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Original article (Orijinal araştırma)

Toxic effects of some acaricides on *Aceria oleae* (Nalepa, 1900) (Acari: Eriophyidae) and its predator *Neoseiulus californicus* (McGregor, 1954) (Acari: Phytoseiidae) under laboratory conditions[1]

Laboratuvar koşullarında *Aceria oleae* (Nalepa, 1900) (Acari: Eriophyidae) ve avcısı *Neoseiulus californicus* (McGregor, 1954) (Acari: Phytoseiidae)'a bazı akarisitlerin toksik etkileri

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Abstract

Olive bud mite, *Aceria oleae* (Nalepa, 1900) (Acari: Eriophyidae) is one of the key pests that regularly needs control with acaricides in olive orchards of Bursa Province, Turkey. For the chemical control of *A. oleae*, it is critical the use of acaricides does not reduce the survival and fecundity of its natural enemies. The toxic effects of three concentrations of seven acaricides were assessed against both *A. oleae* and its predator *Neoseiulus californicus* (McGregor, 1954) (Acari: Phytoseiidae) using a residual method under controlled conditions at Bursa Uludağ University during 2020-2021. The highest recommended concentrations of acequinocyl, azadirachtin, fenbutatin oxide, milbemectin, pyridaben, spirodiclofen and sulfur killed *A. oleae* adults with rates varying from 80 to 100%. Two concentrations of milbemectin, pyridaben and sulfur showed high mortality rates. Nevertheless, highest recommended concentrations of acequinocyl, pyridaben, spirodiclofen and sulfur were found to be highly toxic to *N. californicus* adults with rates varied from 82 to 100%. The high mortalities for mobile immature stages and reducing in the fecundity of *N. californicus* occurred by highest recommended concentrations of all tested acaricides. Based on the scale recommended by the International Organization for Biological Control, some sublethal concentrations of fenbutatin oxide, spirodiclofen and sulfur were found to be slightly harmful to both mature and immature of *N. californicus.*

Keywords: Acaricide, olive bud mite, phytoseiids, side effect, toxicology

Öz

Zeytin tomurcuk akarı, *Aceria oleae* (Nalepa, 1900) (Acari: Eriophyidae) Bursa İli (Türkiye) zeytin bahçelerinde sürekli mücadelesinin yapılması gereken ana zararlılar arasında yer almaktadır. Zararlı akarın kimyasal mücadelesinde kullanılan akarisitlerin, doğal düşmanlarının canlılığını ve üremesini düşürmemesi çok kritiktir. Bu nedenle Bursa Uludağ Üniversitesi'nde 2019-2020 yıllarında 7 akarisitin 3 farklı konsantrasyonunun hem *A. oleae* hem de avcısı *Neoseiulus californicus* (McGregor, 1954) (Acari: Phytoseiidae) üzerindeki toksik etkileri kontrollü koşullarda bir kalıntı metodu kullanılarak belirlenmiştir. Azadirachtin, acequinocyl, fenbutatin oxide, milbemectin, pyridaben, spirodiclofen ve kükürtün Türkiye'de diğer akarlara önerilen en yüksek konsantrasyonları *A. oleae*'nın erginlerini %80 ile 100 arasında öldürmüştür. Milbemectin, pyridaben ve kükürtün iki alt konsantrasyonunun *A. oleae* erginleri üzerindeki öldürücü etkisi çok yüksek bulunmuştur. Buna karşılık, acequinocyl, pyridaben, spirodiclofen ve kükürtün en yüksek konsantrasyonları *N. californicus*'un erginlerinin, %82 ile 100 oranında zehirli bulunmuştur. Tüm akarisitlerin en yüksek konsantrasyonları *N. californicus*'un hareketli ergin öncesi dönemlerinde yüksek ölüm oluşturmuş ve erginlerin yumurta bırakma miktarını çok azaltmıştır. Uluslararası Biyolojik Mücadele Organizasyonu'nun skalasına göre fenbutatin oxide, spirodiclofen ve kükürtün bazı düşük konsantrasyonları *N. californicus*'un hem ergin hem de ergin öncesi dönemleri için hafif zararlı bulunmuştur.

Anahtar sözcükler: Akarisit, zeytin tomurcuk akarı, phytoseiidler, yan etki, toksikoloji

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Introduction

Olive bud mite, *Aceria oleae* (Nalepa, 1900) (Acari: Eriophyidae) damages both flowers and new formed fruits of olive trees, and significantly decreases olive yield. The mite attacks cause flower dropping and the formation of small/premature fruit in olives (Hatzinikolis, 1986; Tzanakakis, 2003). With 26-82% infestation rate of trees and 8-59% injury rate of fruit, this pest is a serious olive pest in some locations with moist conditions in the Mediterranean Basin (Avidov & Harpaz, 1969; Hatzinikolis, 1986; Abou-Awad et al., 2000; Tzanakakis, 2003; Shahini et al., 2009; Chatti et al., 2017). Previous studies reported three eriophyid mite species from olive trees of Turkey: *A. oleae, Aculus olearius* Castagnoli, 1977 and *Tegolophus hassani* (Keifer, 1959) (Kumral & Kovancı, 2004; Çetin & Alaoğlu, 2006; Kaçar et al., 2010; Çetin et al., 2012; Denizhan et al., 2015; Ersin et al., 2020; Kaya, 2020). Among these mites, *A. oleae* was reported as the predominant species in Marmara, Eagan and Mediterranean Regions (Alkan, 1952; Kumral & Kovancı, 2004; Kaçar et al., 2010; Kaya, 2020).

