

## Side Effects of Four Acaricides on the Predatory Mites of *Neoseiulus californicus* McGregor and *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae)

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### Keywords

Phytoseiidae,  
*Neoseiulus californicus*,  
*Phytoseiulus persimilis*,  
Acaricide,  
Side effect

**Abstract:** In the present study determined the side effects of four acaricides of acequinocyl, etoxazole, bifentazate and milbemectin on the predator mites *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus californicus* McGregor. Acaricide concentrations were prepared as a two times the field application dose (2T), field application dose (T), and half of the field application dose (T/2) and were then applied on eggs, nymphs and adults of the *P. persimilis* and *N. californicus*. The side effects of the acaricides applied to the predator mites were determined three, five and seven days after the application. While all doses of acequinocyl and etoxazole showed a higher toxic effect on *N. californicus* eggs, it was found that bifentazate and milbemectin caused similar effects on both predator mite eggs. Furthermore, it was found that the toxic effects of acequinocyl, etoxazole, bifentazate and milbemectin on nymphs and adults of the predator mites were high seventh day after the application. The results of the study showed that four acaricides frequently used against to pest mites, should be used more carefully in the agricultural areas.

## *Neoseiulus californicus* McGregor ve *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) Avcı Türlerine Dört Farklı Akarisitin Yan Etkileri

### Anahtar Kelimeler

Phytoseiidae,  
*Neoseiulus californicus*,  
*Phytoseiulus persimilis*,  
Akarisit,  
Yan etki

**Özet:** Bu çalışmada, acequinocyl, etoxazole, bifentazate ve milbemectin etkili maddeye sahip dört akarisitin, avcı akarlar *Phytoseiulus persimilis* Athias-Henriot ve *Neoseiulus californicus* McGregor'a karşı yan etkileri araştırılmıştır. Tarla uygulama dozunun iki katı (2T), tarla uygulama dozu (T) ve tarla uygulama dozunun yarısı (T/2) olacak şekilde hazırlanan akarisit konsantrasyonları *P. persimilis* ve *N. californicus*'ün yumurta, nimf ve ergin dönemlerine uygulanmıştır. Akarisitlerin avcı akarlaraya etkileri uygulamadan 3, 5 ve 7 gün sonra belirlenmiştir. Acequinocyl ve etoxazole'ün, tüm dozları *N. californicus* yumurtalarında daha fazla toksik etki gösterirken, bifentazate ve milbemectinin ise her iki avcı akar yumurtalarında da benzer etkiye neden olmuştur. Acequinocyl, etoxazole, bifentazate ve milbemectin'in uygulamadan 7 gün sonra avcı akarların nimf ve erginleri üzerinde toksik etkilerinin yüksek olduğu belirlenmiştir. Çalışma sonucunda, üretim alanları içerisinde zararlı kırmızı örümceklere karşı yaygın kullanılan dört akarisitin daha dikkatli bir şekilde kullanılması gerektiğini göstermiştir.

### 1. Introduction

Tetranychid phytophagous mites are a major arthropod pest that is found in greenhouses, orchards, vegetables and ornamentals, particularly during the warm season when populations can reach excessive levels in a short period of time [1, 2]. Owing to its high biotic potential, the pests can quickly inflict economic damage, causing great reductions in the quality and quantity of the plant [3]. Chemical control is the most common strategy for managing spider mite worldwide [4, 5]. However, the use of pesticides

for long periods and frequently causes resistance in pests as well as side effects in natural enemies [6, 7, 8]. As a result, producers are evaluating the use of alternative management strategies for the spider mite that may avoid relying strictly on pesticides [9].

Alternative management strategies include the use of predator mites along with pesticides instead of using pesticides alone in production areas [10]. To identify the most selective pesticides that could be used in combination with biological control strategies it is very important to know the side effects of these

products on the most relevant natural enemies for each specific crop [11]. Phytoseiid mites are important biological control agents for different kinds of pests on various greenhouse, fruit ranches, orchards and citrus groves [12]. According to the habitat and food spectrum, phytoseiid mites are classified as specialists (type I, II) and generalists (type III, IV) [13]. *Phytoseiulus persimilis* A.H. (Acari: Phytoseiidae) is considered a type I predator because it only feeds and is able to survive on *Tetranychus urticae* Koch (Acari: Tetranychidae). In contrast, *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) is a type II predator because it feeds and can survive on alternative prey and pollen in the absence of *T.urticae* [14]. Furthermore, the tolerance of *N. californicus* against low humidity and high temperature is higher than *P. persimilis* [15]. While a 60-80% humidity increase is required for the population to increase in *P. persimilis*, *N. californicus* can maintain its population continuity under low humidity conditions [16].

