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Original scientific paper

Demografic analysis of biopesticide effects on the two-spotted spider mite (Acari: Tetranychidae) following egg treatment

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SUMMARY

The effects of biopesticide products based on oxymatrine, azadirachtin and *Beauveria bassiana* (strain ATCC 74040) on demographic parameters of the two-spotted spider mite, *Tetranychus urticae*Koch (Acari: Tetranychidae), were evaluated in laboratory bioassays. The biopesticides were applied by spraying 24 h old eggs laid on bean leaves, using the following concentrations: 50 µl/l (oxymatrine-based product), 0.75 g/l (azadirachtin-based product) and 3 ml/l (*B. bassiana*-based product). These concentrations were within 95% confidence limits of the LC_{50}s estimated in acute toxicity bioassays. The mites that hatched from treated eggs completed their juvenile development on the same leaf discs, i.e. the toxic effect was caused by topical treatment and residual exposure. When the surviving females entered their preovipositional period, cohorts of 40 control and 40 treated females were transferred to untreated leaf discs (1 female/disc). Females were transferred daily to new discs until the death of the last one. Using the data on their age-specific survival and age-specific fertility (production of female offspring), the following demographic parameters were estimated: gross reproductive rate (*GRR*), net reproductive rate (R_0), intrinsic rate of increase (r_m), finite rate of increase (λ), and mean generation time (T). The biopesticides significantly affected these demographic parameters in females that survived treatments. All three biopesticides significantly reduced the *GRR* and R_0 values. In the bioassays with oxymatrine- and azadirachtin-based products, the r_m and λ values were reduced by 22% and 5%, and 16% and 4%, respectively, due to reduced survival and fertility, as well as extended juvenile developmental time of females that survived treatment, compared to control females. In the bioassay with the *B. bassiana*-based product, the r_m and λ values were reduced by 7% and 2%, respectively, mostly due to the reduced survival of treated females. The oxymatrine- and azadirachtin-based products significantly extended, while *B. bassiana*-based product reduced the T values.

Keywords: *T. urticae*, *B. bassiana*, azadirachtin, oxymatrine, sublethal effects, life tables

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a cosmopolitan species with a very broad spectrum of plant hosts, and its presence in protected environments (greenhouses) across the temperate climatic zone is especially significant as it is a frequent and major pest (Zhang, 2003; Vacante, 2016). The use of synthetic chemical acaricides and insecto-acaricides has been the dominant way of controlling this harmful species for decades. Due to its exceptional natural potential for rapid development of resistance, and a strong selection pressure of acaricides, the resistance of *T. urticae* has become a global phenomenon (Marčić, 2012; Mota-Sanchez & Wise, 2024).

The problem of resistance, the relatively limited possibilities of choosing new active substances and public demands for a reduction in health and environmental risks, have reactualized the importance of biopesticides as an alternative to synthetic compounds. Biopesticides can be broadly defined as pesticides manufactured from living organisms and their biologically active products. Products manufactured from microorganisms (microbial pesticides) and plants (botanical pesticides or botanicals) are two major groups of industrial-scale produced biopesticides for insect and mite pest management in crop protection (Chandler et al., 2011; Marčić, 2021). Microbial products (mycopesticides) based on living propagules of the entomopathogenic fungus *Beauveria bassiana* and botanical products based on the limonoid azadirachtin derived from the neem tree (*Azadirachta indica*) have been probably the most widely used biopesticides. Their toxicity to spider mites has been proven in laboratory bioassays (Castagnoli et al., 2005; Chandler et al., 2005; Duso et al., 2008; Ullah & Lim, 2015). Products based on extracts from *Sophora flavescens* (Fabaceae), a Chinese medicinal herb containing matrine, oxymatrine and other alkaloids, is among the most important newer botanical biopesticides. These active ingredients have shown high toxicity to larvae and adult spider mites (Marčić & Međo 2014; de Andrade et al., 2020).

Collecting baseline data on lethal and sublethal effects of acaricides on *T. urticae* in laboratory bioassays is the first step in optimizing their application to control populations of this harmful species. In addition to the basic biological profile of toxicity to different stages, evaluation of sublethal effects, including population-level response, is necessary to assess the overall performance of acaricides. One of the widely used methods to evaluate population-level response is demographic analysis, based on constructing life tables and calculating the intrinsic rate of increase (r_m) and other demographic parameters of insect and mite populations (Carey, 1993; Stark & Banks, 2003).