In recent years, because the pressure of natural enemies has disappeared due to the intensive use of synthetic chemicals in the olive orchards of Bursa Province, *A. oleae* has become one of the key pests that must be regularly controlled with synthetic acaricides (Kumral et al., 2020). Considering the size of the olive groves, the sole method is the chemical control of *A. oleae*. However, the toxic effects of many acaricides, including botanical and mineral preparations, on *A. oleae* are not known. In a previous study, the effectiveness of a translaminar acaricide, abamectin, against *A. oleae* was determined in an olive grove of Bursa (Kılınç & Kumral, 2016). According to results of that study, three different doses (2.25, 4.5 and 9.0 g ai/100 L water) reduced *A. oleae* density by 87, 93 and 94%, respectively, 7 days after spraying. In addition, the study showed that the dose (4.5 g ai/100 L water) of abamectin reduced density of its predator mites, phytoseiids (mixed population contain 2-3 different species) by 67% under field conditions. The authors suggested that abamectin cannot be used in integrated mite control strategies due to its moderately harmful effect to phytoseiids and should be investigated new environmentally-friendly alternative solutions in the future. According to the European Union Directive 2009/128/EC, member and associated countries should prefer to environmentally-friendly pesticides that are safe for non-target organisms such as biological control agents. Among these agents, phytoseiids can control eriophyid mites successfully (Gerson et al. 2003). Also, several phytoseiid mite species related to eriophyid mite populations in olive trees, *Typhlodromus (Typhlodromus) athiasae* Porath & Swirski, 1965, *Typhlodromus (Typhlodromus) rarus* Wainstein, 1961, *Typhlodromus (Anthoseius) recki* Wainstein, 1958, *Typhlodromus (Anthoseius) psyllakisi* Swirski & Ragusa, 1976, *Typhlodromus (Anthoseius) involutus* Livshitz & Kuznetsov, 1972, *Typhlodromus (Anthoseius) athenas* Swirski & Ragusa, 1976, *Typhlodromus (Anthoseius) rapidus* Wainstein & Arutunjan, 1968, *Neoseiulus californicus* (McGregor, 1954), *Neoseiulus barkeri* Hughes, 1948, *Amblyseius andersoni* (Chant, 1957), *Euseius stipulatus* (Athias-Henriot, 1960) and *Paraseiulus talbii* (Athias-Henriot, 1960), were reported in different locations by some researchers (El-Laithy, 1999; Kumral & Kovanci, 2004; Kumral et al., 2010; Chatti et al., 2017; Ersin et al., 2020; Kaya, 2020; Elhalawany et al., 2021). Also, Abou-Awad et al. (2000) reported that phytoseiids are capable of preventing the outbreak of *A. oleae* in olive groves. *Neoseiulus californicus* is used commercially around the world to control eriophyids and other economically important spider mite species on several crop species (Castagnoli & Simoni, 2003). The predator is widely distributed in the Mediterranean Region of Turkey (Cakmak & Cobanoğlu, 2006; Yorulmaz & Ay, 2012). The phytoseiid is the predominant species in Bursa Province probably due to its adaptation to cold winter conditions and resistance development against some insecticides and acaricides in conventional production areas (Kumral & Kovancı, 2007; Yorulmaz & Ay, 2012). The presence of *N. californicus* in olive orchards was previously reported by Chatti et al. (2017) and Kumral et al. (2021). Additionally, the predation capacity of *N. californicus* fed on *A. oleae* has been shown under laboratory conditions by Kumral et al. (2021), recently.

It is critical that the acaricides used in the chemical control of *A. oleae* do not reduce the survival and fecundity of its natural enemies. If, it is chosen to use any acaricide, their side effects on phytoseiids like *N. californicus*, must be taken into consideration. A sustainable chemical control can be provided, only in cases when biological control agents are not affected negatively (Overmeer & van Zon, 1982; Blümel et al., 2000; Norris et al., 2003). Therefore, laboratory-based side effect studies on female predators should evaluate, not only the toxic effects, but also the negative effects on fecundity (Overmeer & van Zon, 1982). Additionally, the juvenile stages (e.g., larvae and nymphs) of phytoseiid mites are in general more susceptible to pesticides compared with adults and the survival of juveniles is greatly important for maintaining the population of the predator (van Zon & Wysoki, 1978). For that reason, during this study, firstly the effects of different concentrations of seven acaricides having different modes of action (acequinocyl, azadirachtin, fenbutatin oxide, milbemectin, pyridaben, spirodiclofen and sulfur) for *A. oleae* were established. Subsequently, the side effects of these concentrations were shown for *N. californicus* in laboratory conditions based on the scale of International Organization for Biological Control (IOBC).

Materials and Methods

Mite cultures

A field colony of *A. oleae* was used in this experiment. The *A. oleae* colony was collected from Mirzaova Village, Mudanya District, Bursa Province, Turkey. The Turkish *N. californicus* strain was obtained from Gorukle Campus of Bursa Uludağ University (Bursa, Turkey). The identification of both mite species was made by senior author based on following literature: Keifer (1975) and Hatzinikolis (1986) for *A. oleae;* Schuster & Pritchard (1963) and Okassa et al. (2011) for *N. californicus*. Predator mites were mass reared in glass Petri dishes at 27 ± 1ºC, 65% RH and 16:8 h L:D photoperiod in a controlled climate chamber. For mass rearing, the predator mites were fed on individuals of *Tetranychus urticae* Koch, 1836, (Acari: Tetranychidae) and pollen of *Typha latifolia* L., 1753, (Poales: Thyphaceae) (Overmeer, 1985).