Broadly effective pesticides used in agricultural areas cause side effects on all natural enemies as well as predator mites. *P. persimilis* and *N. californicus*, in the Phytoseiidae family, are affected by pesticides used in agricultural areas and cannot maintain their natural population [17]. Identifying the effects of the pesticides to be used on natural enemies is of importance for developing integrated control methods. Therefore, it is necessary to choose the pesticides used for diseases and pests that have the lowest effect on the beneficial insects in integrated control programs. Studies conducted regularly on pesticides and mites with the aim of identifying the side effects are of particular importance in order to develop integrated control programs and determine the compounds to be used in these control programs [18]. At the end of the studies, it is possible to use the pesticides identified as harmless and not very harmful for predator mites in production areas. It is also thought that the development of resistance in pests will reduce based on the decrease in pesticide use.

The study aimed to determine the side effects of four acaricides in different life periods of predator mites, *P. persimilis* and *N. californicus*. Additionally, the effects of acaricides used in the study on egg hatching of *P. persimilis* and *N. californicus* were identified.

## 2. Material and Method

### 2.1. Origin and rearing of predatory mites

In the study, eggs, nymphs and adults of two predator mites, *P. persimilis* and *N. californicus* were used. In Turkey, the *P. persimilis* was first collected from vegetable fields in Hatay province in 1993 [19]; the *N. californicus* was first collected in Isparta from an organic apple garden in 2008 [20]. Both of the

predator mites, so far, were reared in laboratory and were never exposed to a pesticide. The predator mites were reared in a climatically controlled chamber on the *Phaseolus vulgaris* L. var. *Barbunia* plant on which there is *T. urticae*. *P. persimilis* and *N. californicus* populations were reared at  $26 \pm 2^\circ\text{C}$  temperature and  $60 \pm 5\%$  relative humidity in climatically controlled chambers lighted for 16 hours.

### 2.2. Acaricides

Three different doses of acaricides that included a field application dose (T), double the field application dose (2T) and half of the field application dose (T/2) were applied on the eggs, nymph and adults of *P. persimilis* and *N. californicus*. The general features and application doses of the acaricides used in the study are given in Table 1. These acaricides have been chosen because they are widely used in the study.

### 2.3. Obtaining some periods predator mites

A spray tower-leaf disk method was used to determine the side effects of acaricides on *P. persimilis* and *N. californicus*. In the preparation of leaf discs, leaves removed from bean plants (*Phaseolus vulgaris* L. var. *Barbunia*) were placed on moist cotton in 90 mm diameter plastic Petri dishes. To prevent predatory mites from escaping, the leaf discs were made using approximately 3 cm diameter rings with Tangle-Trap adhesive material. The study used eggs, nymphs and adults of *N. californicus* and *P. persimilis*. To ensure that all individuals used in the study were at the same age and developmental stage, female individuals taken from the population were transferred to leaf discs to lay eggs and the eggs taken from these females were used in the study. For nymph and adult experiments, the researchers waited until the eggs hatched and then nymphs and adults of the same age were used.

### 2.4. Application to acaricides on predatory mite eggs

In the study, the Kim & Yoo's [21] method was adopted and used with the purpose of determining the effects of acaricides on *P. persimilis* and *N. californicus* eggs hatching. *N. californicus* and *P. persimilis* eggs were transferred onto leaf discs in the petri dishes and it was confirmed that each leaf disc had 10 individuals. Three doses of acaricides were applied four replications. Only distilled water was applied on predator mite eggs in the control group. The doses prepared acaricides were sprayed on leaf surfaces at 1 bar pressure and  $1.7 \text{ mg cm}^{-2}$  [22] acaricides at every application by spray tower. In daily controls the individuals that hatched and did not hatch from the eggs were determined by using the control group as a reference. Counting procedure continued until all the eggs in the control group were hatched.

**Table 1.** General features and application doses of the acaricides used in the study.

Active substance name	Commercial name	Half of field application dose (ml/100 L water)	Field application dose (ml/100 L water)	Two fold of field application dose (ml/100 L water)
Acequinocly	Kanemite SC g/l	62.5	125	250
Etoxazole	Zoom 10SC g/l	12.5	25	50
Milbemectin	Milbeknock EC g/l	50	100	200
Bifenazate	Floramite SC 240 g/l	30	60	120

