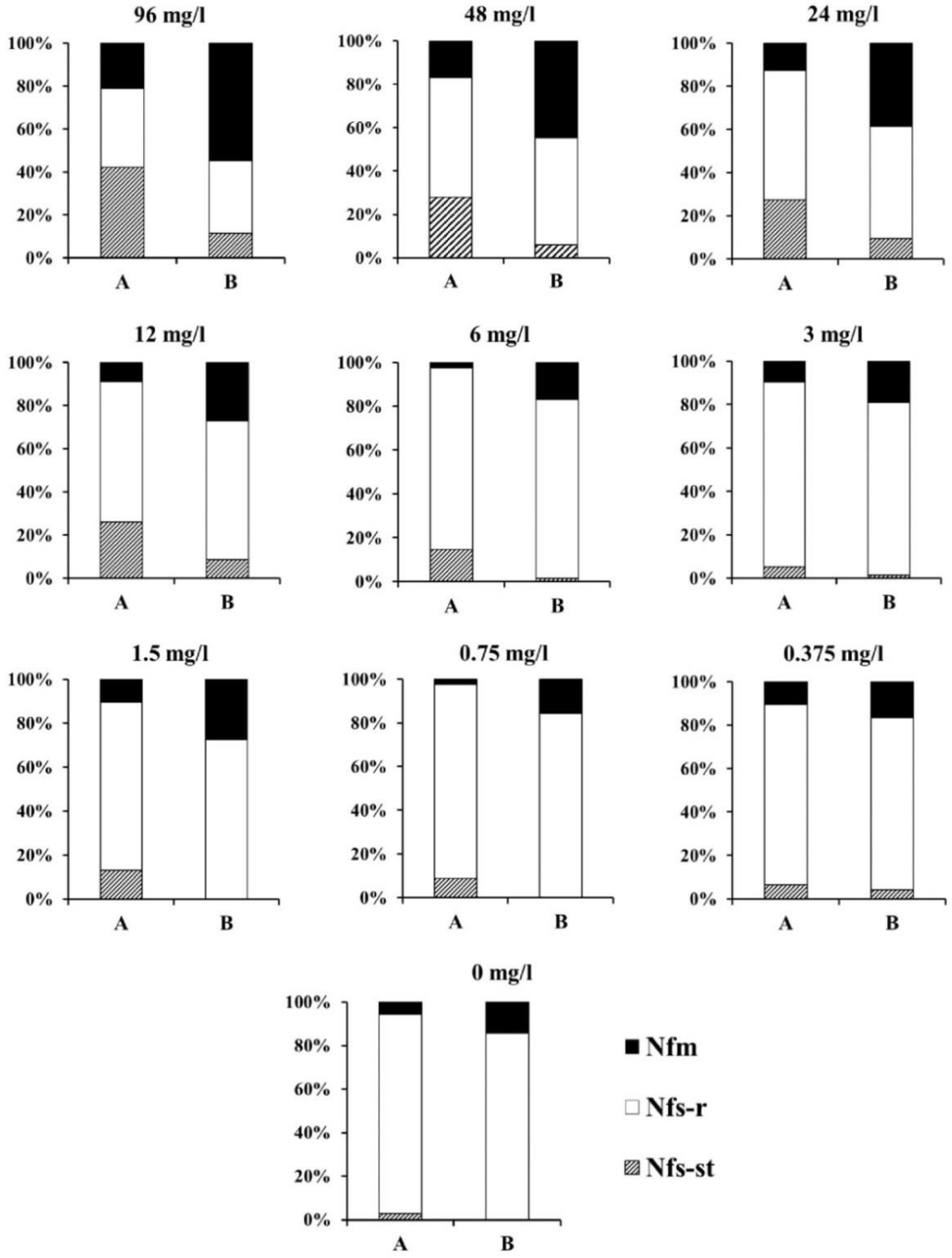


**Fig. 4.11.** Survival and reproduction of *T. urticae* females treated with spirodiclofen (mg/l) at the beginning of the preovipositional period; **Nfm** = dead females after 24-h exposure (A) and total number of dead females 96 h after treatment (B), **Nfs-r** = females that survived 24-h exposure 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

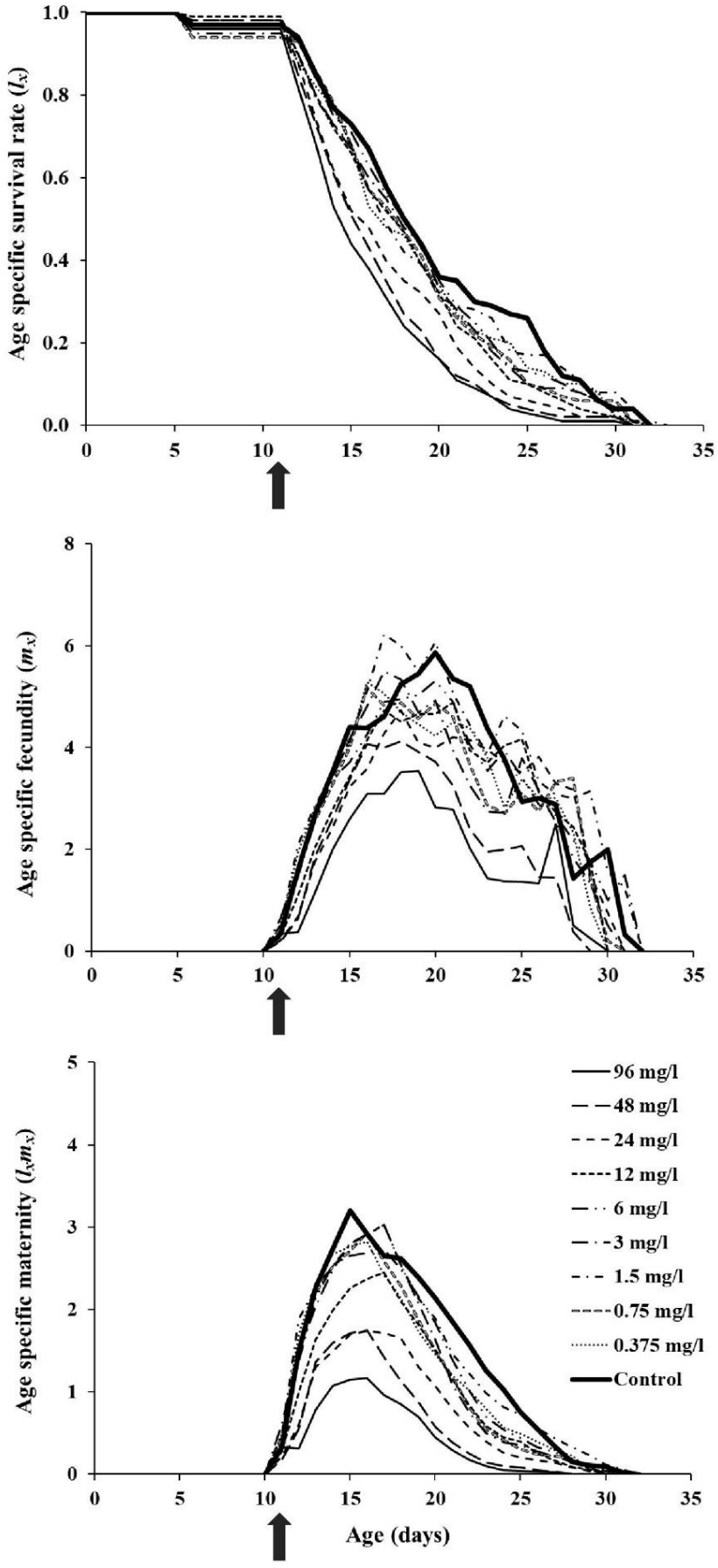


**Fig. 4.12.** Age-specific survival rate ($l_x$), age-specific fecundity ($m_x$) and age-specific maternity ($l_xm_x$) of *T. urticae* after the treatment of females with spirodiclofen (mg/l); arrow indicates the treatment

**Table 4.13.** Population parameters (mean ± SE) of *T. urticae* after the treatment of females with spirodiclofen (mg/l) (fecundity-based life tables)

| mg/l | N | $R_0$ (eggs laid/mite) | $r$ (days$^{-1}$) | $\lambda$ (days$^{-1}$) | $T$ (days) |
|---|---|---|---|---|---|
| 96 | 420 | 8.51 a (± 0.97) | 0.128 a (± 0.007) | 1.136 a (± 0.007) | 16.80 ab (± 0.17) |
| 48 | 390 | 12.21 b (± 1.20) | 0.150 b (± 0.006) | 1.162 b (± 0.006) | 16.71 a (± 0.13) |
| 24 | 240 | 15.45 b (± 1.89) | 0.157 b (± 0.007) | 1.170 b (± 0.008) | 17.44 c (± 0.19) |
| 12 | 240 | 21.41 c (± 2.25) | 0.176 c (± 0.006) | 1.192 c (± 0.007) | 17.41 c (± 0.15) |
| 6 | 147 | 26.09 c (± 3.07) | 0.189 c (± 0.006) | 1.208 c (± 0.008) | 17.27 bc (± 0.19) |
| 3 | 147 | 25.40 c (± 2.92) | 0.191 c (± 0.007) | 1.210 c (± 0.009) | 16.99 ab (± 0.17) |
| 1.5 | 90 | 29.46 c (± 4.72) | 0.195 c (± 0.009) | 1.215 c (± 0.011) | 17.36 bc (± 0.23) |
| 0.75 | 90 | 24.61 c (± 3.56) | 0.189 c (± 0.008) | 1.208 c (± 0.010) | 16.97 ab (± 0.21) |
| 0.375 | 90 | 25.58 c (± 3.87) | 0.191 c (± 0.009) | 1.210 c (± 0.010) | 17.01 abc (± 0.20) |
| 0.0 | 66 | 30.50 c (± 5.01) | 0.195 c (± 0.009) | 1.215 c (± 0.011) | 17.56 c (± 0.20) |

Means in rows followed by different letters are significantly different (the paired bootstrap test)
N = sample size (initial number of eggs in cohort); *GRR* = gross reproductive rate;
$R_0$ = net reproductive rate; $r$ = intrinsic rate of increase; $\lambda$ = finite rate of increase;
$T$ = generation time

The age-specific survival and reproduction of *T. urticae* are presented in Figure 4.12. Spirodiclofen treatment caused a concentration-dependent decrease in age-specific survival rates ($l_x$), compared to the control. Regarding reproduction, age-specific fecundity ($m_x$) in treatments was lower than control data over most of the oviposition period. The only exception was treatment with 1.5 mg/l concentration, where and $m_x$ values were mostly above control data. 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 $l_x m_x$ values exceeding control data.

Population parameters are detailed in Table 4.13. The three highest concentrations significantly reduced $R_0$, $r$ and $\lambda$ (by 49-72%, 20-34%, and 4-6%, respectively), compared to the control. In the treatments with concentration 96 mg/l, these three population parameters were significantly lower also in comparison with treatments with concentrations of 48 mg/l and 24 mg/l. The treatments with concentrations 96, 48, 3, and 0.75 mg/l significantly shortened $T$.

The fertility-based life table gave a similar picture of spirodiclofen impact on the life-history traits and demographic parameters of *T. urticae* (Table 4.14, Table 4.15). 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 of fertility-II was recorded in the treatment with 1.5 mg/l, but it was not significantly different from the control data (Table 4.14).

**Table 4.14.** Life history traits (mean ± SE) of *T. urticae* after the treatment of females with spirodiclofen (mg/l) (fertility-based life tables)

| mg/l | Nf | Fertility-I | Nfr | Fertility-II | Nfr/Nf |
|---|---|---|---|---|---|
| 96 | 234 | 13.62 a (± 1.51) | 88 | 36.22 a (± 2.62) | 0.38 a (± 0.03) |
| 48 | 215 | 20.97 b (± 1.83) | 129 | 34.95 a (± 2.35) | 0.60 b (± 0.03) |
| 24 | 127 | 27.31 b (± 3.00) | 76 | 45.63 b (± 3.74) | 0.60 b (± 0.04) |
| 12 | 126 | 39.19 c (± 3.39) | 88 | 56.11 c (± 3.59) | 0.70 bc (± 0.04) |
| 6 | 76 | 48.38 cd (± 4.17) | 70 | 52.53 bc (± 4.18) | 0.92 d (± 0.03) |
| 3 | 75 | 48.43 cd (± 3.93) | 64 | 56.75 cd (± 3.71) | 0.85 cd (± 0.04) |
| 1.5 | 47 | 54.87 d (± 6.82) | 36 | 71.64 d (± 6.78) | 0.77 cd (± 0.06) |
| 0.75 | 45 | 48.38 cd (± 4.78) | 40 | 54.42 bc (± 4.54) | 0.89 cd (± 0.05) |
| 0.375 | 48 | 46.67 cd (± 5.34) | 41 | 54.63 bc (± 5.34) | 0.85 cd (± 0.05) |
| 0.0 | 35 | 56.29 d (± 6.53) | 32 | 61.56 cd (± 6.39) | 0.91 d (± 0.05) |

Means in rows followed by different letters are significantly different (the paired bootstrap test)
Nf = total number of females; Nfr = number of reproductive females; Nfr/Nf = proportion of reproductive females; Fertility-I = viable eggs/Nf ; Fertility-II = viable eggs/Nfr

Compared to fecundity-I (Table 4.12), fertility-I was somewhat lower as maximum reduction was 11% in treatment with the highest concentration (Table 4.14). Fertility reduction was the 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 weak and 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.