Bioassays on *Aceria oleae*

The following commercial acaricides belonging to seven chemical groups and modes of action were used in bioassays: acequinocyl, azadirachtin, fenbutatin oxide, milbemectin, pyridaben, spirodiclofen and sulfur (IRAC, 2021). Details about acaricides are given in Table 1. The toxic effect of acaricide on *A. oleae* adults was evaluated using a modified residual method under laboratory conditions (Monteiro et al., 2012; Simon, 2014). As a test tool, Plexiglas Munger cells (8 x 10 x 1 cm) with a circular hole (5 cm diameter) in the center were used (Overmeer, 1985). Moistened cotton wool and then black leather (8 x 10 cm) were placed between Plexiglass with or without a circular hole. Stalks of olive flower bud cluster were insert into the layers (Figure 1). At least five concentrations of acaricides were used and randomly applied to *A. oleae*, at concentrations between 50 and 100% that caused death. The highest recommended concentrations (HRC) and two sublethal concentrations resulted in >50% mortality was prepared with distilled water and used for the bioassays. For each bioassay, three replicates were assessed for both treatments and controls (distilled water). Two mL of different acaricide concentrations were sprayed to olive bud clusters for 3 s with spray tower resulting in a deposition of 1.5 mg/cm^2 (Potter precision, Burkard Manufacturing Co. Ltd., Rickmansworth, UK). The plant parts were sprayed with acaricides once during all bioassays. All sprayed olive bud clusters were then dried at 25ºC (room temperature) for 10-15 min (Potter, 1952). Fifty *A. oleae* adults were put onto the olive bud clusters by a paintbrush. To prevent the escape of the mites, the upper Plexiglas lid was closed. Plexiglas plate and whole fragments of Munger cell which were hold together with the aid of four clips. Then, the Munger cells were placed into a controlled climate chamber in Petri dishes at 27 ± 1ºC, 65% RH and 16:8 h L:D photoperiod. The survival of mites was checked daily under a stereomicroscope after the acaricide application for 3 days. It was decided the test duration (3 days) according to mite viability in the control treatments. Mites unable to move when touched with a hairbrush were considered dead. The rates of mortality in control trials did not exceed 10%.

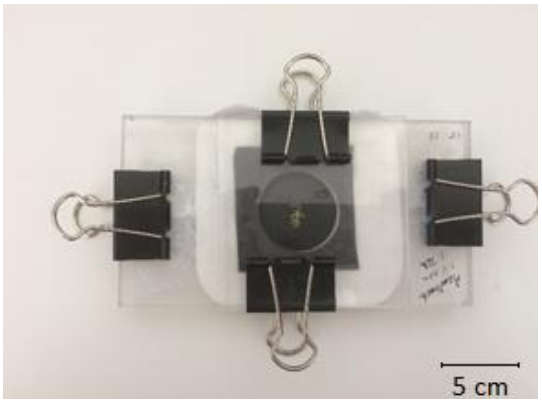

Figure 1. Munger cells used in bioassays.

Table 1. The information about tested acaricides

Active substance	Chemical group[1]	Mode of Action[1]	Mode of action classes[1]	Commercial name	Company	Formulation type[2]	Rate of active substance (g/L)[2]	HRC (mg/L)[2]
Acequinocyl	Unclassified	Mitochondrial complex III electron transport inhibitors	20A	Kanemite	SumiAgro	SC	156	195
Azadirachtin	Botanical acaricides	Compounds of unknown or multible MoA	UN	Nimbecidine	Agrobest	SC	0.3	1.5
Fenbutatine oxide	Organometal	Inhibitors of mitochondrial ATP synthase	12B	Quiz	Hektaş	SC	550	330
Milbemectin	Avermectins	Glutamate-gated chloride channel (GluCl) allosteric modulators	6	Milbeknock	SumiAgro	EC	9.3	9.3
Pyridaben	Pyridazinone	Mitochondrial complex I electron transport inhibitors	21A	Sanmite	SumiAgro	WP	20%	150
Spirodiclofen	Tetronic& Tetramic acid derivates	Inhibitors of acetyl CoA carboxylase	23	Smach	Hektaş	SC	240	60
Sulfur	Minerals	Compounds of unknown or multible MoA	UN	Power sulfur'H	Safa Tarım	WP	80%	3200

[1] The data were obtained from mode of action database of Insecticide Resistance Action Committee (IRAC, 2021);

[2] HRC, highest recommended concentration for other mite pests in Turkey. The data were provided from Turkish Agricultural Ministry Pesticide Registration Database (BKU, 2021).