**Table 2.** Mean egg not hatching rate (%±SE) of predatory mites when exposed to acaricides.

<i>Neoseiulus californicus</i>				
Acaricides	Doses			
	T/2	T	2T	
Acequinocly	49.99±8.61 <sup>bcB</sup>	64.99±3.33 <sup>bcA</sup>	76.66±6.66 <sup>bcA</sup>	
Etoxazole	68.33±6.38 <sup>aB</sup>	83.33±3.85 <sup>aA</sup>	91.66±6.38 <sup>aA</sup>	
Bifenazate	61.66±3.33 <sup>abC</sup>	74.99±3.33 <sup>abB</sup>	89.99±3.85 <sup>abA</sup>	
Milbemectin	44.99±3.33 <sup>cdC</sup>	69.99±3.85 <sup>abcB</sup>	84.99±3.33 <sup>abcA</sup>	
<i>Phytoseiulus persimilis</i>				
Acaricides	Doses			
	T/2	T	2T	
Acequinocly	23.33±3.85 <sup>eC</sup>	34.99±3.33 <sup>eB</sup>	46.66±5.44 <sup>dA</sup>	
Etoxazole	30.48±13.21 <sup>deB</sup>	56.04±15.14 <sup>cdA</sup>	78.82±9.03 <sup>abcA</sup>	
Bifenazate	61.30±4.91 <sup>abC</sup>	77.14±3.86 <sup>abB</sup>	91.44±3.16 <sup>aA</sup>	
Milbemectin	48.33±1.92 <sup>bcC</sup>	63.81±2.77 <sup>bcdB</sup>	77.61±2.93 <sup>abcA</sup>	

\* Lower cases show difference among average of pesticide effect in the same column, upper cases show difference among effect of dose of each pesticide in the same day.  $p < 0.05$

## 2.5. Application to acaricides on thenymphs and adultsof predatory mites

Kaplan et al's [23] and Bernardi et al's [22] methods were used in the study with the aim of determining the effects of acaricides on nymphs and adults of *P. persimilis* and *N. californicus*. Predator mite nymphs or adults at the same period were transferred onto leaf discs; each petri dish had 15 individuals and was sprayed by spraying tower. The spray tower was worked under 1 bar pressure and 1.7 mg cm<sup>-2</sup> acaricides containing compounds were sprayed on the leaf surface. Distilled water were applied on the nymphs and adults of predator mites in the control groups. In the study, three doses of each acaricides (application dose, half of the application dose and double the application dose) were applied four replications. All periods of *T. urticae* individuals were given to hunt in the petri during the counting in order to feed predator mite nymphs or adults. Counting was performed on the first, third, fifth and seventh days and the effects of the acaricides on the nymphs or adults of *N. californicus* and *P. persimilis* were assessed.

## 2.6. Statistical analysis

The mortality rate (M) for the predator mites, as a result of the use of acaricides, was calculated according to Abbott [24]. Therefore, the mortality in the control groups were not given. When the deaths in the control groups were more than 10%, the study was repeated in an experiment. All experiments were conducted in a climatically controlled chamber at 26±2°C, 60±5% relative humidity and 16: 8 hours light: dark photoperiod.

$$Mortality (M) = \frac{CL - DL}{CL} \times 100 \quad (1)$$

Where; CL is the number of living individuals in the control group (%) and DL is the number of the living individuals in the disinfected groups (%)

Pesticides are separated into four groups by the International Organization for Biological Control "Pesticides and Beneficial Organisms" study group as follows: If the mortality rate is <30%, it is accepted as harmless [1], if it is between 30-79%, it is accepted as a little harmful [2], if it is between 80-99% it is as moderately harmful [3] and if it is >99% it is accepted as harmful [4, 25]. In the statistical evaluation of the results, one-way analysis of variance technique (One-Way ANOVA) was applied on the data obtained and the Tukey test was used to determine the difference between the averages [26].

## 3. Results

### 3.1. Effect of acaricides on predatory mites eggs

The effects of acaricides on *N. californicus* and *P. persimilis* egg hatching are given in Table 2. They were found at the highest level of effect at T/2 dose acaricides of etoxazole and bifenazate on *N. californicus* and bifenazate on *P. persimilis*. The highest effect values for of acaricides on eggs hatching at T dose were found in *N. californicus* with etoxazole, bifenazate and milbemectin and in *P. persimilis* with bifenazate. The highest toxic effect of acaricides on eggs hatching at 2T dose was found with etoxazole on *N. californicus* with 91.66%, with bifenazate on *P. persimilis* with 91.44%. The highest

effect of acaricides on eggs hatching in both predator mites at 2T dose was found in etoxazole, bifenazate and milbemectin. In Table 2, the effects of T dose of all acaricides on *N. californicus* and *P. persimilis* were not found statistically different from each other.