Population parameters obtained from fertility-based life tables are detailed in Table 4.15. Spirodiclofen concentration that significantly reduced fertility-II (24 – 96 mg/l) also decreased $r$ and $\lambda$ significantly (by 23-40%, and 4-7%, respectively) compared to the control. The concentrations 96 mg/l and 48 mg/l significantly reduced $R_0$ (by 75% and 61%, respectively), compared to the control. 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). The treatments with concetrations 48 and 0.75 mg/l significantly shortened *T.*

**Table 4.15.** Population parameters (mean ± SE) of *T. urticae* after the treatment of females with spirodiclofen (mg/l) (fertility-based life tables)

| mg/l | N | $R_0$ (viable eggs/mite) | $r$ (days$^{-1}$) | $\lambda$ (days$^{-1}$) | $T$ (days) |
|---|---|---|---|---|---|
| 96 | 420 | 7.59 a (± 0.90) | 0.117 a (± 0.007) | 1.124 a (± 0.008) | 17.28 bcd (± 0.17) |
| 48 | 390 | 11.56 b (± 1.14) | 0.145 b (± 0.006) | 1.156 b (± 0.006) | 16.86 a (± 0.13) |
| 24 | 240 | 14.45 c (± 1.81) | 0.150 b (±0.007) | 1.162 b (± 0.008) | 17.75 d (± 0.19) |
| 12 | 240 | 20.58 c (± 2.18) | 0.173 c (± 0.006) | 1.189 c (± 0.007) | 17.50 cd (± 0.15) |
| 6 | 147 | 25.01 c (± 2.94) | 0.186 c (± 0.006) | 1.204 c (± 0.008) | 17.32 bcd (± 0.20) |
| 3 | 147 | 24.71 c (± 2.83) | 0.188 c (± 0.007) | 1.207 c (± 0.008) | 17.03 abc (± 0.17) |
| 1.5 | 90 | 28.66 c (± 4.59) | 0.194 c (± 0.009) | 1.214 c (± 0.011) | 17.33 bcd (± 0.23) |
| 0.75 | 90 | 24.19 c (± 3.51) | 0.188 c (± 0.008) | 1.207 c (± 0.010) | 16.96 ab (± 0.21) |
| 0.375 | 90 | 24.89 c (± 3.74) | 0.189 c (± 0.009) | 1.208 c (± 0.010) | 17.01 abc (± 0.21) |
| 0.0 | 66 | 29.85 c (± 4.89) | 0.194 c (± 0.009) | 1.214 c (± 0.011) | 17.55 cd (± 0.20) |

Means in rows followed by different letters are significantly different (the paired bootstrap test)
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; $\lambda$ = finite rate of increase; $T$ = generation time

Our demographic analysis showed that spirodiclofen significantly reduced population growth rates of *T. urticae* $F_0$ generation when it was applied against preovipositional 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.

#### *4.3.1.2. $F_1$ generation bioassay*

Table 4.16. shows life history traits of the offspring of *T. urticae* females treated with spirodiclofen. The weak transovarial toxic effect, observed in treatments with the three highest concentratrations (24 – 96 mg/l), caused significant reduction of *Sa*, compared to the control. Additionally, *Pa* of both sexes and TPOP of females were slightly but significantly extended in the treatments with concentrations ranged from 1.5 mg/l to 96 mg/l, while the two highest concentrations significantly reduced adult longevity of both sexes (by 1.6 – 1.7 days, and 1.2 – 1.6 days, respectively, compared to the control). On the other hand, there were no significant differences in fecundity among the treatments.

**Table 4.16.** Life history traits (mean ± SE) of the offspring of *T. urticae* after the treatment of $F_0$ females with spirodiclofen (mg/l)

| mg/l | N | *Sa* | Females | | | | | Males | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Nf | *Pa* | TPOP | Fecundity* | Long | Nm | *Pa* | Long |
| 96 | 90 | 0.73 b (± 0.05) | 39 | 11.05 a (± 0.08) | 12.33 bc (± 0.11) | 44.46 a (± 3.39) | 10.95 b (± 0.54) | 27 | 10.96 a (± 0.06) | 4.41 b (± 0.30) |
| 48 | 80 | 0.86 b (± 0.04) | 36 | 11.00 a (± 0.07) | 12.33 bc (± 0.09) | 47.47 a (± 4.48) | 10.89 b (± 0.70) | 33 | 10.91 a (± 0.05) | 4.09 b (± 0.33) |
| 24 | 80 | 0.86 b (± 0.04) | 38 | 10.95 ab (± 0.06) | 12.38 bc (± 0.10) | 47.32 a (± 3.97) | 11.58 a (± 0.59) | 31 | 10.87 ab (± 0.06) | 5.36 a (± 0.48) |
| 12 | 72 | 0.97 a (± 0.02) | 35 | 10.86 ab (± 0.06) | 12.69 a (± 0.09) | 40.60 a (± 4.53) | 11.20 a (± 0.67) | 35 | 10.86 ab (± 0.06) | 3.97 b (± 0.29) |
| 6 | 75 | 0.96 a (± 0.03) | 39 | 10.92 ab (± 0.04) | 12.46 ab (± 0.10) | 40.67 a (± 3.79) | 11.49 a (± 0.61) | 33 | 10.91 a (± 0.05) | 5.06 a (± 0.43) |
| 3 | 74 | 0.97 a (± 0.02) | 45 | 10.80 b (± 0.06) | 12.14 c (± 0.09) | 45.91 a (± 3.55) | 12.09 a (± 0.55) | 27 | 10.67 b (± 0.09) | 4.59 b (± 0.38) |
| 1.5 | 75 | 0.96 a (± 0.03) | 44 | 10.48 c (± 0.08) | 11.93 c (± 0.08) | 45.86 a (± 3.07) | 11.70 a (±0.50) | 28 | 10.29 c (± 0.09) | 4.75 a (± 0.40) |
| 0.75 | 74 | 0.97 a (± 0.02) | 39 | 10.15 d (± 0.06) | 11.51 e (± 0.08) | 48.08 a (±3.73) | 12.36 a (± 0.58) | 33 | 10.00 d (± 0.02) | 5.18 a (± 0.32) |
| 0.375 | 75 | 0.96 a (± 0.02) | 44 | 10.27 d (± 0.07) | 11.77 d (± 0.09) | 47.64 a (± 4.02) | 12.20 a (± 0.60) | 28 | 10.07 d (± 0.05) | 5.00 a (± 0.35) |
| 0.0 | 75 | 0.96 a (± 0.02) | 39 | 10.15 d (± 0.06) | 11.62 de (± 0.10) | 51.18 a (± 3.94) | 12.56 a (± 0.62) | 33 | 10.04 d (± 0.03) | 5.64 a (± 0.30) |

Means in rows followed by different letters are signficantly different (the paired bootstrap test)
N = sample size (number of eggs in cohort at the beginning of the life table study); Nf = total number of females in cohort; Nm = total number of males in cohort; *Sa* = preadult survival rate (Nf + Nm)/N; *Pa* = preadult developmental time (days); TPOP = total preoviposition period; Long = adult longevity (days); *eggs laid/**N**f

Due to reduction of *Sa*, the $l_x$ values of the offspring of *T. urticae* females treated with the three highest concentrations were lower than in the control almost over the entire lifetime, while in the other treatments they were mostly higher than control values (Fig. 4.13). In all treatments, the $m_x$ values were lower than in the control over the first 3-4 days of oviposition, as well as over a period of several days after the peak of oviposition. Resultingly, all $l_x m_x$ values in the treatment with 96 mg/l were lower than control values, while in the other treatments these values were mostly lower than in the control.

The population parameters are detailed in Table. 4.17. The r and $\lambda$ values were moderately but significantly reduced in the treatments with 96 mg/l due to significant reduction of *Sa* (i.e. transovarial toxicity) and adult longevity, as well as significant extension of *Pa* and *TPOP*. In other treatments, with the exception of 12 mg/l, reduction and//or extension of these parameters did not result in reduction of population growth.

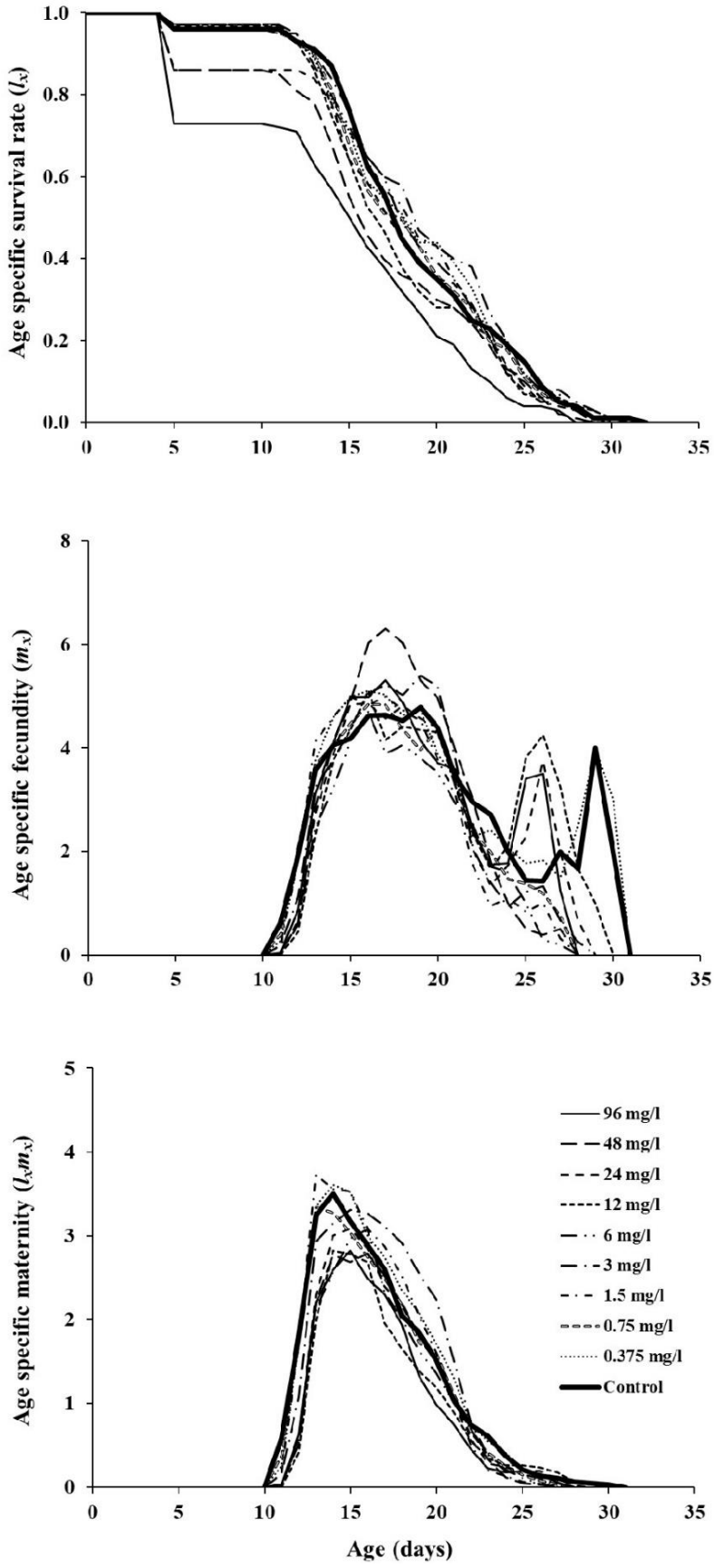


**Fig. 4.13.** Age-specific survival rate ($l_x$), age-specific fecundity ($m_x$) and age-specific maternity ($l_x m_x$) of the offspring of *T. urticae* after the treatment of $F_0$ females with spirodiclofen (mg/l)

**Table 4.17.** Population parameters (mean ± SE) of the offspring of *T. urticae* after the treatment of $F_0$ females with spirodiclofen (mg/l) (fecundity-based life tables)

| mg/l | N | $R_0$ (eggs laid/mite) | $r$ (days$^{-1}$) | $\lambda$ (days$^{-1}$) | $T$ (days) |
|---|---|---|---|---|---|
| 96 | 90 | 19.27 b (± 2.74) | 0.176 b (± 0.008) | 1.192 b (± 0.010) | 16.85 ab (± 0.20) |
| 48 | 80 | 21.36 ab (± 3.30) | 0.180 ab (± 0.009) | 1.197 ab (± 0.011) | 17.05 a (± 0.16) |
| 24 | 80 | 22.48 ab (± 3.23) | 0.182 ab (± 0.008) | 1.200 ab (± 0.010) | 17.06 a (± 0.19) |
| 12 | 72 | 19.74 ab (± 3.23) | 0.174 b (± 0.009) | 1.190 b (± 0.011) | 17.13 a (± 0.29) |
| 6 | 75 | 21.15 ab (± 3.06) | 0.180 ab (± 0.008) | 1.196 ab (± 0.010) | 17.04 a (± 0.16) |
| 3 | 74 | 27.92 a (± 3.38) | 0.196 ab (± 0.007) | 1.216 ab (± 0.009) | 16.99 a (± 0.18) |
| 1.5 | 75 | 26.91 a (± 3.15) | 0.201 a (± 0.007) | 1.222 a (± 0.008) | 16.40 b (± 0.15) |
| 0.75 | 74 | 25.34 ab (± 3.42) | 0.197 ab (± 0.008) | 1.218 ab (± 0.010) | 16.40 b (± 0.18) |
| 0.375 | 75 | 27.95 a (± 3.60) | 0.200 a (± 0.007) | 1.221 a (± 0.009) | 16.65 ab (± 0.19) |
| 0.0 | 75 | 26.61 ab (± 3.60) | 0.199 a (± 0.008) | 1.221 a (± 0.010) | 16.47 b (± 0.22) |

Means in rows followed by different letters are signficantly different (the paired bootstrap test)
N = sample size (number of eggs in cohort); $R_0$ = net reproductive rate; $r$ = intrinsic rate of increase; $\lambda$ = finite rate of increase; $T$ = generation time

### 4.3.2. Egg treatment

#### *4.3.2.1. $F_0$ generation bioassay*

As a consequence of the treatment of *T. urticae* eggs with the three highest spirodiclofen concentrations (2880, 600, and 120 µg/l, that fall within the 95% CLs of $LC_{90}$, $LC_{50}$, and $LC_{10}$ estimates from the acute toxicity bioassay) reduction in $s_{xj}$ was observed at the immature and adult stages (Fig. 4.14).

