

Original article (Orijinal araştırma)

Toxicity and repellency of sage (*Salvia officinalis* L.) (Lamiaceae) and rosemary (*Rosmarinus officinalis* L.) (Lamiaceae) extracts to *Neoseiulus californicus* (McGregor, 1954) and *Phytoseiulus persimilis* Athias-Henriot, 1957 (Acari: Phytoseiidae)

Adaçayı (*Salvia officinalis* L.) (Lamiaceae) ve biberiye (*Rosmarinus officinalis* L.) (Lamiaceae) bitki ekstraktlarının *Neoseiulus californicus* (McGregor, 1954) ve *Phytoseiulus persimilis* Athias-Henriot, 1957 (Acari: Phytoseiidae)'e karşı toksik ve repellent etkileri

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Abstract

In this study the toxicity and repellency of sage and rosemary extracts to two important predator mites were examined. In the toxicity experiments, leaf disc-spray tower method was used with 1, 3, 6 and 12% extract concentrations applied to egg, immature stage and adult stages of the predatory mites. All concentrations of plant extracts and control experiments were conducted with ten replicates with ten individuals in each replicate. The experiments include one control and four concentrations of each extract. For repellency experiments, 0.1, 1, 5 and 10 ml/L concentrations of plant extracts were used. In the toxicity experiments, the greatest effects of the sage and rosemary extracts were 33.3% and 33.3% toxicity to *Neoseiulus californicus* (McGregor, 1954) adults and 62.5% and 38.4% to *Phytoseiulus persimilis* Athias-Henriot, 1957 (Acari: Phytoseiidae) adults, respectively. In the repellency experiments, the greatest effects of the sage and rosemary extracts were 43.7% and 77.7% repellency to *N. californicus* adults and 50.1% and 88.8% for *P. persimilis* adults, respectively. This study indicates the potential to use of sage extract in integrated pest management programs incorporating *N. californicus* and rosemary against *P. persimilis*.

Keywords: *Neoseiulus californicus*, *Phytoseiulus persimilis*, plant extract, repellency, toxicity

Öz

Bu çalışmada, adaçayı ve biberiye ekstraktlarının iki önemli predatör akara toksisitesi ve repellent etkisi incelenmiştir. Toksikite denemelerinde, predatör akarların yumurta, nimf ve ergin dönemlerine %1, 3, 6 ve 12 konsantrasyonları yaprak disk-ilaçlama kulesi yöntemi kullanılarak uygulanmıştır. Tüm ekstrakt ve kontrol denemelerinde konsantrasyonlar; on tekerrür ve her tekerrürde on birey olacak şekilde gerçekleştirilmiştir. Denemelerde bir kontrol ve dört konsantrasyon kullanılmıştır. Repellent etki denemeleri için, bitki ekstraktlarının 0.1, 1, 5 ve 10 ml/L konsantrasyonları kullanılmıştır. Toksik etki çalışmalarında, adaçayı ve biberiye ekstraktlarının en yüksek etkileri sırasıyla, *Neoseiulus californicus* (McGregor, 1954) erginlerinde %33.3 ve %33.3; *Phytoseiulus persimilis* Athias-Henriot, 1957 (Acari: Phytoseiidae) erginlerinde ise %62.5 ve %38.4 olarak bulunmuştur. Repellent etki çalışmalarında ise adaçayı ve biberiye ekstraktlarının en büyük etkileri sırasıyla, *N. californicus* erginlerinde %43.7 ve %77.7; *P. persimilis* erginlerinde ise %50.1 ve %88.8 oranlarında bulunmuştur. Bu çalışma, entegre zararlı yönetim programlarında adaçayı ekstraktının *N. californicus*, biberiye ekstraktının ise *P. persimilis*'e dayalı potansiyel kullanım olanaklarının olduğunu bildirmektedir.