### 3.2. Effect of acaricides on predatory mite nymphs

The different doses of acaricides on *N. californicus* and *P. persimilis* nymphs are given in Table 3. It was detected that the effects of the different doses of acaricides applied on predator mite nymphs increased according to which acaricides was applied and counting the days (Table 3). The results revealed that the acaricides used in the study caused remarkable side effects on predator mite nymphs. In the observations performed seventh day after the application, it was seen that 2T dose caused high side effects at high rate in both predator mite nymphs and acaricidal effects persisted in the same group statistically ( $p < 0.05$ ). At 2T dose, acequinocly showed 100 % effect on *N. californicus* nymphs; acequinocly, etoxazole and bifenazate showed 100% effect on *P. persimilis* nymphs. According to Hassan [25], since the seventh day results showed that acequinocly had more than 99% effect on *N. californicus* nymphs and acequinocly, etoxazole and bifenazate showed more than 99% effect on *P. persimilis* nymphs at 2T dose, they were included in the harmful group. At the 2T dose, for milbemectin<bifenazate<etoxazole effect was between 80-90% on *N. californicus* nymphs and milbemectin showed effect between 80-90% on *P. persimilis* nymphs, they were classified as moderately harmful. In the observations were made seventh days after the application, at T dose of acequinocly, etoxazole on *N. californicus* nymphs and acequinocly, etoxazole and bifenazate in *P. persimilis* nymphs, were classified in the same group ( $p < 0.05$ ). Bifenazate showed less effect against *N. californicus* nymphs and milbemectin showed less effect against to *P. persimilis* predator mite nymphs than the others. Nonetheless, they were not found statistically different from each other ( $p < 0.05$ ). According to Hassan [25], for the results of the seventh day, for etoxazole and bifenazate showed 100% effect on *P. persimilis* nymphs at level of T dose, they were in the harmful group.

For acequinocly < etoxazole < bifenazate showed between 80-90% effect on *N. californicus* nymphs and acequinocly<milbemectin showed between 80-90% effect in *P. persimilis* nymphs at T dose, they were classified as moderately harmful. At T dose, since milbemectin showed between 30-79% effect in *N. californicus* nymphs. It was the only one no placed in very harmful group. In the observations performed seventh days after the application, at T/2 dose, since etoxazole showed 80-90% effect on *N. californicus* nymphs and acequinocly<bifenazate<etoxazole showed between 80-90% effect on *P. persimilis* nymphs, they were not in very harmful group. At T/2

dose, since bifenazate<milbemectin<acequinocly on *N. californicus* nymphs and milbemectin on *P. persimilis* nymphs showed 30-79% effect, they were classified as a little harmful.

### 3.3. Effect of acaricides on predatory mite adults

The effects of the different acaricides doses used in the study on *N. californicus* and *P. persimilis* adults are given in Table 4. It was ascertained that effects of the different acaricides doses applied on predator mite adults increased according to the acaricides and counting daily (Table 4). It was also found that the acaricides used in the study had significant side effects on predator mite adults, particularly at the end of seventh day. In the observations performed seventh days after the application, it was found that all of the acaricides were in the same group statistically in terms of the effect values identified in both predators at 2T dose of the acaricides ( $p < 0.05$ ).

According to Hassan [25], at the seventh day results at 2T dose, for acequinocly and bifenazate showed 100% effect on *P. persimilis* adults, were in the harmful group. At 2T dose, for acequinocly<etoxazole<milbemectin<bifenazate showed 80-99% effect on *N. californicus* adults and also etoxazole<milbemectin showed between 80-99% effect on *P. persimilis* adults, they were classified as moderately harmful. In the observations performed seventh days after the application, for T dose of the acaricides, the effect values of bifenazate and etoxazole identified on *P. persimilis* adults were found statistically similar ( $p < 0.05$ ). All acaricides applied on *N. californicus* at T dose and the effect values identified in acequinocly and milbemectin were found similar and the same group statistically ( $p < 0.05$ ). At T dose, for bifenazate showed 100% effect on *P. persimilis*, they were in the harmful group. According to Hassan [25], at the end of the results of seventh day at T dose, for etoxazole showed between 80-99% effect on *N. californicus* adults and etoxazole<acequinocly<milbemectin showed between 80-99% effect on *P. persimilis* adults, they were in the moderately harmful group. For acequinocly<milbemectin<bifenazate showed between 30-79% effect on *N. californicus* adults at T dose, it was found that they were as little harmful group.

In view of the counting results of seventh day, in acaricide applications at T/2 dose, the highest effect was identified in bifenazate with 89.52% in *P. persimilis* adults and was not found statistically different from the others ( $p < 0.05$ ). At T/2 dose of the acaricides the effects of etoxazole on *N. californicus* adults and the effects acequinocly, etoxazole and milbemectin on *P. persimilis* adults were found similar ( $p < 0.05$ ). At T/2 dose, the effect values of acequinocly, bifenazate, milbemectin on *N. californicus* adults were in the same group.

**Table 3.** Percentage mortality ( $\pm$ SE) of *Neoseiulus californicus* and *Phytoseiulus persimilis* nymphs exposed to acaricides.