The adaptive strategy of *T. urticae* as a colonizing species is based on high progeny production of the youngest females, so that natural populations of that species are often very close to the stable age distribution in which eggs make 65-66%, juveniles 25-26% and adults 8-10% of the population (Carey, 1982; Sabelis, 1985). It is assumed that acaricides are and will continue to be an important component of integrated management of *T. urticae* and other spider mites (Marčić, 2012; Van Leeuwen et al., 2015), Azadirachtin- and *B. bassiana*-based products have been the subject of several demographic studies in which larvae or adult females of *T. urticae* were treated (Martinez-Villar et al., 2005; Seyed-Talebi et al., 2012; Kheradmand et al., 2022) but not eggs, while oxymatrine-based products have not been the subject of such studies so far. The aim of this study was to fill this research gap by conducting a demographic analysis of the effects of these three biopesticides after treatment of eggs as the dominant stage in *T. urticae* populations.

MATERIAL AND METHODS

Spider mite population

A population of *Tetranychus urticae* Koch (Acari: Tetranychidae), set up from samples collected in a ruderal weed habitat in the vicinity of Belgrade, has been reared on bean plants (*Phaseolus vulgaris*) in a climate-controlled room (25-30°C, 16/8 h of light/dark photoperiod) since March 2004 without any pesticide treatments.

Biopesticides

The following commercial products were used:

1) oxymatrine-based product Kingbo, an aqueous solution containing oxymatrine 0.2%, manufactured by Beijing Kingbo Biotech Inc, China;

2) azadirachtin-based product NeemAzal T/S, an emulsifiable concentrate containing azadirachtin 1%, manufactured by Trifolio-M GmbH, Germany;

3) *Beauveria bassiana*-based product Naturalis-L, a suspension concentrate containing 2.3×10^7 conidiospores/ml, strain ATCC 74040.

Demographic bioassays

All bioassays were carried out on primary bean leaves positioned upon moistened cotton pads in Petri dishes with the abaxial surface upward. The Petri dishes were kept in a climate chamber at 27±2°C, under 50-70% RH and 16/8 h of light/dark photoperiod.

The effects of biopesticides on demographic parameters were estimated using life tables constructed for females that survived treatment at the egg stage. Up to 24 h old eggs laid on bean leaves (10 leaves × 25-30 eggs/leaf) were treated with the following biopesticide concentrations: 50 µl/l (oxymatrine-based product), 0.75 g/l (azadirachtin-based product) and 3 ml/l (*B. bassiana*-based product). These concentrations were within 95% confidence limits of the LC_{50}s estimated in acute toxicity bioassays. Control eggs (4 leaves × 25 eggs/leaf) were treated with distilled water. The mites that hatched from treated eggs completed their juvenile development on the same leaf discs. When the surviving females entered their pre-ovipositional period, cohorts of 40 control and 40 treated females were transferred to untreated leaf discs (1 female/disc). Females were transferred daily to new discs until the death of the last female, and the eggs they laid were counted. Ten days after egg laying, adult females that developed from those eggs were counted.

Using data on the age of first reproduction, survivorship and offspring production of females, i.e. *age-specific survival* (l_x = proportion of females alive at age of x days) and *age-specific fertility* (m_x = number of female offspring per female alive), were estimated, assuming that l_x was 1.00 at the age of first reproduction (i.e. juvenile mortality was not included in estimation). Based on these two age-specific functions, demographic parameters (Table 1) were estimated according to Birch (1948) and Carey (1993).

Table 1. Demographic parameters used in the analysis

Demographic parameters	Equations	Definitions
Gross reproductive rate	$GRR = \sum_{x=\alpha}^{\beta} m_x$	the mean number of female offspring that a female can produce during its life span
Net reproductive rate	$R_0 = \sum_{x=\alpha}^{\beta} l_x m_x$	the mean net number of female offspring that a female can produce during its life span (generation growth rate)
Intrinsic rate of increase	$\sum_{x=0}^{\omega} e^{-r_m (x+1)} l_x m_x = 1$	the rate of natural increase in a closed population with constant age-specific mortality and reproduction schedules and a stable age distribution
Finite rate of increase	$\lambda = e^{r_m}$	the multiplication factor of a population at each time unit (daily growth rate)
Mean generation time	$T = \frac{\ln R_0}{r_m}$	the lenght of time that is required by a population to increase R_0-fold

Standard errors of demographic parameters were estimated by using the bootstrap technique (Efron & Tibshirani 1993; Huang & Chi 2012). To obtain stable estimates 100,000 bootstrap replicates were used. Differences between controls and treatments were compared by using paired bootstrap test based on the confidence interval of difference (Tuan et al., 2016). To take the advantage of bootstrap method and paired bootstrap test, the TWOSEX-MSChart software (Chi, 2015) was used to analyze the data for one sex, assuming that all females have the same preadult duration and emerged at the same age.