Toxicity tests on *Neoseiulus californicus*

The toxic effect of the acaricides on both juveniles and adults of *N. californicus* was determined using a modification of the method described by Overmeer & van Zon (1982) in laboratory conditions. Two mL of the three concentrations were sprayed on the undersurface of olive leaves with the spray tower adjusted for the same conditions. Then, the leaves were allowed to air dry for 15 min under room conditions. As a control, the leaves treated with distilled water were used (Potter, 1952). Same Plexiglas Munger cells and

procedures were used for bioassays. An equal amount of eriophyid individuals as prey was put into a cell, followed by the same age females of *N. californicus* (~3 days old) obtained from synchronized population. For juvenile test, freshly deposited eggs (1 day old) were used. The eggs were transferred by paint brush onto sprayed leaves. The trials for immature stages began with egg spraying and continued until the individuals reached adult stage. A total of five female mites and eggs were used for each test. For each acaricide, five replicates were assessed. The cells were put in a climate room at the same conditions. The number of dead female and juvenile mites were recorded daily under a stereomicroscope. Females were considered as dead at the end of 3 days if no movement was observed after a gentle touch by a camel hair brush. Juveniles were recorded as dead when the individuals did not mature or survive during 10 days.

Fecundity inhibition tests on *Neoseiulus californicus*

The inhibition effects of acaricides on the fecundity of *N. californicus* were assessed using the method described by Overmeer & van Zon (1982). The three concentrations were applied to olive leaves based on the above tests. Following this, a mated *N. californicus* female (each newly emerged female was paired with a male adult for 12 h) were put into a Munger cell. An equal amount of prey was put into a cell. The leaves treated with distilled water only served as the control. Five females were treated in each replicate and each treatment was applied to five replicates. The mortality and the number of eggs deposited by females were determined daily and removed until all of the females died naturally.

Data Analysis

Mortality percentages for *A. oleae* and *N. californicus* were corrected using control percentages with Abbott's formula (Abbott, 1925):

$$CM = \frac{CA - TA}{CA} x100$$

where, *CM* is the corrected mortality, *CA* is the proportion alive in control and *TA* is the proportion alive in the treatment (Simon, 2014). A one-way ANOVA was conducted to analyze variation in the corrected mortality data of *N. californicus* juvenile stages and females treated with different acaricides (SAS Institute, 2015). Before the analyses, corrected mortality data were transformed to arcsin. Means obtained by ANOVA were separated using Tukey's HSD post-hoc test. Also, the combined total side effect (E) of the acaricides on *N. californicus* was calculated using the following formula (Overmeer & van Zon, 1982):

$$E = 100 - ((100 - M) \times R)$$

where, *E* is the coefficient of toxicity, *M* is the corrected (Abbott, 1925) mortality effects of acaricides on both juvenile stages or females of *N. californicus* and *R* is the ratio between the mean number of eggs deposited by *N. californicus* females treated acaricides and the mean number of eggs producing by the females exposed to distilled water (control group). The concentrations of each acaricides were classified using these *E* results according to the following IOBC toxicity categories: class I (<30%, harmless), class II (30-79%, slightly harmful), class III (80-99%, moderately harmful), class IV (>99%, harmful) (Sterk et al., 1999). The influence of acaricide exposure on phytoseiid females was analyzed using Kaplan-Meier survival and log-rank test with SPSS 23 version.

Results

Acute toxicity effects on *Aceria oleae*

Table 1 shows the toxicity results for 3 days after application of the highest recommended concentrations (HRC, for other mite pests in Turkey) of seven acaricides to *A. oleae* adults. HRCs of acequinocyl, azadirachtin, fenbutatin oxide, milbemectin, pyridaben, spirodiclofen and sulfur killed *A. oleae* with rates of 97, 80, 91, 100, 100, 80 and 100%, respectively (Table 2). HRCs of acequinocyl, milbemectin,

pyridaben and sulfur were found to be significantly toxic against *A. oleae* adults ($F_{6,20}$ = 93.1; P < 0.01). In addition, *A. oleae* adult mortality rates of their two sublethal concentrations were found to be 54 and 74%, 57 and 66%, 65 and 75%, 93 and 98%, 86 and 98%, 48 and 69%, and 90 and 94%, respectively. Some concentrations of milbemectin (0.29 and 1.26 ai mg/L.), pyridaben (0.75 and 1.5 ai mg/L) and sulfur (3.2 and 12.8 ai mg/L) were significantly more toxic for *A. oleae* adults compared with other acaricides (low conc. $F_{6,20}$ = 69.2; HRC conc. P < 0.01; $F_{6,20}$ = 38.6; P < 0.01). Reducing the concentrations of all tested acaricides was significantly decreased mortality rates of *A. oleae* adults (acequinocyl $F_{2,8}$ = 55.0; P < 0.01; azadirachtin $F_{2,8}$ = 21.6; P < 0.01; fenbutatin oxide $F_{2,8}$= 50.8; P < 0.01; milbemectin $F_{2,8}$= 10.4; P = 0.011; pyridaben $F_{2,8}$ = 15.3; P < 0.01; spirodiclofen $F_{2,8}$= 9.10; P = 0.015; sulfur $F_{2,8}$ = 528; P < 0.01).

Table 2. The bioassay results for *Aceria oleae* adults

Active substance	Number of individuals[a]	Tested conc.	Mean corrected death rates (%)[b]	The tested conc.	Mean corrected death rates (%)[b]	The tested conc.	Mean corrected death rates (%)[b]
Acequinocyl	120	24.4	54.2 bc[d]C[e]	97.5	73.9 bB	195[c]	97.2 aA
Azadirachtin	120	0.75	57.0 bcB	1.5	66.4 bAB	3[c]	80.4 cA
Fenbutatin oxide	120	20.6	65.4 bB	82.5	74.8 bB	330[c]	90.7 bA
Milbemectin	120	0.29	92.5 aB	1.16	98.2 aA	9.8[c]	100 aA
Pyridaben	120	0.75	86.0 aB	1.5	98.1 aAB	150[c]	100 aA
Spirodiclofen	120	7.50	47.8 cC	30	69.4 bB	60[c]	80.4 cA
Sulfur	120	3.20	89.7 aB	12.8	94.4 aAB	3200[c]	100 aA

[a] 120 adult mites were used each concentration; [b] corrected mortality rates by Abbott formula; [c] high recommended concentration for other mite pests in Turkey; [d] means followed by the same lowercase letters within columns are not significantly different (P < 0.01); [e] means followed by the same uppercase letters within rows are not significantly different (P < 0.01).