		Doses	Day 1	Day 3	Day 5	Day 7
<i>Neoseiulus californicus</i>	Acequinocly	T/2	50.00 $\pm$ 2.71 <sup>cdeC</sup>	60.00 $\pm$ 6.70 <sup>dC</sup>	76.32 $\pm$ 1.55 <sup>bcC</sup>	78.74 $\pm$ 2.89 <sup>bcB</sup>
		T	68.33 $\pm$ 3.33 <sup>bcB</sup>	79.40 $\pm$ 5.08 <sup>cdB</sup>	91.31 $\pm$ 3.09 <sup>aB</sup>	96.43 $\pm$ 4.12 <sup>abA</sup>
		2T	83.33 $\pm$ 8.61 <sup>bcA</sup>	98.22 $\pm$ 3.57 <sup>abA</sup>	100.00 $\pm$ 0.0aA	100.00 $\pm$ 0.0aA
	Etoxazole	T/2	52.49 $\pm$ 10.32 <sup>bcdeB</sup>	70.71 $\pm$ 8.21 <sup>bcdA</sup>	77.73 $\pm$ 9.92 <sup>abcA</sup>	82.57 $\pm$ 9.16 <sup>abB</sup>
		T	64.16 $\pm$ 5.00 <sup>bcdAB</sup>	75.23 $\pm$ 7.50 <sup>cdeA</sup>	77.61 $\pm$ 6.52 <sup>bcA</sup>	88.96 $\pm$ 8.75 <sup>abcAB</sup>
		2T	69.25 $\pm$ 3.22 <sup>da</sup>	83.33 $\pm$ 3.74 <sup>cdA</sup>	91.24 $\pm$ 6.18 <sup>abcA</sup>	98.21 $\pm$ 3.57 <sup>aA</sup>
	Bifenazate	T/2	44.99 $\pm$ 6.38 <sup>cdeB</sup>	56.66 $\pm$ 3.85 <sup>deC</sup>	67.26 $\pm$ 2.99 <sup>cdB</sup>	68.45 $\pm$ 3.57 <sup>cC</sup>
		T	63.33 $\pm$ 3.84 <sup>bcdA</sup>	69.99 $\pm$ 3.85 <sup>defB</sup>	75.71 $\pm$ 4.94 <sup>cA</sup>	80.59 $\pm$ 4.04 <sup>cdB</sup>
		2T	71.66 $\pm$ 3.33 <sup>cdA</sup>	78.33 $\pm$ 3.33 <sup>dA</sup>	82.85 $\pm$ 3.29 <sup>cdA</sup>	91.19 $\pm$ 3.66 <sup>bcA</sup>
Milbemectin	T/2	37.38 $\pm$ 7.27 <sup>eC</sup>	44.75 $\pm$ 6.23 <sup>eC</sup>	56.78 $\pm$ 4.71 <sup>dC</sup>	70.23 $\pm$ 2.38 <sup>cC</sup>	
	T	55.83 $\pm$ 7.38 <sup>cdB</sup>	60.35 $\pm$ 2.94 <sup>fB</sup>	68.93 $\pm$ 4.17 <sup>cB</sup>	77.14 $\pm$ 3.86 <sup>dB</sup>	
	2T	74.64 $\pm$ 2.62 <sup>cdA</sup>	77.61 $\pm$ 2.93 <sup>dA</sup>	81.07 $\pm$ 3.16 <sup>dA</sup>	85.95 $\pm$ 0.47 <sup>cA</sup>	
<i>Phytoseiulus persimilis</i>	Acequinocly	T/2	68.81 $\pm$ 9.53 <sup>abA</sup>	75.48 $\pm$ 9.01 <sup>bcA</sup>	77.26 $\pm$ 6.39 <sup>bcB</sup>	87.14 $\pm$ 1.65 <sup>abB</sup>
		T	63.81 $\pm$ 10.05 <sup>bcdA</sup>	85.95 $\pm$ 5.85 <sup>bcA</sup>	91.19 $\pm$ 9.02 <sup>aA</sup>	94.64 $\pm$ 6.84 <sup>abA</sup>
		2T	77.37 $\pm$ 7.98 <sup>cdA</sup>	87.62 $\pm$ 3.81 <sup>cA</sup>	100.00 $\pm$ 0.0aA	100.00 $\pm$ 0.0aA
	Etoxazole	T/2	78.13 $\pm$ 9.80 <sup>aA</sup>	91.43 $\pm$ 3.18 <sup>aB</sup>	90.94 $\pm$ 3.49 <sup>aB</sup>	92.72 $\pm$ 5.84 <sup>aB</sup>
		T	91.18 $\pm$ 6.88 <sup>aA</sup>	98.22 $\pm$ 3.57 <sup>aA</sup>	100.00 $\pm$ 0.0aA	100.00 $\pm$ 0.0aA
		2T	92.97 $\pm$ 5.83 <sup>abA</sup>	100.00 $\pm$ 0.0aA	100.00 $\pm$ 0.0aA	100.00 $\pm$ 0.0aA
	Bifenazate	T/2	78.93 $\pm$ 5.87 <sup>aB</sup>	80.36 $\pm$ 3.57 <sup>abB</sup>	83.93 $\pm$ 3.57 <sup>abB</sup>	89.28 $\pm$ 4.12 <sup>abB</sup>
		T	92.86 $\pm$ 5.83 <sup>aA</sup>	96.43 $\pm$ 4.12 <sup>abA</sup>	100.00 $\pm$ 0.0aA	100.00 $\pm$ 0.0aA
		2T	100.00 $\pm$ 0.0aA	100.00 $\pm$ 0.0aA	100.00 $\pm$ 0.0aA	100.00 $\pm$ 0.0aA
	Milbemectin	T/2	59.16 $\pm$ 6.86 <sup>bcB</sup>	62.61 $\pm$ 4.81 <sup>cdB</sup>	68.93 $\pm$ 4.17 <sup>cdB</sup>	78.92 $\pm$ 0.71 <sup>bcC</sup>
		T	71.07 $\pm$ 4.52 <sup>bcA</sup>	72.86 $\pm$ 0.95 <sup>deA</sup>	77.50 $\pm$ 4.10 <sup>cA</sup>	85.95 $\pm$ 0.48 <sup>bcdB</sup>
		2T	77.00 $\pm$ 3.43 <sup>cdA</sup>	77.85 $\pm$ 4.25 <sup>dA</sup>	82.74 $\pm$ 4.04 <sup>cdA</sup>	91.31 $\pm$ 3.10 <sup>bcA</sup>