Anahtar sözcükler: *Neoseiulus californicus*, *Phytoseiulus persimilis*, bitki ekstraktı, repellent etki, toksik etki

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Introduction

Phytophagous mites are a major cause of economic losses for many plants produced in greenhouses (Cranham & Helle, 1985; Nachman & Zemek, 2002). *Phytoseiulus persimilis* Athias-Henriot, 1957 and *Neoseiulus californicus* (McGregor, 1954) (Acari: Phytoseiidae) are two important predator species used in greenhouses for the biological control of phytophagous mites (McMurtry & Croft, 1997; Raworth, 2001; Oliveira et al., 2007). Acaricides used to control phytophagous mites cause many negative effects including resistance development, side effects in non-target organisms and environmental pollution (Skirvin et al., 2002). Furthermore, the chemicals reduce egg production, preying and reproductive capacity in predatory mites (Kim & Yoo, 2002). However, using only the predators in the control of mites that cause significant economic losses in crops, usually cannot provide the desired control (Ibrahim & Yee, 2000; Fadini et al., 2004). For this reason, the use of *P. persimilis* and *N. californicus* along with some plant extracts in the control against plant parasitic mites in greenhouse cultivation has value (Schmutterer, 1997; Shi & Feng, 2004; Roobakkumar et al., 2010). However, the number of studies of use the plant extracts and phytoseiid predators in the literature is very limited. Choi et al. (2004) determined that essential oils of caraway seed, java citronell, lemon eucalyptus, pennyroyal, and peppermint can cause over 90% mortality of both *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) and *P. persimilis* adults. Miresmailli & Isman (2006) reported that the toxicity of rosemary essential oil is higher on *T. urticae* than *P. persimilis*. El-Sharabasy (2010) determined that the extract from *Artemisia judaica* L. (Compositae) is more toxic to *T. urticae* than *P. persimilis*. Vergel et al. (2011) reported mortality rates for garlic plant extract of 23.81% in *P. persimilis* and 9.82% in *N. californicus* at a concentration of 1.25 ml/L.

Sage (*Salvia officinalis* L.) and rosemary (*Rosmarinus officinalis* L.) are two important medicinal and aromatic plants in the Lamiaceae. Sage and rosemary plants have antioxidant and antimicrobial properties due to the secondary metabolites they contain (Biljana et al., 2007). For this reason, these plants are both consumed and used in medicine in Turkey and around the world. Sage and rosemary plants are grown in many regions of Turkey. Recently, considerable work has been done on the insecticidal and acaricidal functions of many essences from both plants against many pests (Kawka & Tomczyk, 2002; Bozhüyük et al., 2016; Park et al., 2016; Tak et al., 2016). For this reason, sage and rosemary plants were used in the study for determine the possible effects on two natural enemies of phytophagous mites.

Compounds derived from these plants are recommended to be used in the control against insect and mite because of their new mechanism of action, non-target organisms, mammals and environmental effects (Isman, 2006). Another important point is that plant extracts are generally more suitable for use in combination with natural enemies than synthetic pesticides (Erdogan et al., 2012). However, all potential risks need to be addressed in order for these extracts to be used correctly. Therefore, it is absolutely necessary to conduct studies on the toxicity of plant extracts on natural enemies that are being considered for use in integrated pest management programs. In this study, the toxicity and repellency of different concentrations of ethanolic extracts derived from sage and rosemary on *N. californicus* and *P. persimilis* were determined.

Material and Methods

Origin and culture of predatory mites

Phytoseiulus persimilis was collected from vegetable fields in Turkey in 1993 and *N. californicus* was collected from an organic apple orchard in Turkey in 2008 (Sekeroglu & Kazak, 1993; Yorulmaz Salman & Ay, 2013). Predatory mites were cultivated without exposure to any pesticides in an insect rearing room (26±2°C, 60±5% RH and 16:8 h L:D photoperiod). *Tetranychus urticae*, used to feed *P. persimilis* and *N. californicus*, were cultured on beans (*Phaseolus vulgaris* L. cv. Barbania) in a controlled-environment room (26±2°C, 60±5% RH and 16:8 h L:D photoperiod).