Side effects on *Neoseiulus californicus*

The side effects of HRC and two sublethal concentrations of seven acaricides on juveniles and females of *N. californicus* are summarized in Table 3. The corrected mortality for juveniles (JM) varied significantly with exposure to different acaricides ($F_{20,96}$ = 8.76; P < 0.01). JM were 96, 95, 96, 94, 100, 100 and 82% with exposure to HRCs of acequinocyl, azadirachtin, fenbutatin oxide, milbemectin, pyridaben, spirodiclofen and sulfur, respectively. Also, JM of their two concentrations were 76 and 95%, 67 and 71%, 42 and 94%, 31 and 75%, 92 and 94%, 44 and 100% and 22 and 35%, respectively. Significantly low JM values were determined when the juveniles were treated with some concentrations of milbemectin (0.29 ai mg/L) and sulfur (3.2 and 12.8 ai mg/L). The corrected mortality for females (FM) differed significantly with exposure to different acaricides ($F_{20,90}$ = 10.4; P < 0.01). FM were 100, 67, 42, 62, 81, 100 and 92% with exposure to HRCs of acequinocyl, azadirachtin, fenbutatin oxide, milbemectin, pyridaben, spirodiclofen and sulfur, respectively. In addition, FM values of their two sublethal concentrations were found to be 13 and 76%, 13 and 16%, 7 and 21%, 60 and 62%, 44 and 81%, 58 and 96%, and 13 and 83%, respectively. Significantly low FM were detected in treated with some concentrations of acequinocyl (24.4 ai mg/L), azadirachtin (0.75 and 1.5 ai mg/L) and sulfur (3.2 ai mg/L) (Table 3).

Table 3 also shows fecundity reduction (R) of *N. californicus* exposed to the HRCs and two sublethal concentrations of the seven acaricides. The HRCs of the acaricides significantly decreased the fecundity of *N. californicus* females compared untreated females ($F_{20,96}$ = 12.8; P < 0.01). R was considerably less in females exposed to the HRCs of all tested acaricides (from 0 to 0.3). Moderate R (0.4 to 0.6) were observed in some concentrations of azadirachtin (0.75 ai mg/L), fenbutatin oxide (82.5 ai mg/L), milbemectin (1.16 ai mg/L), sulfur (3.2 ai mg/L) and spirodiclofen (7.5 ai mg/L), low to high, respectively.

According to the side effect scale suggested by IOBC, HRCs of acequinocyl, pyridaben, spirodiclofen and sulfur were harmful and others were moderately harmful to females (Table 3). However, HRCs of all tested acaricides were found to be harmful to juveniles except sulfur. The lowest concentrations of acequinocyl, azadirachtin, spirodiclofen and sulfur found to be slightly harmful to females, a few concentrations of fenbutatin oxide (20.6 ai mg/L), spirodiclofen (7.5 ai mg/L) and sulfur (3.2 ai mg/L) were found to be slightly harmful to both juveniles and females of *N. californicus.* Fenbutatin oxide (20.6 ai mg/L) was found to be harmless to females of *N. californicus.*

Table 3. The side effects on *Neoseiulus californicus* of seven acaricides in laboratory

Active substance	Applied concentration (ai mg/L)	Number of individuals	JM[1] (%)		FM[2] (%)		R[3]	E[4] (%)	Juvenile toxicity[6]	E[5] (%)	Female toxicity[6]
Acequinocyl	24.4	50	76.0	a-e[7]	13.3	e	0.3	92.8	III	73.4	II
	97.5	50	95.1	a	76.0	a-c	0.0	100	IV	100	IV
	195.0	50	95.9	a	100	a	0.0	100	IV	100	IV
Azadirachtin	0.75	50	67.2	a-e	12.7	e	0.4	87.9	III	67.7	II
	1.5	50	70.6	a-e	16.2	e	0.2	95.3	III	86.6	III
	3.0	50	95.1	a	66.7	a-e	0.1	99.7	IV	97.7	III
Fenbutatin oxide	20.6	50	42.1	c-e	6.7	e	1.2	42.14	II	6.67	I
	82.5	50	94.1	ab	20.8	de	0.4	97.9	III	72.3	II
	330	50	95.8	a	41.7	a-e	0.2	98.9	IV	86.0	III
Milbemectin	0.29	50	31.3	de	60.1	a-e	0.3	83.5	III	90.5	III
	1.16	50	75.0	a-e	61.9	a-e	0.4	90.5	III	85.5	III
	9.8	50	94.1	ab	61.9	a-e	0.3	98.5	IV	90.5	III
Pyridaben	0.75	50	91.6	a-c	44.4	a-e	0.3	97.7	III	84.4	III
	1.5	50	94.1	ab	71.4	a-d	0.2	99.1	IV	95.7	III
	150	50	100	a	80.9	ab	0.0	100	IV	100	IV
Spirodiclofen	7.5	50	43.9	b-e	58.3	a-e	0.6	65.8	II	74.6	II
	30	50	100	a	96.0	a	0.0	100	IV	100	IV
	60	50	100	a	100	a	0.0	100	IV	100	IV
Sulfur	3.2	50	21.6	e	13.2	e	0.5	59.2	II	54.8	II
	12.8	50	35.3	de	83.3	a	0.2	85.8	III	96.3	III
	3200	50	82.4	a-d	91.7	a	0.1	98.1	III	99.1	IV