\* Lower cases show difference among average of acaricides effect in the same column; upper cases show difference among effect of dose of each pesticide in the same day ( $p < 0.05$ ).

**Table 4.** Mortality percentage ( $\pm$ SE) of *Neoseiulus californicus* and *Phytoseiulus persimilis* adults exposed to acaricides

		Doses	Day 1	Day 3	Day 5	Day 7
<i>Neoseiulus californicus</i>	Acequinocly	T/2	6.9 $\pm$ 0.27 <sup>fC</sup>	28.80 $\pm$ 3.14 <sup>eB</sup>	35.46 $\pm$ 0.47 <sup>eC</sup>	42.08 $\pm$ 5.16 <sup>dC</sup>
		T	21.66 $\pm$ 3.33 <sup>eB</sup>	38.93 $\pm$ 5.85 <sup>fB</sup>	52.73 $\pm$ 12.97 <sup>dB</sup>	70.11 $\pm$ 7.16 <sup>dB</sup>
		2T	34.16 $\pm$ 0.96 <sup>eA</sup>	55.35 $\pm$ 7.66 <sup>fA</sup>	81.43 $\pm$ 6.15 <sup>cdeA</sup>	94.88 $\pm$ 3.42 <sup>aA</sup>
	Etoxazole	T/2	40.83 $\pm$ 1.66 <sup>cdB</sup>	48.16 $\pm$ 3.46 <sup>cdC</sup>	79.64 $\pm$ 0.71 <sup>abA</sup>	81.95 $\pm$ 1.94 <sup>abB</sup>
		T	44.16 $\pm$ 3.18 <sup>cdB</sup>	62.83 $\pm$ 6.33 <sup>cdB</sup>	80.77 $\pm$ 6.84 <sup>bcA</sup>	85.11 $\pm$ 2.61 <sup>bcB</sup>
		2T	59.91 $\pm$ 8.95 <sup>cdA</sup>	75.83 $\pm$ 3.19 <sup>cdA</sup>	86.31 $\pm$ 6.04 <sup>bcdA</sup>	94.76 $\pm$ 3.49 <sup>aA</sup>
	Bifenazate	T/2	29.28 $\pm$ 6.48 <sup>cdeC</sup>	39.64 $\pm$ 2.95 <sup>deC</sup>	52.62 $\pm$ 3.39 <sup>cdB</sup>	59.64 $\pm$ 3.37 <sup>cC</sup>
		T	46.54 $\pm$ 2.91 <sup>cdB</sup>	58.69 $\pm$ 4.62 <sup>defB</sup>	68.45 $\pm$ 3.57 <sup>cdA</sup>	73.69 $\pm$ 3.37 <sup>dB</sup>
		2T	58.69 $\pm$ 4.62 <sup>cdA</sup>	67.26 $\pm$ 2.99 <sup>defA</sup>	75.36 $\pm$ 4.57 <sup>deA</sup>	84.16 $\pm$ 3.75 <sup>bA</sup>
Milbemectin	T/2	26.66 $\pm$ 6.48 <sup>deC</sup>	34.99 $\pm$ 2.95 <sup>deC</sup>	43.09 $\pm$ 6.44 <sup>deC</sup>	54.40 $\pm$ 3.44 <sup>cC</sup>	
	T	43.33 $\pm$ 3.84 <sup>cdB</sup>	51.66 $\pm$ 3.33 <sup>defB</sup>	62.02 $\pm$ 4.26 <sup>dB</sup>	70.23 $\pm$ 6.29 <sup>dB</sup>	
	2T	61.66 $\pm$ 3.33 <sup>cA</sup>	66.66 $\pm$ 0.0 <sup>defA</sup>	74.17 $\pm$ 3.07 <sup>deA</sup>	82.49 $\pm$ 3.76 <sup>bA</sup>	
<i>Phytoseiulus persimilis</i>	Acequinocly	T/2	61.20 $\pm$ 5.63 <sup>bB</sup>	78.16 $\pm$ 0.82 <sup>aB</sup>	80.06 $\pm$ 5.80 <sup>aB</sup>	86.58 $\pm$ 0.95 <sup>abB</sup>