RESULTS

Juvenile development of *T. urticae* females that survived treatment with the oxymatrine-based product at the egg stage lasted one day longer, so they reached the adult stage on the eleventh and achieved their first reproduction on the twelfth day of life, one day later than control females. The treatment reduced the age-specific survival during the first half of adult females' lives, while females partially recovered in the second half of their lives. The age-specific fertility of treated females was reduced compared to the control during the entire reproductive period, the fact being that the last treated female lived two days longer (Figure 1).

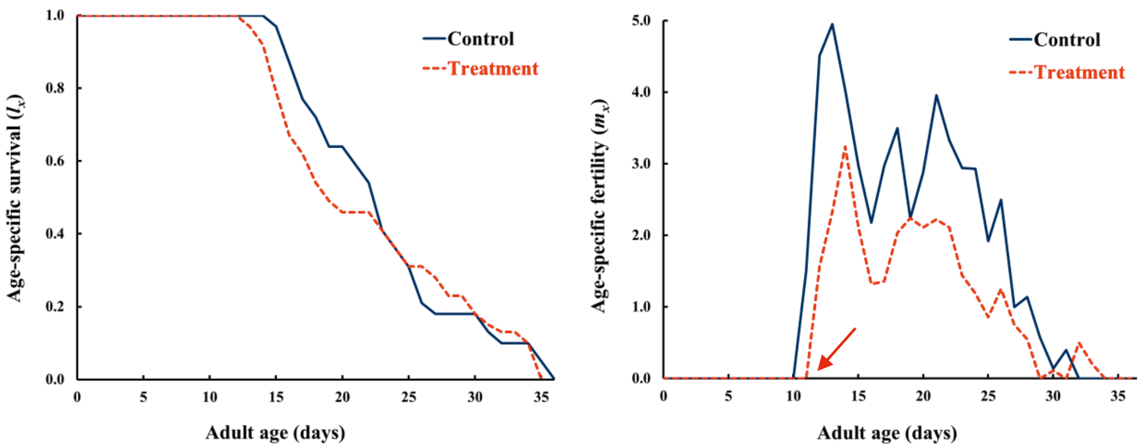

Figure 1. Age-specific survival (l_x) and age-specific fertility (m_x) of *T. urticae*: control = untreated females; treatment = females that survived treatment with the oxymatrine-based biopesticide product (50 μl/l) at the egg stage (red arrow indicates prolonged juvenile development)

As a consequence of survival and fertility reductions, as well as the prolongation of juvenile development, the values of demographic parameters for treated females differed significantly from the corresponding control values (Table 2). The *GRR* was reduced by 44% and R_0 by 48%, while r_m and λ were reduced by 22% and 5%, respenctively. On the other hand, the mean generation time significantly extended.

Table 2. Demographic parameters (means ± SE) of *T. urticae*: control = untreated females; treatment = females that survived treatment with the oxymatrine-based biopesticide product (50 μl/l) at the egg stage

	GRR	R_0	r_m	λ	*T*
Control	52.57 *a* (± 4.02)	35.92 *a* (± 3.29)	0.238 *a* (± 0.004)	1.269 *a* (± 0.006)	15.03 *b* (± 0.23)
Treatment	29.52 *b* (± 3.82)	18.82 *b* (± 2.70)	0.185 *b* (± 0.008)	1.203 *b* (± 0.010)	15.86 *a* (± 0.32)
P	0.00003	0.00007	0	0	0.03677

Means in a column marked by different letters differ significantly (paired bootstrap test, 5% significance level)

GRR = gross reproductive rate (female/female)
R_0 = net reproductive rate (female/female/generation)
r_m = intrinsic rate of increase (female/female/day)
λ = finite rate of increase (female/female/day)
T = mean generation time (days)

Treatment of *T. urticae* eggs with azadirachtin also caused a prolongation in the juvenile development of surviving individuals, which reached the adult stage on the eleventh day and had their first reproduction on the twelfth day of life, one day later than the control females. The age-specific survival in the treatment was reduced compared to the control until the end of life of adult females, while the age-specific fertility of treated females was reduced during most of their adult life, and mostly during their reproductive zenith (Figure 2).