In these treatments, the *Sa* values were significantly reduced, as well as fecundity, female adult longevity and the number of oviposition days, (by 29 - 50%, 2.6 - 4.2 days, and 3.1 - 4.4 days, respectively), while *Pa* and TPOP were slightly but significantly extended, compared to the control (Tab. 4.18). The highest concentration also significantly extended *Pa* of males and reduced their adult longevity. Significant reduction and/or extension of life history traits were observed in some treatments with lower concentrations as well.

On the other hand, the maximum fecundity was recorded in treatment with 3 µg/l, and this value was significantly different from those recorded in all other treatments (except one); however, it was not different from the control (Tab. 4.18).

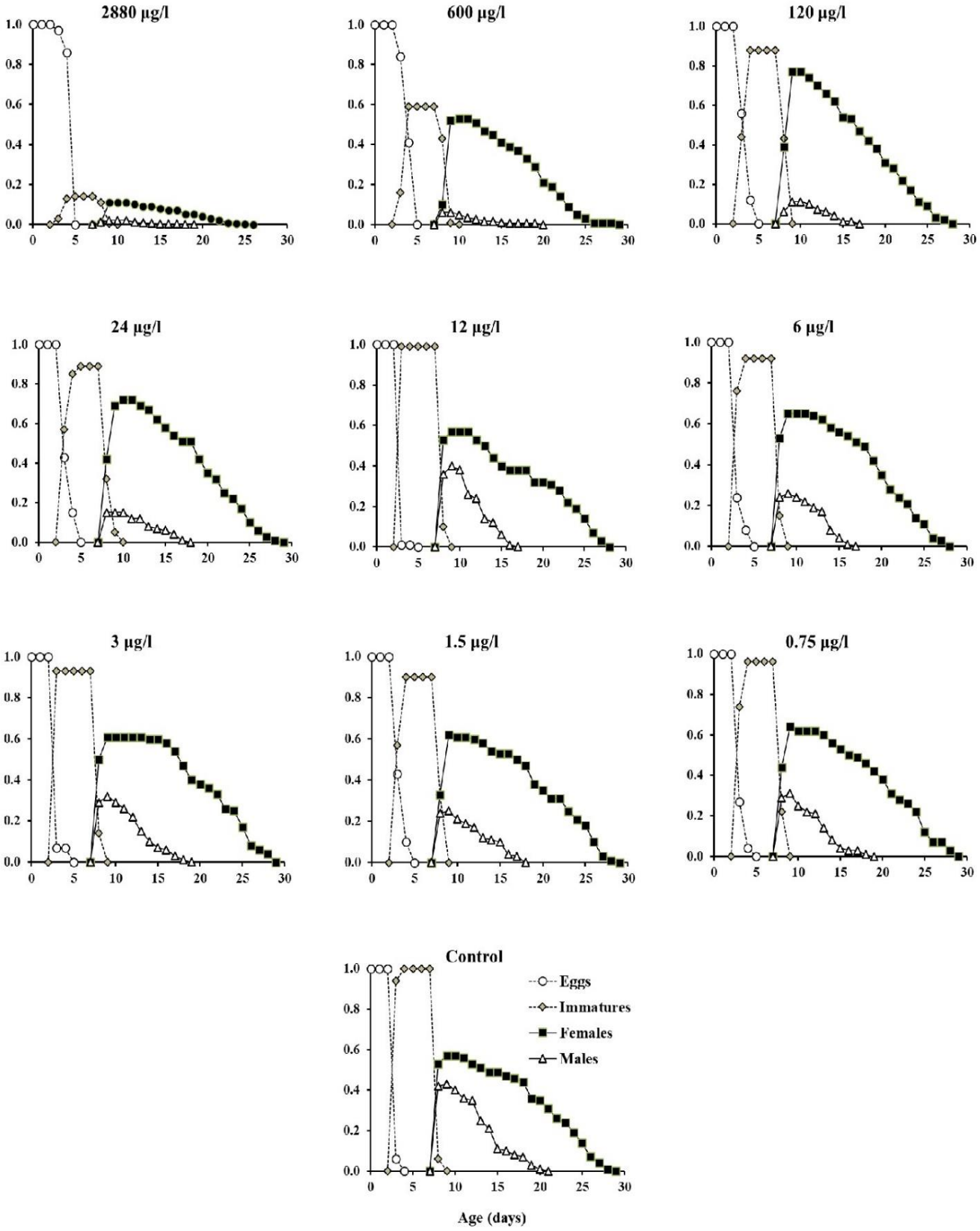


**Fig. 4.14.** Age-stage-specific survival rate ($s_{xj}$) of *T. urticae* after the treatment of eggs with spirodiclofen (µg/l)

**Table 4.18.** Life history traits (mean ± SE) of *T. urticae* after the treatment of eggs with spirodiclofen (μg/l)

| μg/l | N | *Sa* | Females | | | | | | Males | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Nf | *Pa* | TPOP | Fecundity* | Ovd | Long | Nm | *Pa* | Long |
| 2880● | 840 | 0.14 d (± 0.02) | 97 | 8.91 a (± 0.04) | 10.45 a (± 0.08) | 35.43 d (± 2.74) | 7.42 d (± 0.40) | 9.04 d (± 0.43) | 19 | 8.37 a (± 0.11) | 4.89 bc (± 0.61) |
| 600● | 150 | 0.59 c (± 0.04) | 80 | 8.84 a (± 0.05) | 10.20 b (± 0.06) | 50.65 c (± 3.34) | 8.79 c (± 0.45) | 10.59 c (± 0.49) | 9 | 8.00 c (± 0.00) | 5.22 abc (± 1.09) |
| 120● | 90 | 0.88 b (± 0.04) | 69 | 8.50 b (± 0.06) | 9.92 c (± 0.06) | 46.64 c (± 3.47) | 8.73 c (± 0.53) | 10.72 c (± 0.58) | 10 | 8.50 a (± 0.17) | 5.10 abc (± 0.60) |
| 24 | 72 | 0.89 b (± 0.04) | 53 | 8.50 b (± 0.08) | 10.02 bc (± 0.09) | 58.02 bc (± 4.56) | 9.72 bc (± 0.61) | 11.68 bc (± 0.67) | 11 | 8.00 c (± 0.00) | 6.36 ab (± 0.73) |
| 12 | 72 | 0.99 a (± 0.01) | 41 | 8.07 d (± 0.04) | 9.95 c (± 0.07) | 58.59 bc (± 5.51) | 10.02 b (± 0.78) | 12.46 bc (± 0.84) | 30 | 8.13 b (± 0.06) | 4.73 c (± 0.38) |
| 6 | 72 | 0.92 b (± 0.03) | 47 | 8.19 d (± 0.06) | 9.81 cd (± 0.06) | 66.00 ab (± 4.00) | 10.21 b (± 0.57) | 12.62 ab (± 0.62) | 19 | 8.11 bc (± 0.07) | 5.53 abc (± 0.46) |
| 3 | 72 | 0.93 b (± 0.03) | 44 | 8.18 d (± 0.06) | 9.75 d (± 0.09) | 75.36 a (± 4.30) | 11.86 a (± 0.56) | 14.20 a (±0.62) | 23 | 8.09 bc (± 0.06) | 5.65 abc (± 0.48) |
| 1.5 | 72 | 0.90 b (± 0.04) | 45 | 8.47 b (± 0.08) | 9.98 c (± 0.04) | 62.78 b (± 4.56) | 10.93 ab (± 0.64) | 12.89 ab (± 0.73) | 20 | 8.15 abc (± 0.08) | 5.25 abc (± 0.64) |
| 0.75 | 72 | 0.96 a (± 0.02) | 46 | 8.30 c (± 0.07) | 9.98 c (± 0.09) | 66.09 ab (± 5.04) | 10.60 ab (± 0.69) | 12.89 ab (± 0.72) | 23 | 8.09 bc (± 0.06) | 5.04 bc (± 0.53) |
| 0.0 | 72 | 1.00 a (± 0.00) | 41 | 8.07 d (± 0.04) | 9.66 d (± 0.09) | 70.80 ab (± 5.09) | 11.87 a (± 0.65) | 13.29 ab (± 0.76) | 31 | 8.03 bc (± 0.03) | 6.55 a (± 0.52) |

Means in rows followed by different letters are signficantly different (the paired bootstrap test)
N = sample size (number of eggs in cohort at the beginning of the life table study); Nf = total number of females in cohort; Nm = total number of males in cohort; *Sa* = preadult survival rate (Nf + Nm)/N; *Pa* = preadult developmental time (days); TPOP = total preoviposition period; Ovd = Oviposition days; Long = adult longevity (days); *eggs laid/**N**f
● concentrations within the 95% CLs of $LC_{90}$, $LC_{50}$ and $LC_{10}$ estimates from the acute toxicity bioassay

Due to reduction in $s_{xj}$ in the treatments with the three highest concentrations of spirodiclofen, the $l_x$ values were substantially lowered, compared to the control (Fig. 4.15). The $m_x$ and $l_x m_x$ values in these treatments were mostly lower than the control ones, while it was the opposite in the treatments with three lowest concentrations.

The population parameters $R_0$, $r$ and $\lambda$ were significanlty reduced only in the treatments with 2880 μg/l (by 90%, 63%, and 15%) and 600 μg/l (by 33%, 11%, and 3%) (Tab. 4.19) This reduction was mostly a consequence of reduction in *Sa*, as well as reductions in fecundity and longevity. In the treatment with 120 μg/l, in which the population parameters were not significanlty diferent from the control, reduction of *Sa* was not so pronounced. It implies that ovicidal action was decisive for reduction of population growth.

The highest $R_0$ value was recorded in the treatment with 3 μg/l, while the highest $r$ and $\lambda$ values were recorded in the treatment with 6 mg/l. Although significantly higher, compared to the treatments with two highest concentrations, none of these values, however, were significantly diferrent from the control (Tab. 4.19).

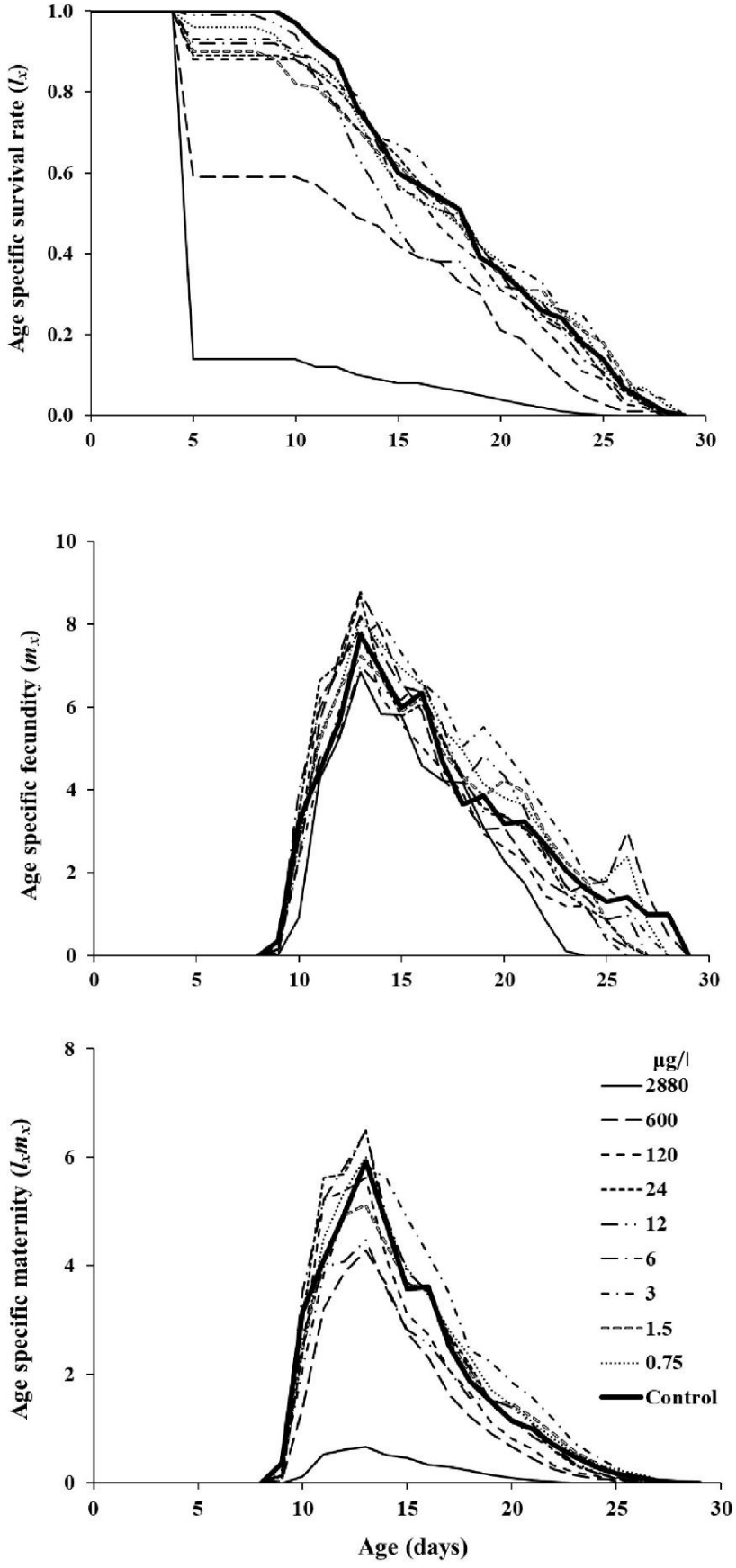


**Fig. 4.15.** Age-specific survival rate ($l_x$), age-specific fecundity ($m_x$) and age-specific maternity ($l_xm_x$) of *T. urticae* after the treatment of eggs with spirodiclofen (µg/l).