		T	70.16 $\pm$ 2.87 <sup>baB</sup>	85.59 $\pm$ 1.57 <sup>abA</sup>	89.01 $\pm$ 4.46 <sup>abB</sup>	92.83 $\pm$ 8.32 <sup>abB</sup>
		2T	82.08 $\pm$ 9.79 <sup>baA</sup>	89.59 $\pm$ 4.02 <sup>abA</sup>	98.33 $\pm$ 3.33 <sup>abA</sup>	100.00 $\pm$ 0.0aA
	Etoxazole	T/2	39.05 $\pm$ 8.69 <sup>cdB</sup>	48.33 $\pm$ 13.67 <sup>cdB</sup>	72.26 $\pm$ 11.71 <sup>abB</sup>	79.76 $\pm$ 5.79 <sup>bB</sup>
		T	48.33 $\pm$ 6.14 <sup>caB</sup>	68.81 $\pm$ 5.22 <sup>cdA</sup>	89.64 $\pm$ 8.68 <sup>abA</sup>	98.07 $\pm$ 3.85 <sup>aA</sup>
		2T	63.33 $\pm$ 11.55 <sup>caA</sup>	73.92 $\pm$ 9.36 <sup>cdA</sup>	91.19 $\pm$ 3.66 <sup>abcA</sup>	98.08 $\pm$ 3.84 <sup>aA</sup>
	Bifenazate	T/2	82.97 $\pm$ 4.29 <sup>aB</sup>	82.62 $\pm$ 4.67 <sup>aB</sup>	84.40 $\pm$ 3.92 <sup>aB</sup>	89.52 $\pm$ 3.87 <sup>aA</sup>
		T	96.66 $\pm$ 3.85 <sup>aA</sup>	98.33 $\pm$ 3.33 <sup>aA</sup>	100.00 $\pm$ 0.0aA	100.00 $\pm$ 0.0aA
		2T	100.00 $\pm$ 0.0aA	100.00 $\pm$ 0.0aA	100.00 $\pm$ 0.0aA	100.00 $\pm$ 0.0aA
	Milbemectin	T/2	41.43 $\pm$ 11.77 <sup>cbB</sup>	58.09 $\pm$ 5.62 <sup>bcC</sup>	65.83 $\pm$ 5.13 <sup>bcB</sup>	80.66 $\pm$ 3.66 <sup>abB</sup>
		T	63.81 $\pm$ 2.77 <sup>baA</sup>	70.71 $\pm$ 0.82 <sup>bcB</sup>	82.62 $\pm$ 4.67 <sup>abcA</sup>	85.92 $\pm$ 0.97 <sup>bcB</sup>
		2T	72.26 $\pm$ 6.46 <sup>bcA</sup>	82.61 $\pm$ 4.67 <sup>bcA</sup>	87.85 $\pm$ 3.67 <sup>abcA</sup>	91.29 $\pm$ 3.11 <sup>abA</sup>

\* Lower cases show difference among average of acaricides effect in the same column, upper cases show difference among effect of dose of each pesticide in the same day ( $p < 0.05$ ).

However, according to Hassan [25], at the end of the counting results of seventh day, at T/2 dose, for etoxazole showed between 80-99% effect on *N. californicus* adults and bifenazate < acequinocly < milbemectin showed between 80-99% effect on *P. persimilis* adults they were classified as moderately harmful. At T/2 dose, for bifenazate < milbemectin < acequinocly showed between 30-79% effect on *N. californicus* adults and

etoxazole showed between 30-79% on *P. persimilis* adults, they were in the little harmful group.