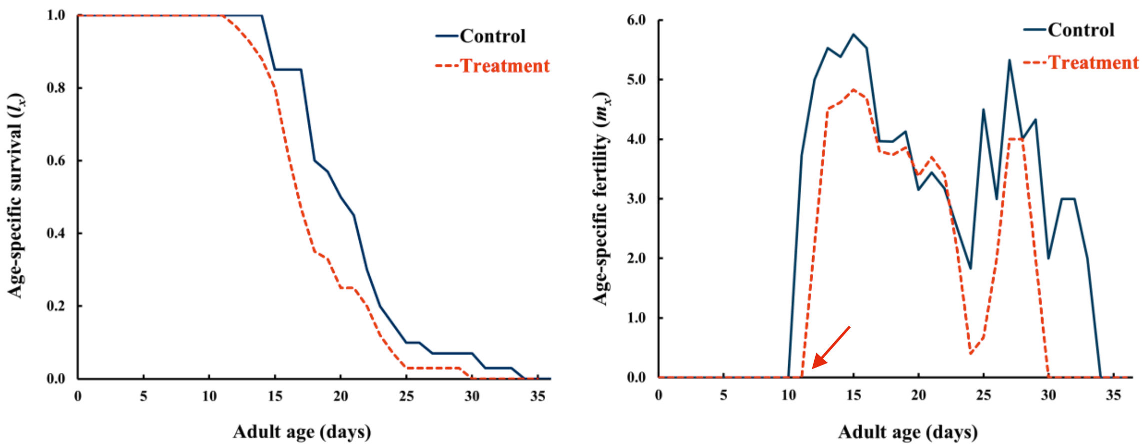

Figure 2. Age-specific survival (l_x) and age-specific fertility (m_x) of *T. urticae*: control = untreated females; treatment = females that survived treatment with the azadirachtin-based biopesticide product (0.75 g/l) at the egg stage (red arrow indicates prolongation of juvenile development)

This reduction in survival and fertility and prolongation of juvenile development of treated females, compared to control females, caused a significant reduction in reproduction and population growth rates (Table 3). The *GRR* was reduced by 34% and the R_0 by 37%, while r_m and λ were reduced by 16 and 4%, respectively. The mean generation time was significantly extended but only by less than 24 h.

Table 3. Demographic parameters (means ± SE) of *T. urticae*: control = untreated females; treatment = females that survived treatment with the azadirachtin-based biopesticide product (0.75 g/l) at the egg stage

	GRR	R_0	r_m	λ	*T*
Control	88.24 *a*	44.32 *a*	0.263 *a*	1.300 *a*	14.43 *b*
	(± 11.34)	(± 3.56)	(± 0.004)	(± 0.005)	(± 0.23)
Treatment	57.95 *b*	28.08 *b*	0.221 *b*	1.248 *b*	15.07 *a*
	(± 7.25)	(± 2.82)	(± 0.005)	(± 0.006)	(± 0.21)
P	0.02911	0.00043	0.00043	0.00043	0.04003

Means in a column marked by different letters differ significantly (paired bootstrap test, 5% significance level)

GRR = gross reproductive rate (female/female)
R_0 = net reproductive rate (female/female/generation)
r_m = intrinsic rate of increase (female/female/day)
λ = finite rate of increase (female/female/day)
T = mean generation time (days)

The treatment of *T. urticae* eggs with the biopesticide Naturalis-L caused a reduction in age-specific survival from the beginning to the end of life of adult females. The age-specific fertility of females in the treatment was also lower than in the control (with the only exception of females aged 25 days) with the greatest differences being noted in females aged 15-23 days (Figure 3).