**Table 4.19.** Population parameters (mean ± SE) of *T. urticae* after the treatment of egg with spirodiclofen (μg/l)

| μg/l | N | $R_0$ (eggs laid/mite) | $r$ (days$^{-1}$) | $\lambda$ (days$^{-1}$) | $T$ (days) |
|---|---|---|---|---|---|
| 2880● | 840 | 4.09 c (± 0.50) | 0.095 c (± 0.008) | 1.099 c (± 0.009) | 14.87 a (± 0.12) |
| 600● | 150 | 27.01 b (± 2.72) | 0.228 b (± 0.007) | 1.256 b (± 0.008) | 14.44 b (± 0.11) |
| 120● | 90 | 35.76 a (± 3.38) | 0.254 a (± 0.006) | 1.289 a (± 0.008) | 14.10 c (± 0.10) |
| 24 | 72 | 42.71 a (± 4.50) | 0.264 a (± 0.007) | 1.302 a (± 0.009) | 14.23 c (± 0.12) |
| 12 | 72 | 33.36 ab (± 4.64) | 0.243 ab (± 0.010) | 1.275 ab (± 0.012) | 14.43 bc (± 0.13) |
| 6 | 72 | 43.08 a (± 4.56) | 0.265 a (± 0.008) | 1.304 a (± 0.010) | 14.18 c (± 0.12) |
| 3 | 72 | 46.06 a (± 5.06) | 0.256 a (± 0.007) | 1.292 a (± 0.009) | 14.94 a (± 0.14) |
| 1.5 | 72 | 39.24 a (± 4.58) | 0.251 a (± 0.008) | 1.285 a (± 0.010) | 14.63 ab (± 0.13) |
| 0.75 | 72 | 42.22 a (± 4.94) | 0.257 a (± 0.008) | 1.292 a (± 0.010) | 14.59 ab (± 0.13) |
| 0.0 | 72 | 40.32 a (± 5.04) | 0.257 a (± 0.009) | 1.294 a (± 0.011) | 14.36 bc (± 0.13) |

Means in rows followed by different letters are significantly different (the paired bootstrap test)
N = sample size (number of eggs in cohort); $R_0$ = net reproductive rate; $r$ = intrinsic rate of increase; $\lambda$ = finite rate of increase; $T$ = generation time
● concentrations within the 95% CLs of $LC_{90}$, $LC_{50}$ and $LC_{10}$ estimates from the acute toxicity bioassay

### *4.3.2.2. $F_1$ generation bioassay*

The treatment of $F_0$ eggs with spirodiclofen significantly affected life history traits of $F_1$ generation (Tab. 4.20). The three highest concentrations (2880, 600, and 120 μg/l) significantly shortened *Pa* of both sexes (but less than by one day) and *TPOP* of females (by 1 - 1.3 days), compared to the control; *TPOP* was also shortened in the treatments with 24, 6, and 3 μg/l. On the other hand, the three highest concentrations significantly reduced female fecundity, longevity, and the number of oviposition days (by 28 - 43%, 2.2 - 3.2 days, and 1.4 - 2.6 days, respectively, compared to the control). Fecundity was significantly reduced in the treatments with 24, 12, and 6 μg/l.

The age-specific survival and reproduction of $F_1$ generation are presented in Figure 4.16. With few exception, the $lx$ values in the treatments were lower than the control values over the entire lifetime. In the treatments with the three highest concentrations of spirodiclofen, the $m_x$ and $l_x m_x$ values were higher, compared to the control, while the values in other treatments were mostly lower than the control values. During the peak oviposition periood, the $m_x$ and $l_x m_x$ values in the control were higher than the treatment values.

The population parameters are presented in Table 4.21. As a result of reduced fecundity and longevity, $R_0$ was significanly lowered in treatments with the three highest concentrations (by 39-55%, compared to the control). However, $r$ and $\lambda$ were significantly reduced only in the treatment with 2880 μg/l. Lack of significant reduction of population growth in the treatments with 600 μg/l and 120 μg/l was probably a consequence of shortened development time and $T$. On the other hand, reduction of $R_0$ resulted in reduction of $r$ and $\lambda$ in the treatments with 6 μg/l and 3 μg/l, in spite of slight but significant shortening of TPOP in these treatments.

**Table 4.20.** Life history traits (mean ± SE) of the offspring of *T. urticae* after the treatment of $F_0$ eggs with spirodiclofen (µg/l)

| | | Females | | | | | | Males | | |
|---|---|---|---|---|---|---|---|---|---|---|
| µg/l | N | Nf | *Pa* | TPOP | Fecundity* | Ovd | Long | Nm | *Pa* | Long |
| 2880● | 75 | 47 | 8.28 c (± 0.07) | 9.72 d (± 0.10) | 35.47 c (± 3.25) | 7.37 c (± 0.49) | 9.34 c (± 0.54) | 28 | 8.39 b (± 0.09) | 4.46 abc (± 0.25) |
| 600● | 75 | 52 | 8.13 bc (± 0.05) | 9.47 e (± 0.08) | 44.63 bc (± 3.85) | 8.27 bc (± 0.52) | 10.29 bc (± 0.57) | 23 | 8.15 c (± 0.07) | 3.87 c (± 0.34) |
| 120● | 75 | 58 | 8.03 b (± 0.02) | 9.40 e (± 0.07) | 42.41 bc (± 4.04) | 8.55 bc (± 0.54) | 9.95 bc (± 0.61) | 17 | 8.06 c (± 0.06) | 5.00 bc (± 0.49) |
| 24 | 75 | 58 | 8.95 a (± 0.03) | 10.35 c (± 0.07) | 49.19 b (± 4.51) | 9.18 ab (± 0.61) | 11.16 ab (± 0.65) | 17 | 8.94 a (± 0.06) | 5.12 ab (± 0.44) |
| 12 | 75 | 55 | 8.97 a (± 0.02) | 10.58 ab (± 0.08) | 50.82 b (± 4.08) | 9.43 ab (± 0.56) | 11.82 ab (± 0.62) | 20 | 8.95 a (± 0.05) | 5.15 ab (± 0.31) |
| 6 | 75 | 45 | 8.95 a (± 0.02) | 10.41 bc (± 0.09) | 46.24 bc (± 4.90) | 9.66 ab (± 0.71) | 11.40 ab (± 0.78) | 30 | 8.97 a (± 0.03) | 4.80 bc (± 0.36) |
| 3 | 75 | 44 | 8.98 a (± 0.02) | 10.45 bc (± 0.08) | 53.50 ab (± 4.56) | 10.12 a (± 0.67) | 12.05 ab (± 0.71) | 31 | 8.97 a (± 0.03) | 5.81 a (± 0.36) |
| 1.5 | 75 | 55 | 8.96 a (± 0.03) | 10.66 ab (± 0.10) | 62.29 a (± 4.49) | 10.64 a (± 0.59) | 12.73 a (± 0.64) | 20 | 8.95 a (± 0.05) | 5.30 a (± 0.33) |
| 0.75 | 75 | 53 | 8.98 a (± 0.02) | 10.80 a (± 0.07) | 56.15 a (± 4.29) | 9.69 ab (± 0.52) | 12.17 a (± 0.61) | 22 | 8.95 a (± 0.05) | 5.23 ab (± 0.38) |
| 0.0 | 75 | 59 | 8.97 a (± 0.02) | 10.68 a (± 0.07) | 62.34 a (± 3.98) | 9.98 a (± 0.53) | 12.53 a (± 0.57) | 16 | 9.00 a (± 0.00) | 5.19 abc (± 0.59) |

Means in rows followed by different letters are signficantly different (the paired bootstrap test)
N = sample size (number of eggs in cohort); Nf = total number of females; Nm = total number of males;
*Pa* = preadult developmental time (days); TPOP = total preoviposition period;
Ovd = oviposition days; Long = adult longevity (days); *eggs laid/**N**f
● concentrations within the 95% CLs of $LC_{90}$, $LC_{50}$ and $LC_{10}$ estimates from the acute toxicity bioassay

**Table 4.21.** Population parameters (mean ± SE) of the offspring of *T. urticae* after the treatment of $F_0$ eggs with spirodiclofen (µg/l)

| µg/l | N | $R_0$ (eggs laid/mite) | $r$ (days$^{-1}$) | $\lambda$ (days$^{-1}$) | $T$ (days) |
|---|---|---|---|---|---|
| 2880● | 75 | 22.23 d (± 2.83) | 0.227 bc (± 0.008) | 1.254 bc (± 0.011) | 13.68 b (± 0.18) |
| 600● | 75 | 30.95 cd (± 3.57) | 0.250 a (± 0.007) | 1.285 a (± 0.009) | 13.70 b (± 0.18) |
| 120● | 75 | 32.80 c (± 3.73) | 0.255 a (± 0.007) | 1.291 a (± 0.009) | 13.66 b (± 0.17) |
| 24 | 75 | 38.04 abc (± 4.22) | 0.242 ab (± 0.007) | 1.273 ab (± 0.008) | 15.05 a (± 0.15) |
| 12 | 75 | 37.27 bc (± 3.97) | 0.238 abc (± 0.007) | 1.268 abc (± 0.008) | 15.22 a (± 0.15) |
| 6 | 75 | 27.75 cd (± 3.91) | 0.219 c (± 0.009) | 1.245 c (± 0.011) | 15.18 a (± 0.20) |
| 3 | 75 | 31.39 cd (± 4.03) | 0.226 bc (± 0.008) | 1.254 bc (± 0.010) | 15.25 a (± 0.12) |
| 1.5 | 75 | 45.68 ab (± 4.57) | 0.245 ab (± 0.006) | 1.278 ab (± 0.008) | 15.59 a (± 0.13) |
| 0.75 | 75 | 39.68 ab (± 4.22) | 0.241 ab (± 0.007) | 1.273 ab (± 0.009) | 15.26 a (± 0.12) |
| 0.0 | 75 | 49.04 a (± 4.30) | 0.254 a (± 0.006) | 1.290 a (± 0.007) | 15.31 a (± 0.12) |

Means in rows followed by different letters are signficantly different (the paired bootstrap test)
N = sample size (number of eggs in cohort); $R_0$ = net reproductive rate; $r$ = intrinsic rate of increase;
$\lambda$ = finite rate of increase; $T$ = generation time
● concentrations within the 95% CLs of $LC_{90}$, $LC_{50}$ and $LC_{10}$ estimates from the acute toxicity bioassay