[1] Corrected death rates of juveniles (survival rate from egg to adult); [2] corrected death rates of females; [3] reproduction reduction rate of treated females compared with untreated ones; [4] total side effect according to juvenile deaths= 100 -((100-JM) x R); [5] total side effect according to female deaths= 100-((100-FM) x R); [6] side effect scale, I = harmless (<%30), II = slightly harmful (30-79%), III = moderately harmful (80-99%), IV = harmful (>99); [7] means followed by the same letter with columns are not significantly different ($P < 0.05$).

Figures 2 and 3 show that the fecundity and lifespan of *N. californicus* females exposed to HRCs and two sublethal concentrations of seven acaricides. Based on Kaplan-Meier survival analysis, HRCs of the acaricides significantly reduced the lifespan of *N. californicus* females ($\chi 2 = 60.2$; $df = 2$, $P < 0.01$). Besides increased concentration of the acaricides, active substance differences resulted in a significant decrease in survivals of *N. californicus* females ($\chi 2 = 187$; $df = 6$, $P < 0.01$).

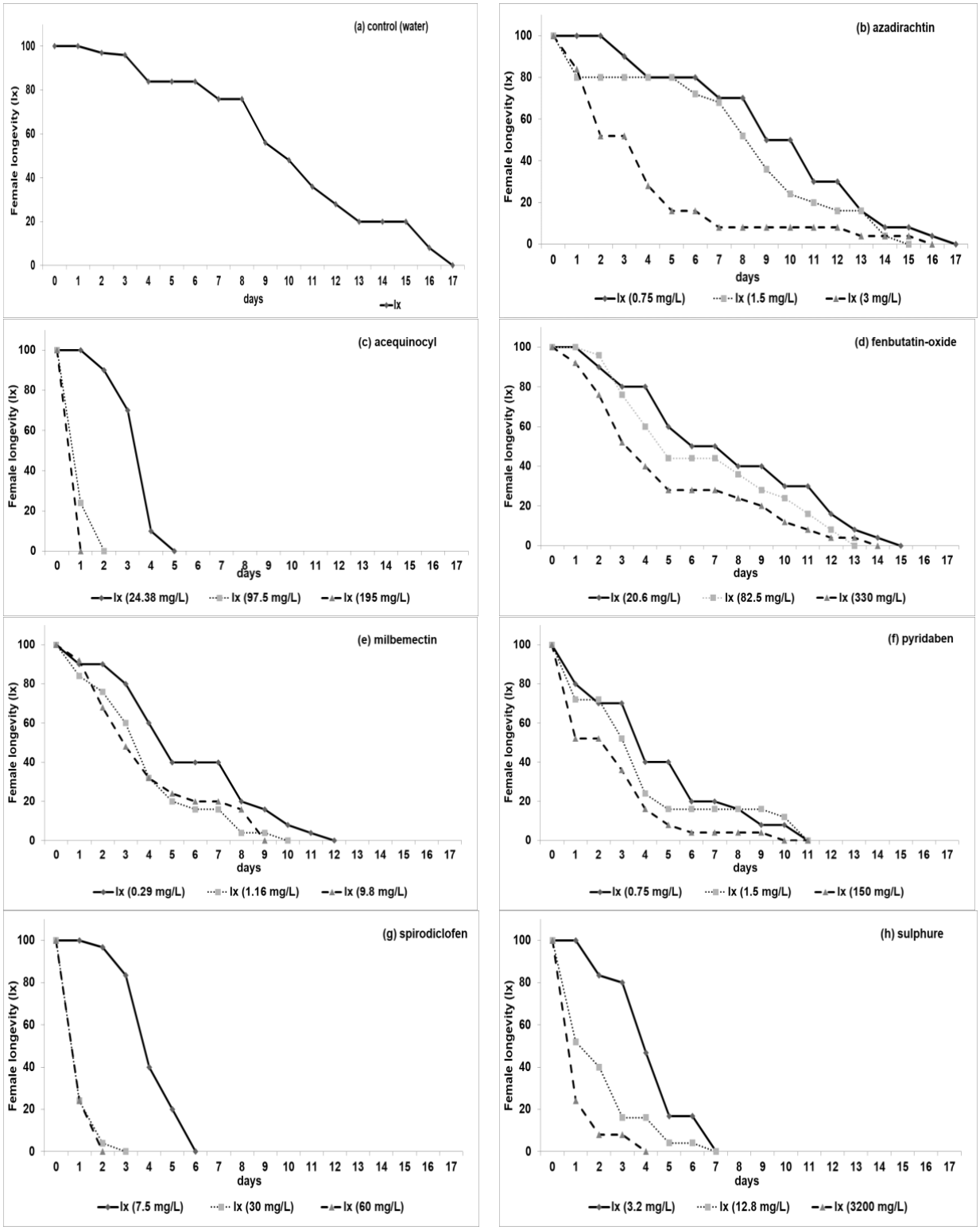

Figure 2. The longevity of a) untreated *Neoseiulus californicus* females versus females exposed to HRCs and two sublethal concentrations of b) azadirachtin, c) acequinocyl and d) fenbutatin oxide, e) milbemectin, f) pyridaben, g) spirodiclofen, and h) sulfur.