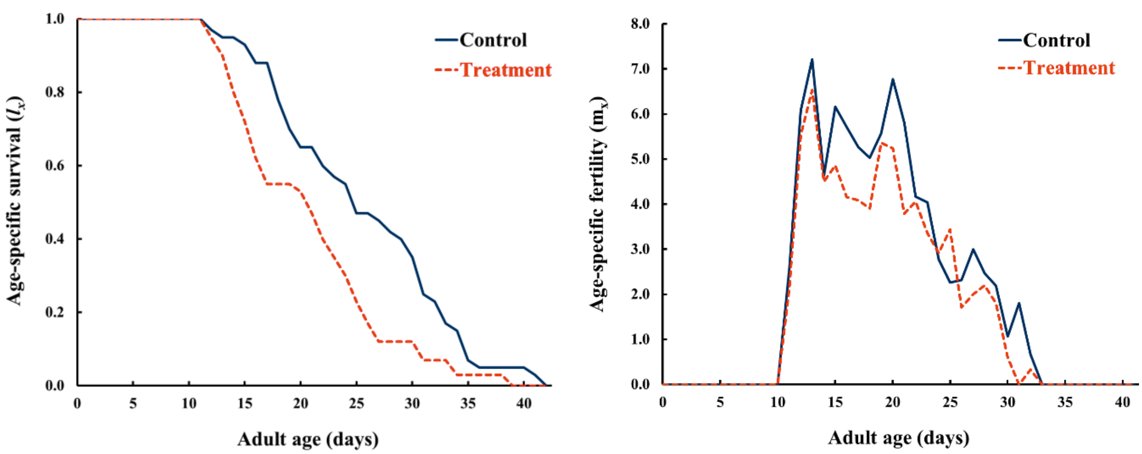

Figure 3. Age-specific survival (l_x) and age-specific fertility (m_x) of *T. urticae*: control = untreated females; treatment = females that survived treatment with the *B. bassiana*-based biopesticide product (3 ml/l) at the egg stage

The reduction in age-specific functions caused a statistically significant reduction in demographic parameters (Table 4). The *GRR* was reduced by 17% and R_0 by 36%, compared to the control; the greater reduction in the latter, compared to the former, is due to the continuous reduction in l_x values throughout the life of treated adult females. Population growth rates in the treatment were reduced by only 7% (r_m) and 2% (λ), compared to the control, but although low, these differences were still statistically significant.

Table 4. Demographic parameters (means ± SE) of *T. urticae*: control = untreated females; treatment = females that survived treatment with the *B. bassiana*-based biopesticide product (3 ml/l) at the egg stage

	GRR	R_0	r_m	λ	*T*
Control	87.57 *a* (± 2.98)	63.80 *a* (± 4.85)	0.269 *a* (± 0.004)	1.309 *a* (± 0.005)	15.45 *a* (± 0.19)
Treatment	72.55 *b* (± 3.80)	40.50 *b* (± 4.41)	0.249 *b* (± 0.005)	1.283 *b* (± 0.007)	14.85 *b* (± 0.21)
P	0.00246	0.00041	0.00253	0.00235	0.03069

Means in a column followed by different letters differ significantly (paired bootstrap test, 5% significance level)

GRR = gross reproductive rate (female/female)
R_0 = net reproductive rate (female/female/generation)
r_m = intrinsic rate of increase (female/female/day)
λ = finite rate of increase (female/female/day)
T = mean generation time (days)

DISCUSSION

All three biopesticides significantly affected demographic parameters in *T. urticae* females that survived topical treatment at the egg stage, as well as residual exposure during juvenile development. The biopesticides significantly reduced their *GRR* and R_0 values but the two botanical biopesticides caused much more pronounced reductions than the mycopesticide. In addition, the R_0 reduction was only 3-4% greater than *GRR* reduction, which means that the contribution of fertility reduction to the overall reduction of population growth rates was considerably higher. On the other hand, treatment with the *B. bassiana*-based product caused a reduction in the R_0 parameter that was twice as high as the reduction in *GRR*, which indicates an equal contribution of reductions in female fertility and length of life to the overall reduction in population growth rates. Reductions in *T. urticae* fecundity, fertility and/or longevity have also been reported in other demographic studies in which larvae (Seyed-Talebi et al., 2012) or adult females (Martinez-Villar et al., 2005; Kheradmand et al., 2022) were treated with these biopesticides. Reductions in fecundity and/or longevity were also recorded in non-demographic studies after treating females (Sundaram & Sloane, 1995; Dimetry et al., 2008; Duso et al., 2008; Marčić & Međo, 2014, 2015) or eggs of *T. urticae* (Marčić & Međo, 2014, 2015).

Demographic bioassays with botanical products showed that these biopesticides prolong the juvenile development of females that survived treatment at the egg stage, which increased their age at the time of first reproduction and thus directly lowered their r_m and λ values (Birch, 1948; Snell, 1978). Prolongation in juvenile *T. urticae* development after egg treatment was also observed in demographic studies with tebufenpyrad (Marčić, 2005) and bifenthrin (Wang et al., 2014).