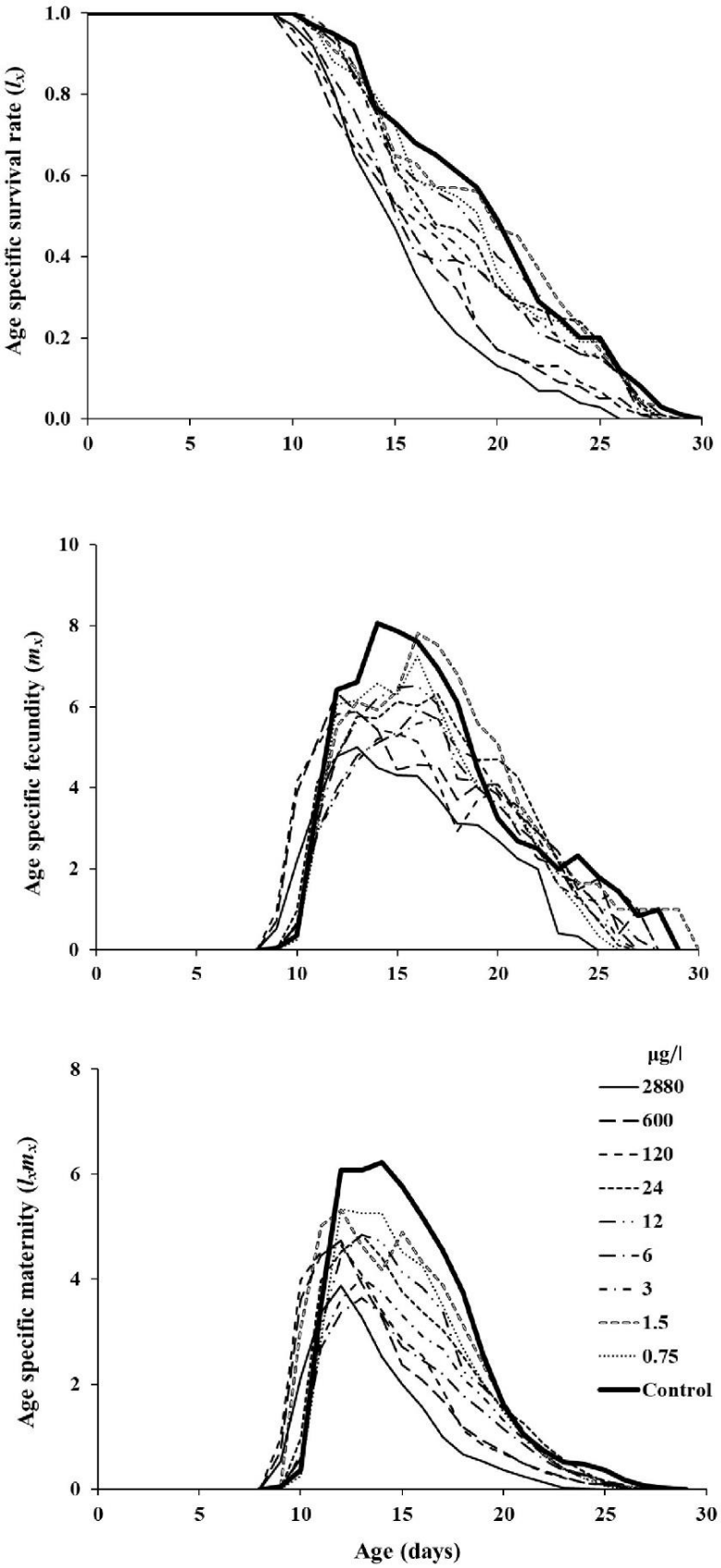


**Fig. 4.16.** Age-specific survival rate ($l_x$), age-specific fecundity ($m_x$) and age-specific maternity ($l_xm_x$) of the offspring of *T. urticae* after the treatment of $F_0$ eggs with spirodiclofen (µg/l)

## 4.4. Hexythiazox vs. spirodiclofen

Considering that *r* integrates lethal and sublethal effects of an acaricide at population level, we compared the effects of hexythiazox and spirodiclofen on this key population parameter. The comparison of *r* reduction following the treatments of *T. urticae* females (Table 4.22) showed that the transovarial toxic action was more important for the hexythiazox's effect.

Application of the fertility-based life tables in $F_0$ generation revealed significant effect of this acaricide applied in a much wider range of concentrations than spirodiclofen; the same was observed in $F_1$ generation.

**Table 4.22.** Comparison of *r* reduction (%)* after the treatment of $F_0$ females of *T. urticae* with hexythiazox and spirodiclofen (mg/l)

| Hexythiazox | | | | Spirodiclofen | | | |
|---|---|---|---|---|---|---|---|
| mg/l | $F_0$(fc) | $F_0$(fr) | $F_1$ | mg/l | $F_0$(fc) | $F_0$(fr) | $F_1$ |
| A | 6.7 | 24.0 | 68.3 | A | 34.4 | 39.7 | 11.6 |
| A/4 | 4.6 | 23.2 | 58.5 | A/2 | 23.1 | 25.2 | 9.5 |
| A/16 | 7.5 | 10.4 | 30.6 | A/4 | 19.5 | 22.7 | 8.5 |
| A/64 | - 1.7 | 11.8 | 17.7 | A/8 | 9.7 | 10.8 | 12.6 |
| A/128 | - 7.1 | 0.0 | 4.9 | A/16 | 3.1 | 4.1 | 9.5 |
| A/256 | 8.3 | 13.5 | 1.1 | A/32 | 2.1 | 3.1 | 1.5 |
| A/1024 | 2.5 | 4.2 | 10.2 | A/64 | 0.0 | 0.0 | - 1.0 |
| A/4096 | 4.2 | 7.6 | 13.6 | A/128 | 3.1 | 3.1 | 1.0 |
| | | | | A/256 | 2.1 | 2.6 | - 0.5 |

*$[1 - (r_{treatment}/r_{control})] \times 100$; the minus sign indicates stimulation

fc = fecundity-based life tables; fr = fertility-based life tables; A = the recommended field rates; Statistically significant reduction compared to the control is highlighted

**Table 4.23.** Comparison of *r* reduction (%)* after the treatment of $F_0$ eggs of *T. urticae* with hexythiazox and spirodiclofen (µg/l)

| Hexythiazox | | | Spirodiclofen | | |
|---|---|---|---|---|---|
| µg/l | $F_0$ | $F_1$ | µg/l | $F_0$ | $F_1$ |
| A● | 63.6 | 2.8 | A● | 63.0 | 10.7 |
| A/8● | 33.1 | 6.4 | A/4.8● | 11.3 | 1.6 |
| A/80● | 14.9 | - 7.6 | A/24● | 1.1 | - 0.4 |
| A/160 | 13.0 | 10.4 | A/120 | - 2.7 | 4.7 |
| A/320 | 7.4 | 0.8 | A/240 | 5.4 | 6.3 |
| A/640 | 11.5 | - 0.4 | A/480 | - 3.1 | 13.8 |
| A/1280 | 0.7 | 1.6 | A/960 | 0.4 | 11.0 |
| A/2560 | 0.0 | - 2.0 | A/1920 | 2.3 | 3.5 |
| A/5120 | - 0.4 | - 1.2 | A/3840 | 0.0 | 5.1 |

*$[1 - (r_{treatment}/r_{control})] \times 100$; the minus sign indicates stimulation

● concentrations within the 95% CLs of $LC_{90}$, $LC_{50}$ and $LC_{10}$ estimates

Statistically significant reduction compared to the control is highlighted

The *r* reduction following the egg treatments is presented in Table 4.23. The range of concentrations that caused significant *r* reduction in $F_0$ generation was much more wider for hexythiazox than spirodiclofen. This is consistent with the finding that hexythiazox is more toxc to the eggs of *T. urticae* than spirodiclofen. On the other hand, no significant reduction in $F_1$ generation was observed after the treatment with hexythiazox, while spirodiclofen caused significant reduction in the treatments with the highest and two other concentrations.

Taking into account the range of concentrations causing significant effects, the impact of hexythiazox on population growth of *T. urticae* can be evaluated as a stronger than the impact of spirodiclofen.

# 5. DISCUSSION

## 5.1. Acute toxicity of the acaricides

### 5.1.1. Hexythiazox

Hexythiazox, a thiazolidinone compound introduced more than 35 years ago, is still extensively used due to its broad spectrum of acaricidal activity against spider mites and other phytophagous mites, its long lasting control capacity and favorable ecotoxicological profile (Van Leeuwen et al. 2015; Bergeron and Schmidt-Jeffris 2020). As a mite growth inhibitor (MGI) targeting enzyme chitin synthase, hexythiazox has excellent toxic activity against eggs and immature stages (Yamada et al. 1987; Welty et al. 1988; Rathman et al. 1990).

Results obtained in our acute toxicity bioassays should be compared to other studies in which similar application method (direct spraying of eggs/females on leaf discs) was used. In order to assess acute toxicity of hexythiazox to the egg stage, Marris (1988) produced aqueous deposit 0.9 mg/cm$^2$ by spraying, and found $LC_{50}$ to be 1.6 mg/l, while Herron et al. (1993) estimated $LC_{50}$ to be 0.2 mg/l in a bioassay with the deposit 3.2 mg/cm$^2$. On the other hand, Adesanya et al. (2018) produced the deposit 2 mg/cm$^2$ after spraying and found $LC_{50}$ to be 2 mg/l. The deposit produced in our bioassay (2.2 mg/cm$^2$) was close to that of the previous study, but the estimated $LC_{50}$ (0.06 mg/l) was more than 30 times lower. These differences can be explained by different aqueous deposits, as well as natural variations in susceptibility among populations.

Due to its mode of action, hexythiazox is considered ineffective against adult female spider mites, but field recommended concentrations of this acaricide may still cause low-to-moderate toxicity. Moreover, by including a broad range of concentrations in bioassay, it is possible to estimate $LC_{50}$ values, bearing in mind that only the data showing the toxic effect of recommended and/or lower concentrations have practical consequence. Using the slide-dip technique (females were affixed dorsally on scotch double-sided tape on microscope slides, then the slides were shortly dipped in acaricide solution), Chapman and Marris (1986) estimated the $LC_{50}$ for *T. urticae* females after 24 h exposure to be 1.48 g/l (concentration 59× higher than the recommended rate). In recent studies with the same species, Bergeron and Schmidt-Jeffris (2020) sprayed females on leaf discs with 225 mg/l (maximum label rate) and observed 56% mortality after 48 h. Leviticus et al. (2020) used the leaf disc dip bioassay (the discs bearing mites were shortly dipped in acaricide solution) and Havasi et al. (2021) a residual bioassay (females were placed on the discs that were previously dipped in acaricide soluton) - both with 24-h exposure - and found $LC_{50}$ to be 187.52 mg/l and 2.35 g/l, respectively. In our demographic bioassay, female mortality of 18–23% following 24 h exposure was caused by hexythiazox concentrations ranging from the recommended rate (50 mg/l) to a 16× lower concentration (3.125 mg/l). The differences that were recorded are due to different methods of the acaricide application, duration of exposure and natural variations in susceptibility among the test populations.

During the development and early commercialization of hexythiazox it had been noted that *T. urticae* females treated with the acaricide lay eggs that later failed to hatch. This reduction of fertility, assumed to be caused by transovarial biotransference of the uptaken acaricide through the maternal body to eggs, was found to be relatively short-lived. Spraying of females with its recommended field concentration (25 mg/l), and 1 h exposure to residues on leaf discs reduced by 66% the hatching of eggs laid over 3 weeks of oviposition on untreated leaf discs. A 10× lower concentration of hexythiazox caused 41% reduction, whereas 18% of control eggs failed to hatch. The hatching pattern revealed that eggs laid during the first 2 days did not hatch at all, whereas the number of hatched eggs increased later. Over the following 2 days, hatching in the group treated with the lower concentration reached the level of control hatching, whereas the same level was

achieved by the higher concentration only during the last 2 days of the trial (Chapman and Marris 1986; Marris 1988). A similar pattern was observed by Yamada et al. (1987), who treated females with 31 and 7.8 mg/l of hexythiazox, a difference being that the control-level hatching in both treatments was reached after 4 days. In another recent study, Adesanya et al. (2018) sprayed *T. urticae* females on leaf discs with a series of hexythiazox concentrations, then transferred them to unsprayed leaf discs after 24 h exposure, and allowed them to oviposit for additional 24 h. Based on the number of unhatched eggs, the authors determined a concentration-mortality response and estimated the $LC_{50}$ for egg mortality at 24 mg/l.

In our $F_0$ bioassay, the recovery of egg viability occurred under a pattern similar to the one described by Marris (1988): transovarial toxic effect of hexythiazox was the most evident on eggs laid on the first day after treatment, and weaker on eggs laid on the second and third day, whereas the fourth-day egg viability in most treatments approached the viability of control eggs. Hatching reduction of eggs laid on the first day, which was 15–84% (values corrected for the percentage of unhatched control eggs) was achieved in treatments with concentrations that ranged from label concentration to 4167-fold lower concentration. In the $F_1$ generation bioassay, hatching reduction for eggs laid on the first day after treatment was 13–87% (corrected values). Based on the results of both trials, the estimated $LC_{50}$ would probably be between 12.5 and 3.125 mg/l, which would be below the $LC_{50}$ estimated by Adesanya et al. (2018). On the other hand, maximum reduction in our trial was below the 100% reduction reported by Yamada et al. (1987) and Marris (1988) after treatments with lower concentrations. Such variability is most probably caused by natural differences among test populations.

### 5.1.2. Spirodiclofen

Spirodiclofen, a tetronic acid derivative, has been the most widely sold acaricide in the world (Sparks et al. 2020). As an inhibitor of acetyl coenzyme carboxylase (ACC), spirodiclofen is highly active against all developmental stages of spider mites. Acute toxicity bioassays yielded different estimation of LCs for eggs, depending on spirodiclofen application methods and natural variations among *T. urticae* populations. In a bioassay performed by spraying the whole plant with 100 ml of liquid, $LC_{90}$ estimated to be 0.75 mg/l (Wachendorff et al. 2002; Nauen 2005). After spraying eggs on leaf discs, producing 1.5 mg/cm$^2$ aqueous deposit, Van Pottelberge et al. (2009b) found $LC_{50}$ to be 8.7 mg/l, while Saryazdi et al. (2013) sprayed 3 ml of liquid under 50 kPa air pressure and estimated $LC_{50}$ to be 0.64 mg/l (deposit was not measured). The latter $LC_{50}$ was close to our estimation.

Spirodiclofen causes unique symptoms in treated *T. urticae* females: their bodies grow to unusually large size and weight, due to inability to lay eggs which accumulate in the body (a part of females is being sterilized i.e. didn't lay eggs), and it take most females several days to die (Nauen 2005; Marčić 2007; Van Pottelberge et al. 2009a). The unique symptomology of poisoning was also observed in treated females in our demographic bioassay. In this bioassay, female mortality >50% was found only in the treatment with 96 mg/l, after 24-h exposure followed by 96 h on untreated surface. On the other hand, spraying the whole plants with 100 ml of liquid yielded $LC_{90}$ estimation to be 4.9 mg/l, after 48-h exposure (Wachendorff et al. 2002; Nauen 2005). Using leaf disc dip method, Saber et al. (2018) and Sani et al. (2019) estimated $LC_{50}$ to be 9.07 mg/l and 3.11 mg/l, respectively, after 24-h exposure. In a bioassay carried out by spraying females on leaf disc with concentrations ranged 6 – 96 mg/l, producing 4 mg/cm$^2$ aqueous deposit, Marčić (2007) found female mortality of 29 – 75%, after 72-h of exposure.