At the end of the study, the aim was to determine the side effects of selected acaricides on both predator mites by the same method. It was found that all of the acaricides used in the study showed high levels of toxic effect on *N. californicus* and *P. persimilis* nymphs and adults at the end of seventh day. However, in

relation to the effects of the acaricides on eggs hatching, the toxic effect of acaricides, except for bifentazate and milbemectin, were found to be higher for *N. californicus*. Bifentazate and milbemectin showed similar effects on eggs hatching on both predator mites. Some studies that investigated the side effects of some pesticides on some predator mites in the Phytoseiidae family are available. Oomen et al. [28], revealed that 51 insecticides/acaricides, 33 fungicides, 12 herbicides and four growing regulators had side effects on *P. persimilis*. James [29] determined that application dose of bifentazate is moderately harmful on *Galendromus occidentalis* (Nesbitt) (Acari: Phytoseiidae). Kim and Seo [30], found that bifentazate showed even toxic effect after 168 hours of application on *P. persimilis*. Kim and Yoo [21], determined that bifentazate, acequinocyl, chlorfenapyr, flufenoxuron and fenbutatin oxide were very toxic against to *P. persimilis* adults and milbemectin and fenazaquin were very toxic against to this predator's adults and nymphs. Similarly, in our study, bifentazate, acequinocyl and milbemectin were found harmful for *P. persimilis* nymphs and adults. A study by Sterk et al. [31] performed under laboratory and semi-field conditions found that thiamethoxam was moderately harmful on adults of *P. persimilis*. It was also found that imidacloprid was harmful. Castagnoli et al. [32] reported that rotenone showed high levels of toxic effect on *N. californicus*, pyrethrins and imidacloprid reduced fertility rate, *Beauveria bassiana* showed high level of mortality on the generations of the predator and azadirachtin and pymetrozine showed a little toxic effect. Ahn et al. [33], determined that abamectin caused toxic effect on *P. persimilis* eggs. Abamectin and milbemectin are the acaricides that are in the same group having the same effect mechanism. Similarly, our study illustrated that milbemectin reduced eggs hatching rates of *P. persimilis* and *N. californicus*. Kim et al. [34] identified that abamectin showed even toxic effect 168 hours after applying on *Amblyseius cucumeris* (Oudemans) (Acari: Phytoseiidae) and reduced egg efficiency. Similarly, it was also found that seventh day after the application, milbemectin was highly toxic on *P. persimilis* and *N. californicus*. A study by Bostanian and Akalach [35] conducted on *P. persimilis*, *Amblyseius fallacies* Schicha (Acari: Phytoseiidae) and *Orius insidiosus* (Say) (Hemiptera: Anthracoridae) revealed that indoxacarb did not show toxic effects on the individuals but reduced fertility rate for *P. persimilis* with the rate of 26.7%, S-kinoprene and endosulfan had effect on one predator species at least, and dimethoate, abamectin and insecticidal soap have the highest toxicity levels in three beneficial organisms. Cloyd et al. [10] found that chlorfenapyr, spiromesifen and bifentazate are more toxic on *P. persimilis* compared to *N. californicus*. Similarly, our study revealed that bifentazate is more harmful on the *P. persimilis* nymphs and adults compared to the nymphs and adults of *N. californicus*. Irigaray and Zalom [36] determined that fenpyroximate application reduced

the life span of female adults of *G. occidentalis* and spiromesifen and acequinocyl application reduced the life span of female adults to four days. Furthermore, as the reproduction and fertility rates decreased, etoxazole and bifentazate application did not decrease the life span of female adults; however, these female adults were not able to reproduce.

Irigaray et al. [37] found that 100% mortality rate was observed following the application of fenpyroximate and etoxazole on *P. persimilis*, abamectin increased the mortality rates of female adults after six days and acequinocyl increased mortality rates of the female adults after three days. Our study similarly determined that etoxazole showed 100% and 98.08% effect on *P. persimilis* nymphs and adults of after seven days. A study by Alzoubi and Cobanoglu [38] that investigated the effects of some pesticides on *T. urticae* and predators *P. persimilis* and *Amblyseius californicus* identified that hexythiazox was harmless after 24 hours but harmful on predator mites after 72 hours. Nadimi et al. [39] found that hexythiazox was not harmful for *P. persimilis* with the low effect of all application doses and found that all doses of fenpyroximate and field application dose of abamectin were toxic against predator mites.

The results of the study revealed that acequinocyl, etoxazole, bifentazate and milbemectin acaricides showed high levels of toxicity on the nymphs and adults of *P. persimilis* and *N. californicus* at seventh days after applying. Additionally, it was seen that acequinocyl, etoxazole, bifentazate and milbemectin reduced egg hatching for both predator mites. According to the results of the study, four acaricides that are used frequently against to the pest spider mites should be used more carefully in agricultural areas. The use of the results regarding side effect studies conducted with predator mites in the pesticide lists to be updated in the future will make contribution to the practice. The identification of the side effects of the pesticides used will enable the use of preparations that are harmless or a little harmful to natural enemies. Thus, the feasibility of biological control will be facilitated, the use of excessive pesticide doses will be prevented, and the environment and the health of living individuals will be protected.

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