Preparation of plant extracts

Sage and rosemary plants were collected from production areas of the Agricultural Application and Research Centre, Faculty of Agriculture, Süleyman Demirel University during the 2016 growing period and extracts were obtained from leaves of the plants. The plant materials were dried at room temperature until they reached equilibrium moisture content. The sage and rosemary plants (100 g) were separately weighed and transferred to the Erlenmeyer flasks. For organic solvent, 500 ml of 99% ethanol, was added to the plant material and mixed 24 h on an orbital shaker. The ethanol in the mixture was removed with a rotary evaporator. The extracts were stored in glass tubes at +4°C until used.

Toxicity experiments

The method of El-Sharabasy (2010) was used for the determine of the toxicity of the plant extracts on *N. californicus* and *P. persimilis*. A preliminary study was conducted on eggs, immature stages and adult mites of the same age. Fifteen individual adult females were transferred to 3 cm bean leaf discs in separate 9 cm Petri dishes. In the egg experiments, eggs in the same age were used to determine the effect of plant extracts on egg hatch. Eggs were kept for 24 h to obtain immature stages of the same age for experiments to determine the effects of plant extracts on immature stages. In addition, female individuals of the same age, which matured a few days after the eggs were laid, were used in the experiments on adults.

In the toxicity studies, the leaf disc-spray tower method was used. For this purpose, 3 cm bean leaf discs were placed in 9 cm Petri dishes on moistened cotton. Due to the mobility of the mites and the stay in the leaf disc along the experiment, the leaf was surrounded by the Tangle Trap. Concentrations of 1, 3, 6 and 12% of plant extracts were applied to the mites of different life stages. Concentrations of plant extracts were determined by the concentrations of plant extracts applied to *P. persimilis* by El-Sharabasy (2010). Pure water was used as a control. TritonX100 (0.01%) was added to the purified water and the extracts. Each toxicity assay had one control and four extract concentrations replicated ten times. There were ten individuals in each replicate. Different concentrations of plant extracts were applied to the leaf surface at 2 ml at 100 kPa in the spray tower. After spraying, a large number of *T. urticae* were added as prey to leaf discs to prevent the predatory mites sticking to Tangle Trap. The toxicity of plant extracts was determined at 24, 48 and 72 h by dead-live counts of immature and adult stages of the predatory mites. For the egg hatch experiment, observations continued until all eggs in the control group had hatched. In the experiments, *T. urticae* were added to each Petri dish to feed predatory mites until the end of the assessment period.

Repellency experiments

To determine the repellency of the plant extracts on different life stages of the predatory mites, the methods of Miresmailli et al. (2006) and Nerio et al. (2009) were used. In the repellency experiments, the same age immature stages and adult individuals of predatory mites were used. Predatory mites were obtained according to the method described in the toxicity experiments of immature stage and adult individuals of the same age. Concentrations of 0.1, 1, 5 and 10 ml/L of plant extracts were applied to the predatory mites in the repellency experiments. For each concentration, the repellency experiments were carried out in four repetitions. In each repetition, 10 individuals were available. In the experiments, one half was transferred to a 9 cm Petri dish containing cotton with a base of 3 cm diameter bean leaf disks immersed in 0.01% TritonX100 solution (control portion) and the other half in plant extract solution. The predatory mites, for prevent their escape along the experiment the leaf was surrounded by the Tangle-Trap in repellent experiments. In addition, a large number of *T. urticae* individuals were added as prey in order to reduce the movement of predatory mites in the leaf disc. The immature stage or adult individuals of the predatory mites to be used in the middle part of the bean leaf disc dissection were transferred with a brush. In the repellency experiments, individuals in control and plant extract sections of leaf discs were counted after 24, 48 and 72 h.

Statistical analysis

The mortality percentages from the toxicity experiments were calculated using the Abbott formula (Abbott, 1925). The results from the repellency experiments are given using the repellency percent index according to Obeng-Ofori et al. (1997):

$$\text{Repellency (\%)} = [(Nc - Nt) / (Nc + Nt)] \times 100$$

where, Nc is the number of individuals moving to control direction on leaf surface, and Nt is the number of individuals in the direction of the application on the leaf surface.