Spirodiclofen also reduces fertility due to a short-lived transovarial toxic action to the eggs laid by treated females (Wachendorff et al. 2002; Nauen 2005). Marčić (2007) found that fertility of *T. urticae* females treated with 6, 12 and 24 mg/l was reduced by 42, 84 and 97%, respectively. Fertility reduction was at its highest over the initial three days following treatment. Van Pottelberge et al. (2009a) exposed females for 12 h to the residues on leaf discs sprayed with 200 mg/l and

observed viability reduction of 59% in eggs laid on the first day after the treatment, while Saryazdi et al. (2013) found fertility reduction of 33 – 42% in females treated with $LC_{25}$ (0.36 mg/l) over three days after the treatment. Short-lived reductions in fertility (with a maximum of 32% on the first day) has been also revealed in our demographic bioassay, after the treatments of *T. urticae* females with 24, 48 and 96 mg/l of spirodiclofen.

## 5.2. The acaricides' effects on life history traits and population growth

### 5.2.1. Hexythiazox: toxicity to the eggs is decisive

The effects of hexythiazox on population growth of *T. urticae* have been evaluated in few studies. Chapman and Marris (1986) tested the label and 10× lower concentrations of hexythiazox and noted that transovarial toxicity can contribute to population growth reduction but there was no further in-depth research. Sekulić (1995) assessed *T. urticae* population growth after hexythiazox treatment of eggs and juveniles (at $LC_{90}$), using the female age-specific life table. Recently, Havasi et al. (2021) assessed population growth in the offspring of *T. urticae* females treated with hexythiazox, using the age-stage two-sex life table, and found no significant effect on $r$ and $\lambda$. The authors dipped leaf discs in acaricide solutions (0.9–1.6 g/l) and exposed adult females to fresh acaricide residues for 24 h. The surviving females were transferred to untreated discs and laid eggs for an additional 24 h, and the eggs were then used for providing initial cohorts for the life table study. According to the $l_x$ curves presented in the paper, no significant reduction in survival (i.e. no transovarial toxicity to eggs) was observed after the first five days. Although the applied concentrations of hexythiazox were considerably higher than recommended, the residual exposure of females was not sufficient to achieve significant effects. This finding is consistent with a report by Chapman and Marris (1986), who compared direct spraying and residual exposure of *T. urticae*, and found that the latter did not markedly affect egg viability. As for the other MGIs, Havasi et al. (2019) reported no significant effects of diflovidazin on population growth of the offspring of *T. urticae* females exposed to the acaricide's residues.

The life table studies with hexythiazox and other plant-inhabiting mites are scarce. Using the female age-specific life table, El-Sharabasy et al. (2015) assessed life history traits and population parameters of predatory mite *Phytoseiulus persimilis* treated with hexythiazox at the egg stage, while Havasi et al. (2021) compared population growth of the offsprings of predatory mite *Amblyseius swirskii* and *T. urticae*, following treatments of the $F_0$ females.

In our female treatment bioassays with direct spraying, the mortality of preovipositional and young ovipositional *T. urticae* females caused in the $F_0$ generation by hexythiazox concentrations of 50, 12.5 and 3.125 mg/l lowered their $l_x$ values and crucially affected $R_0$, but not population growth rates. Unlike the fecundity-based life table, the fertility-based life table revealed significant reduction in fertility and population growth in the treatments with a wide range of concentrations (50–0.195 mg/l), caused mainly by the acaricide transovarial toxicity. The fertility-based variant of age-stage two-sex life table is undoubtely more labour-intensive. However, its application is necessary when evaluating acaricides that exhibit transovarial toxicity. Besides hexythiazox, transovarial toxicity to *T. urticae* eggs has also been observed in the MGIs clofentezine, diflovidazin, and etoxazole (Chapman and Marris 1986; Pap et al. 1996; Van Leeuwen et al. 2012; Adesanya et al. 2018), flucycloxurone, an inhibitor of chitin biosynthesis (Grosscurt et al. 1988), as well as in spirodiclofen and spiromesifen, the inhibitors of acetyl CoA carboxylase (ACC) (Marčić 2007; Van Pottelberge et al. 2009a; Marčić et al. 2010). Otherwise, the use of fecundity-based life tables would underestimate population-level effects of these acaricides on spider mites.

The demographic rule that initial reproduction primarily determines the population rate of increase of *r*-selected species (Birch 1948; Snell 1978) was confirmed by the results of our bioassays. Relatively short-lived but significant transovarial toxicity that occurred in the initial 4

days after treatment of preovipositional and young ovipositional females (when around 50% of eggs were laid), crucially affected the *r* and $\lambda$ in the $F_0$ generation. This transgenerational effect was most evident regarding eggs laid within the initial 24 h after treatment but it may be assumed to stay on as significant over the following 3–4 days of oviposition, primarily in treatments with higher concentrations. Apart from the decisive impact of transovarial toxicity that reduced $s_{xj}$ at the egg stage, population growth reduction in the $F_1$ generation was also contributed by transgenerational sublethal effects on life-history traits – causing a small but significant extension of TPOP and reduction in fecundity (associated with reduction in oviposition duration in days and female longevity). TPOP determines population growth in such a way that the earlier an egg is laid the greater is its contribution to *r*. The relation between the age of first reproduction and fecundity is such that a 10% decrease in the former generally has the same effect as a 100% increase in the latter (Snell 1978).

The egg treatment bioassay showed that ovicidal action of hexythiazox was decisive for reduction in population growth of the $F_0$ generation of *T. urticae*, and this reduction was much stronger, compared to the female treatment bioassay. On the other hand, population growth of the $F_1$ generation was considerably reduced in the latter, while no significant effects were observed in the former, where shortening of *Pa* and TPOP compensated reduction in fecundity and longevity. To the best of our knowledge, there have been no the age-stage two-sex demographic studies in which the eggs of *T. urticae* were directly treated with hexythiazox or the other MGIs.

### 5.2.2. Spirodiclofen: sterilization makes a difference

Demographic analyses of the effects of spirodiclofen on population growth of plant-inhabiting mites have been carried out in several studies so far. Using the female age-specific life table, Marčić (2007) found significant reduction in $R_0$, *r* and $\lambda$ following treatment of preovipositional *T. urticae* females with 12 mg/l. The age-stage two-sex life table have been used in recent studies. Saber et al. (2018) treated *T. urticae* females at the $LC_{25}$ level and observed significant reductions in $R_0$, *r* and $\lambda$ of the offspring of treated females. Sani et al. (2019) found that treatment of *T. urticae* females at the $LC_{15}$ and $LC_{35}$ levels reduced significantly the same parameters in their offspring. Exposure of females of the citrus red mite, *Panonychus citri* McGregor, to $LC_{20}$ also caused significant reduction in population growth of the $F_1$ generation (Ahmed and Abdelwines 2021). In studies with predatory mites *A. swirskii* (Alinejad et al. 2016) and *Neoseiulus californicus* (Sarbaz et al. 2017), population-level effects of spirodiclofen were assessed on the $F_1$ generation following treatment of parental females with 1-2 lethal acaricide concentrations. The effects of spiromesifen, another ACC inhibitor, have also been assessed on the offspring of treated *T. urticae* females (Bozhgani et al. 2019; Rajaee et al. 2022).

In our $F_0$ generation bioassay with spirodiclofen, the fecundity-based life table revealed that a significant reduction in population growth was mainly due to sterilization of treated 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, accompanied by accumulation of eggs in the genital tract and body swelling, had been reported in previous studies with spirodiclofen (Nauen 2005; Marčić 2007; Van Pottelberge et al. 2009a). 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 toxic effect did not reduce significantly the fertility, nor consequently the population parameters, compared to the same parameters acquired in the fecundity-based life table. The $F_1$ generation bioassay showed that extension of *Pa* and *TPOP* was the most striking transgenerational effect in the treatments with a wider range of concentrations. However, reduced population growth was observed only in two treatments, as the result of combination of extended developmental time with transovarial toxicity and reduced longevity (the highest concentration), or reduced fecundity

(12 mg/l).

In the egg treatment bioassay with the $F_0$ generation, ovicidal action of the two highest concentrations of spirodiclofen, as well as extended *Pa* and TPOP, and fecundity and longevity reduction, resulted in reduction of population growth. In the $F_1$ generation, shortening of *Pa* and TPOP compensated fecundity and longevity reductions. There have been no the age-stage two-sex demographic studies in which the eggs of *T. urticae* were directly treated with spirodiclofen or spiromesifen.

### 5.2.3. Do the acaricides stimulate *T. urticae*?

Testing a wide range of concentrations in life table bioassays is important because it may reveal hormesis i.e. the concentration-response relationship in which low-concentrations cause stimulating effect on life history traits and population parameters, whereas high concentrations act inhibitory. Besides this basic qualitative characteristic, hormetic response also has its quantitative properties: maximum magnitude of stimulating response is mostly 30–60% greater than control magnitude and occurs within a concentration range (hormetic zone) which is up to 10-fold lower than the highest concentration which has no significant effect (Guedes and Cutler 2014).

Stimulation of fecundity and/or population growth of spider mites have been observed in a number of studies (e.g. Iftner and Hall 1984; Jones and Parella 1984; He et al. 2011; Wang et al. 2016; Međo et al. 2017; Zanardi et al. 2018). However, only Cordeiro et al. (2013) carried out a study with a wide range of acaricide concentrations and demonstrated that the effect of deltamethrin on population growth rate of *Oligonychus ilicis* fit hormetic concentration-response model.

Our testing of a wide range of hexythiazox and spirodiclofen concentrations revealed that the concentration-response relationship regarding fecundity, fertility, longevity, as well as $R_0$, $r$, and $\lambda$ in several cases was non-linear. The higher concentrations significantly reduced these parameters, treatments with the mid-range concentrations resulted in maximum values of these parameters, whereas the lower concentrations caused reductions which were not necessarily significant, compared to the control. These maximums were significantly greater than the values in treatments with higher and lower concentrations (not all of them) but did not differ significantly from control values. Such examples of stimulation were mostly caused by the concentrations entering the hormetic zone. However, besides the lack of significant differences from the control, the maximum magnitude of stimulation was no more than 17%. Taking into account the qualitative and quantitative properties of hormesis, our results evidently divert from that type of concentration-response relationship. On the other hand, considering the importance of proper experimental design (Cutler 2013; Calabrese et al. 2019), it is possible that a bioassay with more concentrations within the hormetic zone would have revealed response that fit the hormetic model i.e. a response comprising at least one concentration that causes a stimulating response significantly greater than the control.

## 5.3. Implications to the pest management

High reproduction of young and fertilized adult females is crucial for the population biology of *T. urticae* as a colonizing species. Its natural populations are often close to the stable age distribution in which eggs, immatures, and adults account for around 66%, 26%, and 8%, respectively (Carey 1982; Sabelis 1985; Li and Margolies 1993). Fecundity reduction in the females as the main dispersers weakens the recovery potential of a population on poorly covered or uncovered plant surfaces, assuming heterogeneity of spray coverage in the field (Martini et al. 2012).

In order to assess the implications to the management of *T. urticae*, the profiles of biological activity (acaricide toxicity to the eggs and females, and the effects on $r$) obtained in this study

should be discussed in the context of population biology of this species. Taking into account the biological profile of hexythiazox, it can be presumed that the treatment with 0.781 mg/l – the concentration above the upper limit of the $LC_{90}$ estimation in egg toxicity bioassay and 64-fold lower than the recommended field rate – will eliminate 90% of eggs (i.e. around 60% of population with the stable age distribution) and reduce *r* of survivors from female treatment up to 20%. Regarding spirodiclofen, the corresponding concentration is 6 mg/l (16-fold lower than the recommended), but it is not capable of significant *r* reduction. The treatment with 24 mg/l (4-fold lower than the recommended concentration) is needed to achieve both egg elimination and significant *r* reduction.

These results and presumptions have a practical value. Application of the acaricides at reduced concentrations leaves a possibility for combining them with predatory mites (as biological control agents of spider mites) within an integrated spider mite management program (Duso et al. 2020). The most significant natural enemies of spider mites are predatory mites in the family Phytoseiidae, which include more than 30 species commercialized as biological control agents (Knapp et al. 2018). The choice of acaricides compatible with phytoseiid mites implies assessing an acceptable level of their detrimental effects. Several studies with hexythiazox and spirodiclofen (Alinejad et al. 2016; Sarbaz et al. 2017; Döker and Kazak 2019; Bergeron and Schmidt-Jeffris 2020; Havasi et al. 2021; Schmidt-Jeffris et al. 2021) have shown more favourable impact on some predatory mites of the former than the latter. Nevetheless, considering that not all predators are equal, there is often a need to evaluate compatibility of acaricides with predatory species and/or strains important in local environments. Considering that testing under field conditions is more realistic, a complementary approach to the evaluation of selectivity, which integrates laboratory, semi-field and field data, is needed as a sustainable solution.