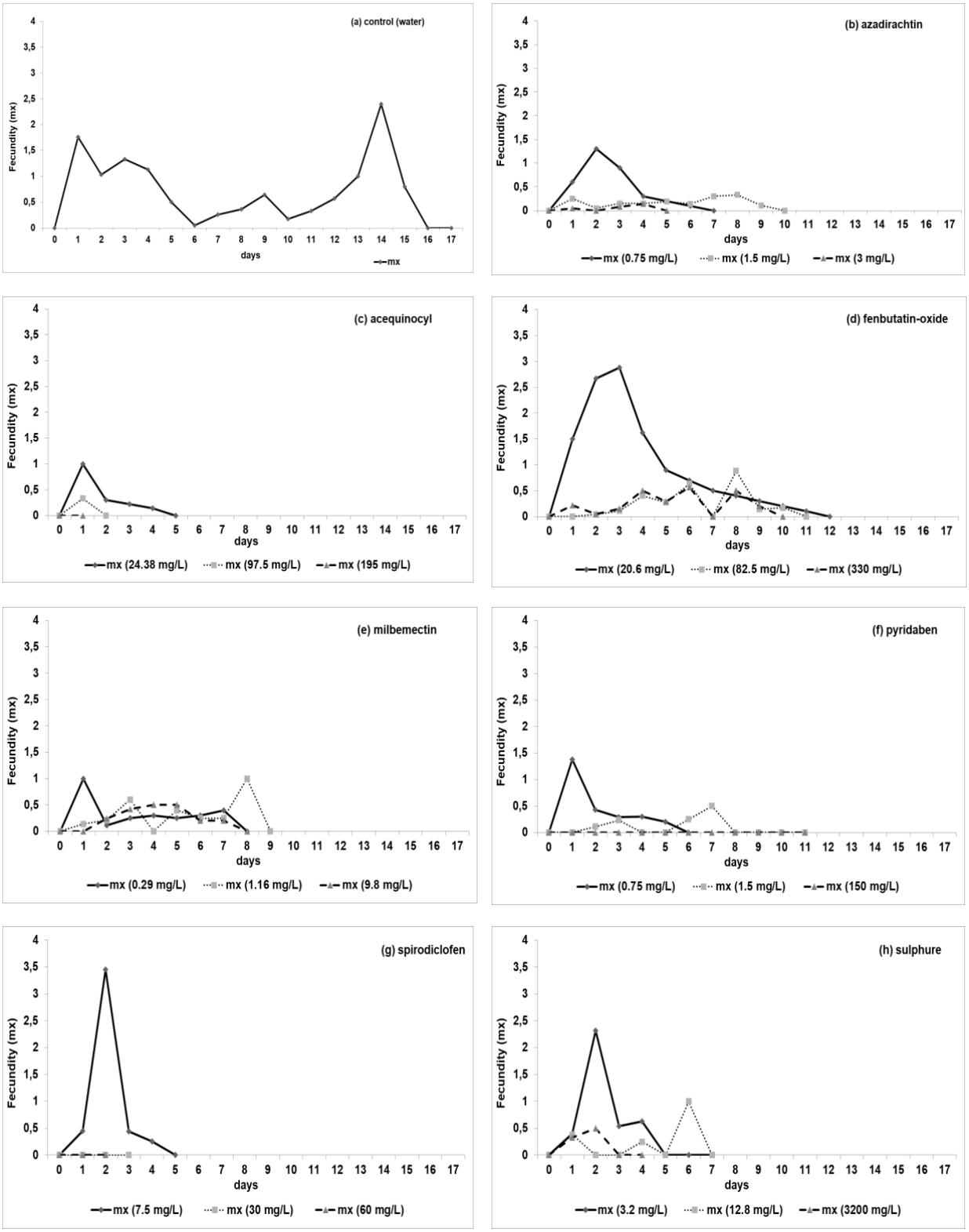

Figure 3. The fecundity of a) untreated *Neoseiulus californicus* females versus females exposed to HRCs and two sublethal concentrations of b) azadirachtin, c) acequinocyl and d) fenbutatin oxide, e) milbemectin, f) pyridaben, g) spirodiclofen and h) sulfur.

The lifespans of females were 1, 2, 4, 9, 10, 14 and 16 days for acequinocyl, spirodiclofen, sulfur, milbemectin, pyridaben, fenbutatin oxide and azadirachtin, respectively. Females exposed to the lowest concentrations of fenbutatin oxide (20.6 ai mg/L) and azadirachtin (0.75 ai mg/L) survived at a significantly higher rate than those exposed to other acaricides (Figure 2). HRCs of the acaricides significantly reduced oviposition of *N. californicus* females compared untreated females ($F_{20,96}$ = 11.6; P < 0.01). The fecundity of females was 0, 0, 0, 0.3, 0.8, 2.1 and 2.5 eggs/days for acequinocyl, pyridaben, spirodiclofen, azadirachtin, sulfur, milbemectin and fenbutatin oxide, respectively. The lowest concentrations of fenbutatin oxide (20.6 ai mg/L) did not affect the fecundity of females compared with untreated females (Figure 3).