The results were arcsin transformed (Zar, 1999). Toxicity and repellency were analyzed by three-way repeated measures ANOVA (there were three factors in experiments with immature and adult stages: extracts, concentrations and observation time). Ovicidal effects were analyzed by two-way ANOVA (for: extracts and concentrations). Tukey's test was used to determine the differences between the means.

Results

Toxicity bioassay results

The toxicity the plant extracts on *N. californicus* and *P. persimilis* adults are given in Table 1. The toxicity of extracts to adult and immature stages of predatory mites varied depending on the treatment, dose and time (for adults $F = 36.25$, $df = 6$, $p < 0.05$; for immature stages $F = 36.45$, $df = 6$, $p < 0.05$). There were interactions between these variables. It was found that the toxicity of the sage and rosemary extract to adults increased with time. Also, at all concentrations the toxicity of the rosemary extract to *N. californicus* was statistically higher than the toxicity of sage extract ($p < 0.05$). The highest toxicity to *N. californicus* adults was 33.3 and 62.5% for 12% sage and rosemary extracts, respectively ($F = 35.23$, $df = 6$, $p < 0.05$). For *P. persimilis* adults, the mortalities at all concentrations of sage and rosemary extracts were similar. The highest toxicity to *P. persimilis* was 33.3% for the sage extract and 38.4% for the rosemary extract ($F = 28.52$, $df = 6$, $p < 0.05$).

Table 1. Toxicity of the different concentrations of sage and rosemary extracts on the adults of *Neoseiulus californicus* and *Phytoseiulus persimilis* (mean±SE)

| Time (h) | Concentration (ml/L) | Mortality (%) | | | |
|----------|----------------------|----------------------------------|--------------|----------------------------------|--------------|
| | | <i>Neoseiulus californicus</i> * | | <i>Phytoseiulus persimilis</i> * | |
| | | Sage | Rosemary | Sage | Rosemary |
| 24 | 1 | 2.0±0.25 bE | 20.5±0.35 aD | 10.4±0.88 aE | 8.2±0.48 aE |
| | 3 | 4.1±0.78 bD | 28.5±1.57 aC | 20.2±0.25 aC | 22.2±0.56 aC |
| | 6 | 12.5±0.88 bC | 38.7±2.02 aB | 25.3±0.68 bB | 30.4±0.77 aB |
| | 12 | 16.6±1.05 bB | 51.0±0.35 aA | 27.5±0.98 bA | 31.6±0.26 aB |
| 48 | 1 | 2.0±0.33 bE | 25.6±0.78 aC | 14.4±0.33 aD | 10.2±0.25 bD |
| | 3 | 6.3±0.45 bD | 35.8±0.25 aB | 22.6±0.57 aC | 23.1±1.05 aC |
| | 6 | 13.6±1.02 bC | 59.1±0.56 aA | 28.3±1.05 bA | 34.5±1.05 aA |
| | 12 | 19.1±0.88 bB | 61.2±0.66 aA | 30.5±0.33 bA | 34.8±0.88 aA |
| 72 | 1 | 2.2±0.28 bE | 30.6±0.13 aC | 18.2±2.05 aC | 12.2±0.54 bD |
| | 3 | 13.3±0.55 bC | 40.8±0.55 aB | 25.4±1.09 aB | 25.6±0.33 aC |
| | 6 | 15.5±0.45 bB | 61.2±1.05 aA | 29.1±0.35 bA | 36.3±0.45 aA |
| | 12 | 33.3±0.78 bA | 62.5±1.08 aA | 33.3±0.45 bA | 38.4±1.09 aA |

* For each predator mite, different lowercase letters in the same line and different uppercase letters in the same column indicate that the means are significantly different according to plant extract and dose, respectively ($p < 0.05$).