# 6. Conclusions

1) This disertation is the first demographic analysis of the effects of acaricides hexythiazox and spirodiclofen on the $F_0$ and $F_1$ generations of *T. urticae* carried out using the age-stage two-sex life table.

2) Acute toxicity bioassays and a pairwise comparison of LCs between the acaricides showed that hexythiazox was significantly more toxic to directly treated eggs of *T. urticae* than spirodiclofen: 9-fold times at the $LC_{50}$ level, and 6-fold at the $LC_{90}$ level.

3) Although both acaricides caused a short-lived transovarial toxic effect on the eggs laid by treated females (i.e. reduction in their fertility), this effect was decisive for the impact on population growth rates after the treatment with hexythiazox, but not with spirodiclofen.

4) Application of the fecundity-based variant of age-stage two-sex life table in the female bioassay underestimated population-level effects of hexythiazox on the $F_0$ generation of *T. urticae*. The values of population growth rates $r$ and $\lambda$ in the treatments with the concentrations ranged from the recommended one to 64-fold lower - obtained from this variant of life table (which ignores transovarial toxicity) - were significantly higher, compared to those obtained from the fertility-based variant of age-stage two-sex life table. The population growth rates obtained from the latter were significantly lowered, compared to the control, while those obtained from the former were not. Transovarial toxic effect had a decisive contribution to reducing the population growth rates of the $F_1$ generation as well, while the influence on life history traits was less important.

5) Spirodiclofen significantly reduced the $r$ and $\lambda$ values of the $F_0$ generation of *T. urticae* in the female bioassay 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 the population growth rates of *T. urticae*, compared to those obtained from the fecundity-based variant. This effect contributed to population growth reduction of the $F_1$ generation, together with extension of development time and longevity reduction, but only in treatment with the highest concentrations.

6) Application of the fertility-based variant of age-stage two-sex life table (although more labour-intensive) is necessary in bioassays with acaricides that exhibit transovarial toxicity in order to achieve a more precise assessment of their population-level effects on spider mites.

7) Ovicidal action of hexythiazox was decisive for reduction in the population growth rates of the $F_0$ generation of *T. urticae*, following the egg treatment with the concentrations ranged from the recommended one to 640-fold lower. No significant effects on population growth of the $F_1$ generation were observed, in which shortening of development time compensated reduction in fecundity and longevity.

8) Ovicidal action of the two highest concentrations of spirodiclofen contributed to reduction in the population growth rates of the $F_0$ generation of *T. urticae*, following the egg treatment, together with longevity reduction and development time extension. In the $F_1$ generation, shortening of development time compensated fecundity and longevity reductions.

9) Considering the range of concentrations of hexathiazox and spirodiclofen causing significant reduction in the population growth rates of *T. urticae*, it can be concluded that the impact of the former is stronger than that of the latter.

10) Testing of a wide range of hexythiazox and spirodiclofen concentrations revealed that the maximum values of some life history traits and the population growth rates can be observed in the treatments with the mid-range concentrations. This stimulative effects, however, diverted from the hormetic model of concentration-response relationship.

11) The results of demographic analysis of hexathiazox and spirodiclofen effects have practical implications to the management of *T. urticae* populations. These acaricides can be applied at reduced concentrations leaving a possibility for combining them with the predatory mites as biological control agents within an integrated spider mite management program.

# 7. References


**Adesanya A.W., Lavine M., Moural T.W., Lavine L., Walsh D.B., Zhu F. 2021.** Mechanisms and management of acaricide resistance for *Tetranychus urticae* in agroecosystems. J. Pest Sci. 94: 639-663.

**Adesanya A.W., Morales M.A., Walsh D.B., Lavine M., Lavine L., Zhu F. 2018.** Mechanisms of resistance to three mite growth inhibitors of *Tetranychus urticae* in hops. Bull. Entomol. Res. 108: 23-34.

**Ahmed M.M., Abdelwines M.A. 2021.** Sublethal effects of cyflumetofen and spirodiclofen on biological parameters of citrus red mite, *Panonychus citri* McGregor (Acari: Phytoseiidae). Pers. J. Acarol. 10: 467-480.

**Alinejad M., Kheradmand K., Fathipour Y. 2015.** 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. Acarina 23: 172-180.

**Alinejad M., Kheradmand K., Fathipour Y. 2016.** Assessment of sublethal effects of spirodiclofen on biological performance of the predatory mite *Amblyseius swirskii*. Syst. Appl. Acarol. 21: 375-384.

**Bayu M.S., Ullah M.S., Takano Y., Gotoh T. 2017.** Impact of constant versus fluctuating temperatures on the development and life history parameters of *Tetranychus urticae* (Acari: Tetranychidae). Exp. Appl. Acarol. 72: 205–227.

**Bergeron P.E., Schmidt-Jeffris R.A. 2020.** Not all predators are equal: miticide non-target effects and differential selectivity. Pest Manag. Sci. 76: 2170-2179.

**Birch L.C. 1948.** The intrinsic rate of natural increase of an insect population. J. Animal Ecol. 17: 15-26.

**Bozhgani N.S.S., Ghobadi H., Riahi E. 2018.** Sublethal effects of chlorfenapyr on the life table parameters of two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae). Syst. Appl. Acarol. 23: 1342-1351.

**Bozhgani N.S.S., Kheradmand K., Talebi A.A. 2019.** The effects of spiromesifen on life history traits and demographic parameters of predatory mite *Neoseiulus californicus* (Acari: Phytoseiidae) and its prey *Tetranychus urticae* Koch (Acari: Tetranychidae). Syst. Appl. Acarol. 23:1512-1525.

**Calabrese E.J., Agathokleous E., Kozumbo W.J., Stanek III E.J., Leonard D. 2019.** Estimating the range of the maximum hormetic stimulatory response. Environ. Res. 170: 337-343.

**Carey J.R. 1982**. Demography of the twospotted spider mite *Tetranychus urticae* Koch. Oecologia 52: 389-395.

**Carey J.R. 1993.** Applied demography for biologists, with special emphasis on insects. New York – Oxford: Oxford University Press.

**Chapman R.B., Marris J.W.M. 1986.** The sterilising effect of clofentezine and hexythiazox on female twospotted mite. Proceedings of 39th New Zealand Weed and Pest Control Conference, Canterbury, New Zealand, pp 237-240.

**Chi H. 1988.** Life table analysis incorporating both sexes and variable development rates among individuals. Environ. Entomol. 17: 26-34.

**Chi H. 2021.** TWOSEX-MSChart: a computer program for the age-stage, two-sex life table analysis. http://140.120.197.173/Ecology/

**Chi H., You M., Atlihan R., Smith C.L., Kavousi A., Özgökçe M.S. *et al*. 2020.** Age-stage, two-sex life table: an introduction to theory, data analysis, and application. Entomol. Gen. 40: 103-124.

**Chi H., Güncan A., Kavousi A., Gharakhani G., Atlihan R., Özgökçe M.S. *et al.* 2022.** TWOSEX-MSChart: the key tool for life table research and education. Entomol. Gen. 42: 845-849.

**Cordeiro E.M.G., de Moura I.L.T., Fadim M.A.M., Guedes R.N.C. 2013.** Beyond selectivity: are behavioral avoidance and hormesis likely causes of pyrethroid-induced outbreaks of the southern red mite *Oligonychus ilicis*? Chemosphere 93: 1111-1116.

**Cutler G.C. 2013.** Insects, insecticides and hormesis: evidence and consideration for study. Dose-Response 11: 154-177.

**Döker I., Kazak C. 2019.** Non-target effects of five acaricides on a native population of *Amblyseius swirskii* (Acari: Phytoseiidae). Int. J. Acarol. 45: 69-74.

**Douris V., Steinbach D., Panteleri R., Livadaras I., Pickett J.A., Van Leeuwen T. *et al.* 2016.** Resistance mutation conserved between insects and mites unravels the benzoylurea insecticide mode of action on chitin biosynthesis. PNAS 113: 14692-14697.

**Duso C., Van Leeuwen T., Pozzebon A. 2020.** Improving the compatibility of pesticides and predatory mites: recent findings on physiological and ecological selectivity. Curr. Opin. Insect Sci. 39: 63-68.

**Efron B., Tibshirani R.J.1993.** An introduction to the bootstrap. New York: Chapman & Hall.

**El-Sharabasy H.M. 2015.** Effects of some acaricides on life table parameters of the predatory mite, *Phytoseiulus macropilis* fed on the two-spotted spider mites. J. Appl. Plant Prot. 4: 23-26.

**Finney D.J. 1971.** Probit analysis, 2nd edition. Cambridge, UK: Cambridge University Press.

**Goodman D. 1982.** Optimal life histories, optimal notation, and the value of reproductive value. Am. Nat. 119: 803-823.

**Grosscurt A.C., ter Haar M., Jongsma B., Stoker A. 1988.** PH 70-23: a new acaricide and insecticide interfering with chitin deposition. Pestic. Sci. 22: 51-59.

**Guedes R.N.C., Cutler G.C. 2014.** Insecticide-induced hormesis and arthropod pest management. Pest Manag. Sci. 70: 690-697.

**Guedes R.N.C., Smagghe G., Stark J.D., Desneux N. 2016.** Pesticide-induced stress in arthropod pests for optimized integrated pest management programs. Annu. Rev. Entomol. 61: 43-62.

**Havasi M., Bozhgani N.S.S., Golmohmmadi G., Kheradmand K. 2021.** Impact of hexythiazox on life table parameters of the *Amblyseius swirskii* (Acari: Phytoseiidae) and its prey *Tetranychus urticae*. J. Crop Prot. 10:.295-308.

**Havasi M., Kheradmand K., Mosallanejad H., Fathipour Y. 2019.** Sublethal effects of diflovidazin on life table parameters of two-spotted spider mite *Tetranychus urticae* (Acari: Tetranychidae). Int. J. Acarol. 44: 115-120.

**He H.G., Jiang H.B., Zhao Z.M., Wang J.J. 2011.** Effects of a sublethal concentration of avermectin on the development and reproduction of citrus red mite, *Panonychus citri* (McGregor) (Acari: Tetranychidae). Int. J. Acarol. 37: 1-9.

**Herron G.A., Edge V., Rophail J. 1993.** Clofentezine and hexythiazox resistance in *Tetranychus urticae* Koch in Australia. Exp. Appl. Acarol. 17: 433-440.

**Huang Y.B., Chi H. 2012.** Age-stage two-stage life tables of *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) with a discussion on the problem of applying female age-specific life tables to insect populations. Insect Sci. 19: 263-273.

**Hoy M.A. 1998.** Myths, models and mitigation of resistance to pesticides, Phil. Trans. R. Soc. Lond. B, 353: 1787-1795.

**Hoy M.A. 2011.** Agricultural acarology. Boca Raton, FL: CRC Press.

**Iftner D.C., Hall F.R. 1984.** The effects of fenvalerate and permethrin residues on *Tetranychus urticae* Koch fecundity and rate of development. J. Agric. Entomol. 1: 191-200.

**IRAC 2022.** Mode of Action Classification Scheme, Version 10.4. IRAC International MoA Working Group, Insecticide Resistance Action Committee http://www.irac-online.org/modes-of-action/

**Jones V.P., Parella M.P. 1984.** The sublethal effects of selected insecticides on life table parameters of *Panonychus citri* (Acari: Tetranychidae). Can. Entomol. 116: 1033-1040.

**Knapp M., van Houten I., van Baal E., Groot T. 2018.** Use of predatory mites in commercial biocontrol: current status and future prospects. Acarologia 58: 72-82.

**Leviticus K., Cui L., Ling H., Jia Z.Q., Huang Q.T., Han Z.J., Zhao C.Q., Xu L. 2020.** Lethal and sublethal effects of fluralaner on the two-spotted spider mites, *Tetranychus urticae* Koch (Acari: Tetranychidae). Pest Manag. Sci, 76:888–893

**Li J., Margolies D.C. 1993.** Effects of mite age, mite density, and host quality on aerial dispersal behavior in the twospotted spider mite. Entomol. Exp. Appl. 68: 79-86.

**Li Y.Y., Fan X., Zhang G.H., Liu Y.Q., Chen H.Q., Liu H. et al. 2017.** Sublethal effects of bifenazate on life history and population parameters of *Tetranychus urticae* (Acari: Tetranychidae). Syst. Appl. Acarol. 22: 148-158.

**Lindquist E.E., Krantz G.W., Walter D.E. 2009.** Classification. In: A manual of acarology, 3rd edition (Krantz G.W. and Walter D.E. eds.). Lubbock, Texas Tech University Press, pp. 97-103.

**Maleknia B., Fathipour Y., Soufbaf M. 2016.** How greenhouse cucumber cultivars affect population growth and two-sex life table parameters of *Tetranychus urticae* (Acari: Tetranychidae). Int. J. Acarol. 42: 70-78.

**Marčić D. 2007.** Sublethal effects of spirodiclofen on life history and life-table parameters of twospotted spider mite (*Tetranychus urticae*). Exp. Appl. Acarol. 42: 121-129.

**Marčić D. 2012.** Acaricides in modern management of plant-feeding mites. J. Pest Sci. 85: 395-408.

**Marčić D. 2022.** Akaricidi u savremenoj fitomedicini. Beograd: Društvo za zaštitu bilja Srbije.

**Marčić D., Ogurlić I., Mutavdžić S., Perić P. 2010.** The effects of spiromesifen on life history traits and population growth of two-spotted spider mite (Acari: Tetranychidae). Exp. Appl. Acarol. 50: 255-267.