The data obtained in this study represent a starting point for improvements in the management of *T. urticae* populations. Considering the stable age distribution of this species in which eggs were the dominant stage, it is clear that treatment with a concentration at the LC_{50} level could eliminate a significant part of the population, and reduce its recovery potential due to reduced population growth rates. From this point of view, the most significant effect can be expected from the oxymatrine-based product, whose LC_{50} for eggs was 40 times lower than the recommended concentration, while the consequence of egg treatment is a reduction of r_m by 22%. The azadirachtin-based product applied at the concentration seven times lower than recommended reduced r_m by 16%, while the weakest effect was achieved by the *B. bassiana*-based product whose LC_{50} for eggs, equal to the recommended concentration, reduced r_m by 7%. These results indicate a possibility of applying oxymatrine- and azadirachtin-based products at reduced rates, integrated with the use of predatory mites as biological control agents. Integration of predators with these bioacaricides could be the topic of our future research. Field trials have shown that this approach is a viable solution for the application of other acaricides (Rhodes et al., 2006; Bilbo & Walgenbach, 2020).

Biopesticides are a potentially useful tool within the strategy of sustainable management of spider mite populations, which requires inclusion of a broad spectrum of acaricides with different mechanisms of action and low risk for humans and the environment (Marčić, 2012; Adesanya et al., 2021). Considering the existing obstacles in commercialization and adoption of biopesticides as stand-alone products (Chandler et al., 2011), the solution for their wider use is their inclusion in integrated programs of chemical, biological and other methods of spider mite control.

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Demografska analiza delovanja biopesticida na običnu grinju paučinara (Acari: Tetranychidae) nakon tretmana jaja

REZIME

Efekti biopesticidnih proizvoda na bazi oksimatrina, azadirahtina i *Beauveria bassiana* (soj ATCC 74040) na demografske parametre grinja paučinara, *Tetranychus urticae* Koch (Acari: Tetranychidae), procenjeni su u laboratorijskim testovima. Biopesticidni proizvodi su primenjeni prskanjem jaja starih 24 sata, koja su bila položena na listovima pasulja, koristeći sledeće koncentracije: 50 µl/l (proizvod na bazi oksimatrina), 0.75 g/l (proizvod na bazi azadirachtina) i 3 ml/l (proizvod na bazi *B. bassiana*). Ove koncentracije su bile unutar 95% granica poverenja LC_{50} vrednosti procenjenih u ogledima akutne toksičnosti. Grinje koje su se izlegle iz tretiranih jaja završile su svoj juvenilni razvoj na istim listovima, tj. toksični efekat je izazvan direktnim tretmanom i residualnim izlaganjem. Kada su preživele ženke ušle u period preovipozicije, kohorte od 40 kontrolnih i 40 tretiranih ženki premeštene su na netretirane listove (1 ženka/disk). Ženke su svakodnevno premeštane na nove diskove dok poslednja ženka nije uginula. Na osnovu podataka o njihovoj starosno specifičnom preživljavanju i starosno specifičnoj plodnosti (stvaranje ženskih potomaka), procenjeni su sledeći demografski parametri: bruto reproduktivna stopa (*GRR*), neto reproduktivna stopa (R_0), intrinzična stopa rasta (*rm*), konačna stopa rasta (λ) i prosečno vreme generacije (*T*). Biopesticidi su značajno uticali na demografske parametre kod ženki koje su preživele tretman. Sva tri biopesticida su značajno smanjila vrednosti *GRR* i R_0. U ogledu sa proizvodima na bazi oksimatrina i azadirahtina, vrednosti *rm* i λ smanjene su za 22% i 5%, odnosno 16% i 4%, zbog smanjenog preživljavanja i plodnosti, kao i produženog vremena juvenilnog razvoja preživelih ženki u poređenju sa kontrolom. U ogledu sa proizvodom na bazi *B. bassiana*, vrednosti *rm* i λ smanjene su za 7% i 2%, uglavnom zbog smanjenog preživljavanja tretiranih ženki. Proizvodi na bazi oksimatrina i azadirahtina značajno su produžili, dok je proizvod na bazi *B. bassiana* smanjio vrednosti *T*.

Ključne reči: *T. urticae, B. bassiana*, azadirahtin, oksimatrin, subletalni efekti, životne tabele