The toxicity of different concentrations of the plant extracts on *N. californicus* and *P. persimilis* immature stages are given in Table 2. The toxicity of the rosemary extract in all concentrations of *N. californicus* immature stages was statistically greater than the toxicity of the sage extract ($p < 0.05$). The highest toxicity to *N. californicus* immature stages was 37.5 and 67.3% for 12% sage and rosemary extracts, respectively. The toxicity of the sage extracts to *P. persimilis* was statistically higher than the toxicity of the rosemary extract at all concentrations ($F = 36.83$, $df = 6$, $p < 0.05$). The highest toxicity to *P. persimilis* was 50.2% for the sage extract and 44.6% for the rosemary extract ($F = 27.56$, $df = 6$, $p < 0.05$).

Table 2. Toxicity of the different concentrations of sage and rosemary extracts on *Neoseiulus californicus* and *Phytoseiulus persimilis* immature stages (mean \pm SE)

| Time (h) | Concentration (ml/L) | Mortality (%) | | | |
|----------|----------------------|----------------------------------|--------------------|----------------------------------|--------------------|
| | | <i>Neoseiulus californicus</i> * | | <i>Phytoseiulus persimilis</i> * | |
| | | Sage | Rosemary | Sage | Rosemary |
| 24 | 1 | 8.1 \pm 0.75 bE | 18.7 \pm 0.33 aF | 15.5 \pm 0.35 aF | 5.7 \pm 0.25 bF |
| | 3 | 18.3 \pm 1.75 bC | 22.9 \pm 1.55 aE | 30.1 \pm 0.28 aC | 15.2 \pm 0.25 bE |
| | 6 | 20.4 \pm 0.25 bC | 29.1 \pm 0.15 aD | 41.2 \pm 0.22 aB | 32.4 \pm 0.35 bC |
| | 12 | 28.5 \pm 0.44 bB | 37.5 \pm 0.45 aC | 48.3 \pm 0.55 aA | 36.1 \pm 1.08 bC |
| 48 | 1 | 10.2 \pm 0.55 bE | 20.7 \pm 0.58 aE | 20.2 \pm 0.64 aE | 9.1 \pm 1.22 bF |
| | 3 | 20.4 \pm 1.15 bC | 29.1 \pm 0.75 aD | 33.3 \pm 0.98 aC | 18.2 \pm 0.45 bD |
| | 6 | 22.4 \pm 2.02 bC | 35.4 \pm 0.63 aC | 46.4 \pm 0.45 aA | 33.3 \pm 0.22 bC |
| | 12 | 30.6 \pm 0.33 bB | 45.8 \pm 0.48 aB | 49.6 \pm 0.69 aA | 40.1 \pm 0.75 bB |
| 72 | 1 | 16.6 \pm 0.28 bD | 22.9 \pm 0.35 aE | 25.2 \pm 0.58 aD | 12.1 \pm 0.78 bE |
| | 3 | 27.0 \pm 0.75 bB | 33.3 \pm 0.75 aC | 35.7 \pm 0.75 aC | 20.4 \pm 0.85 bD |
| | 6 | 33.3 \pm 0.48 bA | 39.5 \pm 2.05 aC | 48.4 \pm 0.67 aA | 35.3 \pm 0.98 bC |
| | 12 | 37.5 \pm 0.88 bA | 67.3 \pm 1.05 aA | 50.2 \pm 1.09 aA | 44.6 \pm 1.05 bA |

* For each predator mite, different lowercase letters in the same line and different uppercase letters in the same column indicate that the means are significantly different according to plant extract and dose, respectively ($p < 0.05$).

The results for different concentrations of the sage and rosemary extracts on *N. californicus* and *P. persimilis* egg development are given in Table 3. As the concentration both plant extracts increased, the effect on *N. californicus* hatch also increased. The greatest effect on *N. californicus* egg hatch was 51.1% with 12% rosemary extract ($F = 10.58$, $df = 4$, $p < 0.05$). The greatest effect on *P. persimilis* egg hatch was 18.6% at 12% for both sage and rosemary extracts ($F = 14.12$, $df = 4$, $p < 0.05$).