**Marić I., Marčić D., Petanović R., Auger P. 2018.** Biodiversity of spider mites (Acari: Tetranychidae) in Serbia: a review, new records and key to all known species. Acarologia 58: 3-14.

**Marić I., Međo I., Marčić D., Petanović R., Jovanović S., Ueckermann E.A. 2021.** Spider mites (Acari: Tetranychidae) from Serbia: new species for the country and the Balkan Peninsula, with a key to all known Serbian species. Syst. Appl. Acarol. 26: 304-316.

**Marris J.W.M. 1988.** The toxicity of hexythiazox to twospotted spider nite (*Tetranychus urticae* Koch) adults and eggs. M.Sc.Thesis, University of Canterburry, New Zealand

**Martini X., Kincy N., Nansen C. 2012.** Quantitative impact assessment of spray coverage and pest behavior on contact pesticide performance. Pest. Manag. Sci. 68: 1471-1477.

**Migeon A., Dorkeld F. 2022.** Spider Mites Web: a comprehensive database for the Tetranychidae. Montpellier, France: INRA-CBGP. http://www.montpellier.inra.fr/CBGP/spmweb

**Međo I., Stojnić B., Marčić D. 2017.** Acaricidal activity and sublethal effects of the microbial pesticide spinosad on *Tetranychus urticae* (Acari: Tetranychidae). Syst. Appl. Acarol. 22: 1748–1762.

**Mota-Sanchez D., Wise J.C. 2020.** The Arthropod Pesticide Resistance Database. Michigan State University. www.pesticideresistance.org

**Musa A., Međo I., Marić I., Marčić D. 2017.** Acaricidal and sublethal effects of a *Chenopodium*-based biopesticide on the two-spotted spider mite (Acari: Tetranychidae). Exp. Appl. Acarol. 71: 211–226.

**Nauen R. 2005.** Spirodiclofen: mode of action and resistance risk assessment in tetranychid pest mites. J. Pestic. Sci. 30: 272-274.

**Pap L., Hajimichael J., Bleicher E. 1996.** Biological evaluation of SZI-121, a new miticide. J. Environ. Sci. Health Part B 31: 521-526.

**Rajaee F., Ghane-Jahromi M., Maroofpour N., Sedaratian-Jahromi A. 2022.** Sublethal effects of spiromesifen on life table traits of *Tetranychus urticae* (Acari: Tetranychidae) and *Neoseiulus californicus* (Acari: Phytoseiidae). Acarologia 62: 772-785.

**Rathman R.J., Beers E.H., Flexner J.L., Riedl H., Hoyt S.C., Westigard P.H., Knight A.L 1990.** Baseline bioassays with hexythiazox and clofentezine of three mite species (Acari: Tetranychidae) occurring on Washington and Oregon tree fruits. J. Econ. Entomol. 83: 1711-1714.

**Rimy S.J., Das G., Gotoh T., Ullah M.S. 2021.** Lethal and sublethal effects of bifenazate on the biological parameters of *Tetranychus truncatus* Ehara (Acari: Tetranychidae). Syst. Appl. Acarol. 26: 2118-2132.

**Robertson J.L., Jones M.M., Olguin E., Alberts B. 2017.** Bioassays with arthropods (3nd edition). Boca Raton, FL: CRC Press.

**Robertson J.L., Worner S.P. 1990.** Population toxicology: suggestions for laboratory bioassays to predict pesticide efficacy. J. Econ. Entomol. 83: 8-12.

**Sabelis M.W. 1985.** Reproductive strategies. In: Helle W., Sabelis M.W. (Eds). Spider mites, their biology, natural enemies and control. Amsterdam: Elsevier, pp. 265-278.

**Saber M., Ahmadi Z., Mahdavinia G. 2018.** Sublethal effects of spirodiclofen, abamectin and pyridaben on life-history traits and life-table parameters of two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae). Exp. Appl. Acarol. 75: 55–67.

**Sáenz-de-Cabezón Irigaray F.J., Zalom F.G. 2012.** Transovarial biotransference of etoxazole through a terrestrial trophic web. Pest Manag. Sci. 68: 1467-1470.

**Sani N.S, Kheradmand K., Talebi A.A. 2019.** Sublethal effects of spirodiclofen on the demographic parameters of *Tetranychus urticae* Koch (Acari: Tetranychidae), Arch. Phyt. Plant Prot. 52: 938-952.

**Sarbaz S., Goldasteh S., Zamani A.A., Solymannejadiyan E., Shoushtari R.V. 2017.** Side effects of spiromesifen and spirodiclofen on life table parameters of the predatory mite, *Neoseiulus californicus* McGregor (Acari: Phytoseiidae). Int. J. Acarol. 43: 380-386.

**Savi P.J., de Moraes G.J., Melville C.C., Andrade D.J. 2019.** Population performance of *Tetranychus evansi* (Acari: Tetranychidae) on African tomato varieties and wild tomato genotypes. Exp. Appl. Acarol. 77: 555–570.

**Schmidt-Jeffris R., Beers E., Sater C. 2021.** Meta-analysis and review of pesticide non-target effects on phytoseiids, key biological control agents. Pest Manag. Sci. 77: 4848-4862.

**Sekulić D. 1995.** The effects of hexythiazox, pyridaben, and flucycloxuron on the population growth of *Tetranychus urticae* Koch. (Acari, Tetranychidae). Pesticidi, 10: 25-34.

**Snell T.W. 1978.** Fecundity, developmental time and population growth rate. Oecologia 32: 119–125.

**Sparks T.C., Crossthwaite A.J., Nauen R., Banba S., Cordova D., Early F. *et al*. 2020.** Insecticides, biologics and nematicides: updates to IRAC's mode of action classfication – a tool for resistance management. Pestic. Biochem. Physiol. 167: 104587.

**Sparks T.C., Nauen R. 2015.** IRAC: Mode of action classification and insecticide resistance management. Pestic. Biochem. Physiol. 121: 122-128.

**Stark J.D., Banks J.E. 2003.** Population-level effects of pesticides and other toxicants on arthropods. Annu. Rev. Entomol. 48: 505-519.

**Stork N.E. 2018.** How many species of insects and other terrestrial arthropods are there on Earth? Annu. Rev. Entomol. 63: 31-45.

**Taghizadeh R., Chi H. 2022.** Demography of *Tetranychus urticae* (Acari: Tetranychidae) under different nitrogen regimes with estimations of confidence intervals. Crop Prot. 155: 105920.

**Tuan S.J., Lin Y.H., Yang C.M., Atlihan R., Saska P., Chi H. 2016.** Survival and reproductive strategies in twospotted spider mites: demographic analysis of arrhenotokous parthenogenesis of *Tetranychus urticae* (Acari: Tetranychidae). J. Econ. Entomol. 109: 502-509.

**Ullah M.S., Kamimura T., Gotoh T. 2020.** Effects of temperature on demographic parameters of *Bryobia praetiosa* (Acari: Tetranychidae). J. Econ. Entomol. 113: 211-221.

**Vacante V. 2016.** The handbook of mites of economic plants. Wallingford, UK: CABI Publishing.

**Van Leeuwen T., Demaeght P., Osborne E.J., Dermauw W., Gohlke S., Nauen R. *et al*. 2012.** Population bulk segregant mapping uncovers resistance mutations and the mode of action of a chitin synthesis inhibitor in arthropods. PNAS 109: 4407-4412.

**Van Leeuwen T., Tirry L., Yamamoto A., Nauen R., Dermauw W. 2015.** The economic importance of acaricides in the control of phytophagous mites and an update on recent acaricide mode of action research. Pestic. Biochem. Physiol. 121: 12-21.

**Van Leeuwen T., Vontas J., Tsagkarakou A., Tirry. L. 2009.** Mechanisms of acaricide resistance in the two-spotted spider mite *Tetranychus urticae.* In: Biorational control of arthropod pests (Ishaaya I., Horowitz A.R. eds.), Dordrecht, The Netherlands: Springer, pp. 347-393.

**Van Pottelberge S., Khajehali J., Van Leeuwen T., Tirry L. 2009a.** Effects of spirodiclofen on reproduction in a susceptible and resistant strain of *Tetranychus urticae* (Acari: Tetranychidae). Exp. Appl. Acarol. 47: 301-309.

**Van Pottelberge S., Van Leeuwen T., Khajehali J., Tirry L. 2009b.** Genetic and biochemical analysis of a laboratory-selected spirodiclofen-resistant strain of *Tetranychus urticae* (Acari: Tetranychidae). Pest Manag. Sci. 65: 358-366.

**Van de Vrie M., McMurtry J.A., Huffaker C.B. 1972.** Ecology of tetranychid mites and their natural enemies: a review. III. Biology, ecology and pest status, and host-plant relations of tetranychids. Hilgardia 41: 343-432.

**Wachendorff U., Nauen R., Schnorbach H.J., Rauch N., Elbert A. 2002.** The biological profile of spirodiclofen (Envidor®) - a new selective tetronic acid acaricide. Pflanzenschutz-Nachrichten Bayer 55: 149–176.

**Walter D.E., Proctor H.C. 2013.** Mites: ecology, evolution and behaviour, 2nd edition. Dordrecht, the Netherlands: Springer.

**Wang S., Tang X., Wang L., Zhang Y., Wu Q., Xie W. 2014.** Effects of sublethal concentrations of bifenthrin on the two-spotted spider mite *Tetranychus urticae* (Acari: Tetranychidae). Syst. Appl. Acarol. 19: 481–490.

**Wang L., Wang Y., Xie W., Wu Q., Wang S. 2016.** Sublethal effects of spinetoram on the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae). Pestic. Biochem. Physiol. 132: 102-107.

**Welty C., Reissig W.H., Dennehy T.J., Weires R.W. 1988.** Susceptibility to hexythiazox of eggs and larvae of European red mite (Acari: Tetranychidae). J Econ. Entomol. 81: 586-592.

**Yamada T., Kaeriyama M., Matsui N. 1987.** Development of a new miticide, hexythiazox. J. Pestic. Sci. 12: 327-335

**Yin W.D., Qiu G.S., Yan W.T., Sun L.N., Zhang H.J., Ma C.S., Adaobi U.P. 2013.** Age-stage two-sex life tables of *Panonychus ulmi* (Acari: Tetranychidae) on different apple varieties. J. Econ. Entomol. 106: 2118-2125.

**Zanardi O.Z., Bordini G.P., Franco A.A., Morais M.R. de, Yamamoto P.T. 2018.** Spraying pyrethroid and neonicotinoid insecticides can induce outbreaks of *Panonychus citri* (Trombidiformes: Tetranychidae) in citrus groves. Exp. Appl. Acarol. 76: 339-354.

**Zhang Z.Q. 2003.** Mites of greenhouses: identification, biology and control. Wallingford, UK: CABI Publishing.

**Прилог 1.**

# Изјава о ауторству

Потписани-а **Asma Ahmed Musa**

број индекса **53023/2016**

**Изјављујем**

да је докторска дисертација под насловом

**„Demographic analysis of acaricide effects on the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae)“**

- резултат сопственог истраживачког рада,
- да предложена дисертација у целини ни у деловима није била предложена за добијање било које дипломе према студијским програмима других високошколских установа,
- да су резултати коректно наведени и
- да нисам кршио/ла ауторска права и користио интелектуалну својину других лица.

**Потпис докторанда**

У Београду, новембар 2023.

Asma. Musa

**Прилог 2.**

# Изјава о истоветности штампане и електронске верзије докторског рада

Име и презиме аутора : **Asma Ahmed Musa**

Број индекса : **53023/2016**

Студијски програм: **Биологија, Ентомологија**

Наслов рада: **„Demographic analysis of acaricide effects on the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae)“**

Ментор: **проф.др Жељко Томановић и др Дејан Марчић**

Потписани/а **Asma Ahmed Musa**

Изјављујем да је штампана верзија мог докторског рада истоветна електронској верзији коју сам предао/ла за објављивање на порталу **Дигиталног репозиторијума Универзитета у Београду.**

Дозвољавам да се објаве моји лични подаци везани за добијање академског звања доктора наука, као што су име и презиме, година и место рођења и датум одбране рада.

Ови лични подаци могу се објавити на мрежним страницама дигиталне библиотеке, у електронском каталогу и у публикацијама Универзитета у Београду.

**Потпис докторанда**

У Београду, новембар, 2023.

Asma. Musa

**Прилог 3.**

# Изјава о коришћењу

Овлашћујем Универзитетску библиотеку „Светозар Марковић“ да у Дигитални репозиторијум Универзитета у Београду унесе моју докторску дисертацију под насловом:

**„Demographic analysis of acaricide effects on the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae)“**

која је моје ауторско дело.

Дисертацију са свим прилозима предао/ла сам у електронском формату погодном за трајно архивирање.

Моју докторску дисертацију похрањену у Дигитални репозиторијум Универзитета у Београду могу да користе сви који поштују одредбе садржане у одабраном типу лиценце Креативне заједнице (Creative Commons) за коју сам се одлучио/ла.

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5. Ауторство – без прераде

6. Ауторство – делити под истим условима

(Молимо да заокружите само једну од шест понуђених лиценци, кратак опис лиценци дат је на полеђини листа).

**Потпис докторанда**

У Београду, новембар, 2023.

Asma Musa

1. Ауторство - Дозвољавате умножавање, дистрибуцију и јавно саопштавање дела, и прераде, ако се наведе име аутора на начин одређен од стране аутора или даваоца лиценце, чак и у комерцијалне сврхе. Ово је најслободнија од свих лиценци.

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