Discussion

The current study showed that different concentrations of acequinocyl, azadirachtin, fenbutatin oxide, milbemectin, pyridaben, spirodiclofen and sulfur were toxic to *A. oleae* adults. Although the toxicity effect of acaricides on some other species of the family Eriophyidae has been widely reported in several crops, there is no record about their biological effects on *A. oleae* (Ky & Shepherd, 1988; Silva et al., 1988; van Leeuwen et al., 2010; Fischer & Klötzli, 2015; Kashyap et al., 2015; Kolcu & Kumral, 2018). In our study, HRCs of acequinocyl, milbemectin, pyridaben and sulfur were found to be significantly more toxic to *A. oleae* compared with other tested acaricides. When the concentrations of milbemectin, pyridaben and sulfur were reduced, these low concentrations were still highly toxic with mortality of more than 90%. Similarly, some researchers reported that different concentrations of milbemectin, pyridaben and sulfur are highly toxic to eriophyid species such as *Aculops lycopersici* (Massee, 1937) (Acari: Eriophyidae) (Royalty & Perring, 1987; Silva et al., 1988; van Leeuwen et al., 2010; Fischer & Klötzli, 2015; Kolcu & Kumral, 2018).

This study also provided laboratory findings about the side effects of acaricides at three concentrations to *N. californicus*. HRCs of all tested acaricides were harmful to phytoseiid juveniles. In spite of the fact that azadirachtin, fenbutatin oxide and milbemectin were found to be moderately harmful to adults, their high toxicity to juveniles showed that the acaricides are unfavorable for *N. californicus*. The current study also showed that some low concentrations of fenbutatin oxide, milbemectin, sulfur and spirodiclofen were slightly toxic to both females and juveniles of *N. californicus*. Based on R, low concentrations of fenbutatin oxide, spirodiclofen and sulfur slightly reduced the fecundity of females. In addition, their low concentrations were highly toxic to *A. oleae*. Therefore, acequinocyl and pyridaben are not a favorable acaricide for *N. californicus* and they cannot be recommended to use with this predatory mite in integrated management (Ghadim Mollaloo et al., 2018). Similar to our results, some researchers reported that minor side effects on phytoseiids were observed from evaporation and dusts arising from application of sulfur (Pijnakker & Ramakers, 2009; Gazquez et al., 2011). Also, previous studies also showed the compatibility of sulfur with phytoseiids in different agricultural ecosystems (Pijnakker & Ramakers, 2009; Gazquez et al., 2011; Fiedler & Sosnowska, 2012). In contrast, some researchers who reported that HRC of spirodiclofen was harmful to some phytoseiids due to considerably negative effects on their fecundity and lifespan (Maroufpoor et al., 2016; Evans et al., 2018), while only sublethal doses were slightly harmful (Kaplan et al., 2012; Audenaert et al., 2014; Alinejad et al., 2016; Döker & Kazak, 2019). Our results indicated that low concentrations of fenbutatin oxide have no negative effect on *N. californicus*, which is consistent with the results of other studies on the effects of the acaricide on *Phytoseiulus persimilis* (Athias-Henriot, 1957) and *N. californicus* (Kim & Yoo, 2002; Evans et al., 2018).

In the present study, the lowest concentration of azadirachtin was slightly harmful to *N. californicus* females whereas it was moderately harmful to its juveniles. Similar with the findings of Kurubal & Ay (2015), the lifespan of females was not affected when applied the concentration of azadirachtin. Our findings on adult toxicity of azadirachtin are similar with the results of some studies demonstrated that, the botanical insecticide/acaricide did not cause high mortality of phytoseiid mites due to quick degradation of the botanical acaricide under field conditions (Castagnoli et al., 2005; Audenaert et al., 2014). When making

comparisons with other studies, this variation in mortality of phytoseiid juveniles might be a result of different formulations and concentration as well as test conditions (Castagnoli et al., 2005; Audenaert et al., 2014). Our results are similar to those of some authors who showed azadirachtin properties stem from strong antifeedant activity against many arthropod species including mites, which is supplemented also by marked insect growth regulatory and sterility effects (Mordue & Blackwell, 1993; Sundaram & Sloane, 1995).

In conclusion, in order to conserve biological control agents, the most important factor for controlling the key pests is to reduce the concentrations applied by using sublethal doses, which do not have biological, behavioral and/or demographic effects on natural enemies (Roush, 1989; Dent, 2000; Desneux et al., 2007). Consequently, the results of our study show that the low concentrations of tested acaricides may conserve populations of *N. californicus*. The findings are consistent with the findings of Dent (2000) who reported that acaricides at reduced rates might be used in combination with biological control agents for IPM, reducing the selection pressure and development of acaricide resistance. Among tested acaricides, the low concentration of sulfur was highly toxic to *A. oleae*, but this concentration was not harmful to females and juveniles of *N. californicus.* For *A. oleae* control, a low concentration of sulfur is compatible with *N. californicus.* Alternatively, when *A. oleae* population reaches high population densities in olive orchards, low concentrations fenbutation oxide and spirodiclofen, which have moderately toxic effects, can be applied, and so the phytoseiid population could be conserved due to only slightly harmful effects to them. Azadirachtin could be used due to its quick degradation potential under field conditions. However, this hypothesis should be tested under field conditions. This integrated strategy could both reduce the chemical residue in olives while also conserving the population of the predatory mite. The tested acaricides and their determined concentrations have potential for the control of *A. oleae* in olive orchards, although more studies are needed for verification of the results under field conditions.

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