Table 3. Egg hatching effect of plant extracts with different concentration on *Neoseiulus californicus* and *Phytoseiulus persimilis* eggs (mean \pm SE)

| Concentration (ml/L) | Mortality (%) | | | |
|----------------------|----------------------------------|--------------------|----------------------------------|--------------------|
| | <i>Neoseiulus californicus</i> * | | <i>Phytoseiulus persimilis</i> * | |
| | Sage | Rosemary | Sage | Rosemary |
| 1 | 1.2 \pm 0.22 bD | 11.6 \pm 0.25 aD | 2.1 \pm 0.55 aD | 2.1 \pm 0.78 aD |
| 3 | 4.1 \pm 0.55 bC | 18.6 \pm 0.65 aC | 6.8 \pm 1.12 aC | 4.16 \pm 0.46 aC |
| 6 | 10.4 \pm 1.05 bB | 37.2 \pm 0.78 aB | 12.4 \pm 0.98 aB | 10.4 \pm 0.15 aB |
| 12 | 16.6 \pm 0.88 bA | 51.1 \pm 0.15 aA | 18.6 \pm 0.45 aA | 18.6 \pm 0.33 aA |

* For each predator mite, different lowercase letters in the same line and different uppercase letters in the same column indicate that the means are significantly different according to plant extract and dose, respectively ($p < 0.05$).

Repellency bioassay results

Repellency of the different concentrations of the sage and rosemary extracts to *N. californicus* and *P. persimilis* adults is shown in Table 4. The repellency of plant extracts to adult and immature stages of predatory mites varied depending on the dose and time. Similarly, to the toxicity experiment, there were interactions between these explanatory variables. The repellency of sage and rosemary extracts to *N. californicus* adults showed that the rosemary extract was statistically more repellent than the sage extract at 5 and 10 ml/L ($p < 0.05$). The highest repellency to *N. californicus* adults was 43.7 and 77.7% at the 10 ml/L for the sage and rosemary extracts, respectively ($F = 35.85$, $df = 6$, $p < 0.05$). Repellency of the plant extracts to *P. persimilis* adults were similar to that for *N. californicus* adults. Especially at 5 and 10 ml/L, both extracts had increasing repellency to *P. persimilis* adults. The highest repellency to *P. persimilis* was 50.1 and 88.8% at 10 ml/L for the sage and rosemary extracts, respectively ($F = 33.24$, $df = 6$, $p < 0.05$).

The repellency of the different concentrations of the sage and rosemary extracts to *N. californicus* and *P. persimilis* immature stages is shown in Table 5. The highest repellency to *N. californicus* immature stages was 38.8 and 44.1% for the sage and rosemary extracts, respectively ($F = 35.84$, $df = 6$, $p < 0.05$). For *P. persimilis* immature stages, the highest repellency of plant extracts was at 10 ml/L. At 10 ml/L, the repellency of the sage extract was statistically higher than that of the rosemary extract to *P. persimilis* immature stages ($p < 0.05$).

Table 4. Repellency of different concentrations of sage and rosemary extracts on *Neoseiulus californicus* and *Phytoseiulus persimilis* adults (mean±SE)

| Concentration (ml/L) | Time (h) | Repellency (%) | | | | | | | |
|-------------------------|-------------|----------------------------------|----|-----------|----|----------------------------------|----|-----------|----|
| | | <i>Neoseiulus californicus</i> * | | | | <i>Phytoseiulus persimilis</i> * | | | |
| | | Sage | | Rosemary | | Sage | | Rosemary | |
| 0.1 | 24 | 6.25±0.43 | bD | 13.5±0.85 | aE | 8.32±1.33 | bF | 15.3±0.36 | aF |
| | 48 | 18.7±0.38 | aC | 13.5±0.75 | bE | 16.4±0.75 | bE | 29.7±0.45 | aE |
| | 72 | 12.5±0.55 | bC | 18.9±0.33 | aE | 27.9±0.45 | bD | 40.2±0.55 | aD |
| 1 | 24 | 21.2±0.78 | aB | 15.3±0.48 | bE | 22.6±0.69 | aD | 12.5±1.45 | bF |
| | 48 | 21.2±1.05 | aB | 15.3±1.65 | bE | 31.1±0.58 | aC | 17.3±1.13 | bF |
| | 72 | 9.09±1.38 | bD | 33.3±1.89 | aD | 37.5±1.14 | aB | 33.3±0.78 | bE |
| 5 | 24 | 8.1±0.89 | bD | 43.5±1.15 | aC | 23.7±1.45 | bD | 43.5±0.65 | aD |
| | 48 | 24.3±0.33 | bB | 53.8±0.36 | aB | 25.5±0.88 | bD | 53.8±0.54 | aC |
| | 72 | 29.7±0.45 | bB | 48.7±0.64 | aC | 33.3±0.95 | bC | 58.6±0.35 | aC |
| 10 | 24 | 42.8±1.45 | bA | 66.6±0.55 | aA | 45.9±0.45 | bA | 63.3±1.98 | aB |
| | 48 | 37.5±1.15 | bA | 72.2±1.15 | aA | 50.1±0.65 | bA | 68.2±1.65 | aB |
| | 72 | 43.7±0.75 | bA | 77.7±1.25 | aA | 50.1±1.05 | bA | 88.8±0.35 | aA |

* For each predator mite, different lowercase letters in the same line and different uppercase letters in the same column indicate that the means are significantly different according to plant extract and dose, respectively ($p < 0.05$).

Table 5. Repellency of different concentrations of sage and rosemary extracts on *Neoseiulus californicus* and *Phytoseiulus persimilis* immature stages (mean±SE)

| Concentration (ml/L) | Time (h) | Repellency (%) | | | |
|-------------------------|-------------|----------------------------------|--------------|----------------------------------|--------------|
| | | <i>Neoseiulus californicus</i> * | | <i>Phytoseiulus persimilis</i> * | |
| | | Sage | Rosemary | Sage | Rosemary |
| 0.1 | 24 | 5.5±0.56 bE | 11.7±0.59 aE | 8.2±0.66 bF | 11.1±0.48 aE |
| | 48 | 11.1±0.89 aD | 11.7±0.46 aE | 16.4±0.95 aE | 11.1±0.65 bE |
| | 72 | 11.1±0.78 aD | 11.7±0.75 aE | 25.5±0.35 aD | 27.7±0.52 aC |
| 1 | 24 | 10.5±1.56 aD | 12.4±0.68 aE | 21.3±0.75 aE | 23.0±1.54 aD |
| | 48 | 14.2±0.98 aC | 12.1±1.55 aE | 24.4±1.56 bD | 28.2±1.78 aC |
| | 72 | 14.2±1.45 bC | 17.0±0.52 aD | 27.7±1.35 bD | 33.3±1.45 aB |
| 5 | 24 | 18.7±1.05 bC | 23.0±1.45 aC | 57.1±0.85 aA | 26.0±0.95 bC |
| | 48 | 31.2±1.89 aB | 28.2±1.85 bB | 40.4±0.47 aB | 21.3±0.35 bD |
| | 72 | 35.2±0.55 aA | 33.3±1.09 aB | 45.5±0.69 aB | 31.2±0.75 bB |
| 10 | 24 | 27.7±0.36 aB | 10.0±1.45 bE | 33.3±0.58 bC | 43.5±0.33 aA |
| | 48 | 27.7±0.76 bB | 39.5±0.45 aA | 36.9±0.78 aC | 38.8±0.89 aA |
| | 72 | 38.8±0.85 bA | 44.1±0.78 aA | 47.7±1.84 aB | 38.8±1.15 bA |

* For each predator mite, different lowercase letters in the same line and different uppercase letters in the same column indicate that the means are significantly different according to plant extract and dose, respectively ($p < 0.05$).

Discussion

Plants can protect themselves from herbivores and pathogenic attack using secondary metabolites (Miresmailli & Isman, 2014; Pavela, 2016). This feature of plants gives suggests that these metabolites can be used to control pests. However, for invertebrate pest control it is also important to know the effects of the herbal substances on the natural enemies. This study demonstrates toxicity and repellency of sage and rosemary extracts to two major predators (*P. persimilis* and *N. californicus*), which are widely used in the control against phytophagous mites. The toxicity to both predatory mites increased with increasing extract concentration and exposure time. The effect of rosemary extract on *N. californicus* adults was greater than the sage extract. For *P. persimilis* adults, however, the effect of both extracts was similar. The toxicity of the rosemary extract was much higher for *N. californicus* immature stages than that of the sage extract. However, the toxicity of the rosemary extract to *P. persimilis* immature stages was less than the sage extract. In addition, the toxicity of plant extracts to the immature stages at higher concentrations was greater than on the adults. The reason for this might be that the immature stages have not yet completed their development and therefore, the toxicity of plant extracts was greater due to the lower amount of chitin in their bodies. The effect of the extracts on *N. californicus* egg hatch was greater with the rosemary extract than the sage extract. Whereas, the ovicidal effects of rosemary and sage extracts on *P. persimilis* were similar. It was observed that the effects of the sage extract on *N. californicus* and *P. persimilis* were moderate and similar. However, the effect of the rosemary extract on *P. persimilis* was much less than on *N. californicus*. This suggests that the active components of the rosemary extract are more effective on *N. californicus* than *P. persimilis*. Another possibility is that *P. persimilis* is more resistant to the active components or essences in the rosemary extract than *N. californicus*. However, in order to reach a definite judgment, the toxicity of all the active components of rosemary plant extract need to be determined for *P. persimilis*.

There have been many studies on the toxicity of plant extracts to phytophagous mites (Mateeva et al., 2003; Liu et al., 2004; Rasikari et al., 2005; Antonious et al., 2006; Shi et al., 2006; Wang et al., 2007; Moneim et al., 2011; Topuz & Madanlar, 2011). Yorulmaz Salman et al. (2014) found that the extracts of sage and rosemary were toxic to *T. urticae* with nymph and adult mortality of 79 and 62%, respectively, for sage extract and 58 and 82% for rosemary extract, respectively. In the present study, *P. persimilis* was more resistant than *N. californicus* when exposed to the same extracts. Hence, it is concluded that *P. persimilis* for the control of two spotted spider mite can be used in an integrated management programs using sage and rosemary extracts. However, the number of studies in which the effects of plant extracts on natural enemies have been identified, especially in integrated management programs, is rather limited. Vergel et al. (2011) reported that the garlic plant extract resulted in a mortality rate of 23.81% for *P. persimilis* and 9.82% for *N. californicus* at 1.25 ml/L. Bernardi et al. (2013) reported that azadirachtin, a plant-based preparation, was highly toxic to *T. urticae*, while the toxicity to *N. californicus* and *Phytoseiulus macropolis* (Banks, 1904) (Acari: Phytoseiidae) were much lower, so the combined use of azadirachtin and predatory mites was suitable. In our study, it was also shown, based on the published reports, that the toxicity of the plant extracts to *T. urticae*, were higher than the two predatory mites in the Phytoseiidae. This indicates the potential for combined use of plant extracts and predatory mites in integrated pest management programs.

When the results of repellency were evaluated, it was determined that the repellency of the rosemary extract to *N. californicus* and *P. persimilis* adults was higher than that of the sage extract. Also, the repellency of the rosemary extract to *N. californicus* immature stages was higher than that of the sage extract. In the case of *P. persimilis* immature stages, the repellency of the sage and rosemary extracts were varied according to the concentration and exposure time. The extracts of sage and rosemary showed repellency at certain rates to both predatory mites. There are many studies in which plant extracts have been found to repel phytophagous mites (Antonious & Snyder, 2006; Antonious et al., 2006; Kumral et al., 2010; Mozaffaria et al., 2012). However, there are no studies reporting repellency of plant extracts on predatory mites in the Phytoseiidae. This study is very important because it is the first study to determine the repellency of sage and rosemary extracts on the Phytoseiidae. It is also thought that the toxic and repellency of plant extracts and use of natural enemies combined, should be evaluated together in integrated management programs. The repellency of the sage and rosemary extracts to *N. californicus* and *P. persimilis* needs to be considered if these extracts are to be used in integrated control programs that use these predatory mites.

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