Türk. entomol. derg., 2014, 38 (4): 365-376
ISSN 1010-6960

*Orijinal araştırma (Original article)*

# Contact and repellency effects of *Rosa damascena* Mill. essential oil and its two major constituents against *Tetranychus urticae* Koch (Acari: Tetranychidae)

*Rosa damascena* Mill. uçucu yağı ve iki önemli bileşeninin *Tetranychus urticae* Koch (Acari: Tetranychidae)'de kontakt ve repellent etkileri

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## Summary

*Tetranychus urticae* Koch (Acari: Tetranychidae) is a harmful pest of plants worldwide. Essential oils and their components are potential alternatives to the use of synthetic pesticides for two-spotted spider mite control. The aim of this study was to investigate the contact and repellency effects of Rosa *damascena* essential oil and its two major constituents, geraniol and citronellol, on *Tetranychus urticae*. A total of 22 compounds were identified by GC-MS analysis, and citronellol (23.43%) and geraniol (34.91%) were the main scent compounds of the fresh rose flowers. To determine the contact effect, 1, 5, 10 and 20 ml/l concentrations of rose essential oil, geraniol and citronellol were applied to egg, nymph and adult life stages of two-spotted spider mites using the leaf disc-spray tower method. To determine the repellency effect, 0.1, 1, 5 and 10 ml/l concentrations of rose essential oil, geraniol and citronellol were applied to nymphs and adults of *Tetranychus urticae*. The study showed that rose oil, geraniol and citronellol had contact, repellent and ovicidal effects on the different life stages of *T. urticae*. In addition, geraniol was found to have the highest contact, repellent and ovicidal activity, followed by rose oil and citronellol. The results suggest that *Rosa damascena* essential oil and its two major constituents, geraniol and citronellol, can potentially be used for the management of *Tetranychus urticae*.

**Keywords:** *Tetranychus urticae*, *Rosa damascena* oil, geraniol, citronellol, acaricidal effect

## Özet

*Tetranychus urticae* Koch (Acari:Tetranychidae) dünyada bitkiler için önemli bir zararlıdır. İki noktalı kırmızı örümcek mücadelesinde uçucu yağ ve bileşenlerinin kullanımı sentetik pestisitlere alternatif olarak görülmektedir. Bu çalışmada, *Rosa damascena* uçucu yağı ile iki önemli bileşeni geraniol ve citronellol'ün *Tetranychus urticae*'de kontakt ve repellent etkileri araştırılmıştır. Taze gül çiçeğinde citronellol (%23.43) ve geraniol (%34.91) olmak üzere toplam 22 bileşik belirlenmiştir. Kontakt etki için, gül yağı, geraniol ve citronellol'ün 1, 5, 10 ve 20 ml/l konsantrasyonları yaprak disk ilaçlama kulesi metodu kullanılarak iki noktalı kırmızı örümceğin yumurta, nimf ve ergin dönemlerine uygulanmıştır. Repellent etki için, gül yağı, geraniol ve citronellol'ün 0.1, 1, 5 ve 10 ml/l konsantrasyonları iki noktalı kırmızı örümceğin yumurta, nimf ve ergin dönemlerine uygulanmıştır. Çalışma sonucunda *Tetranychus urticae*'nin farklı dönemleri üzerinde gül yağı ve iki önemli bileşeni geraniol ve citronello'ün kontakt, repellent ve ovisidal etkilerinin bulunduğu belirlenmiştir. Buna ilaveten en yüksek kontakt, repellent ve ovisidal etki geraniol'de belirlenirken, bunu gül yağı ve citronellol izlemiştir. Çalışma sonucunda elde edilen veriler *Rosa damascena* uçucu yağı ile iki önemli bileşeni geraniol ve citronellol'ün *Tetranychus urticae* mücadelesinde potansiyel olarak kullanılabileceğini göstermektedir.

**Anahtar sözcükler**: *Tetranychus urticae*, gül yağı, geraniol, citronellol, akarisidal etki

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Alınış (Received): 18.04.2014 Kabul ediliş (Accepted): 06.11.2014

## Introduction

The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is a ubiquitous species that is present worldwide on a wide variety of plants (Helle & Sabelis, 1985). *T. urticae* feeds by puncturing cells and draining the contents, subsequently producing a characteristic yellow speckling on leaf surfaces. They also produce a silk webbing, which is clearly visible at high infestation levels (Jeppson et al., 1975). The host plant can be affected in different ways, including a decrease in photosynthesis or by an injection of phytotoxic substances when feeding (Jhonson & Lyon, 1991). This mite species has been reported to attack approximately 1200 species of plants, of which more than 150 are economically significant (Zhang, 2003). *T. urticae* is commonly controlled by applications of synthetic pesticides (Van Leeuwen et al., 2004). However, the spider mite's short life cycle, which results in large populations, presents a major challenge in controlling their proliferation. Another challenge is the reduced efficacy of most synthetic pesticides, in part because resistance to synthetic pesticides is quickly selected (Stumpf & Nauen, 2001; Herron & Rophail, 2003; Van Leeuwen et al., 2009). Spider mites have evolved resistance to more than 80 acaricides to date, and resistance has been reported in more than 60 countries (Miresmailli et al., 2006). Due to the adverse effects of pesticide use, alternative control methods are being sought for *T. urticae*. A variety of natural bioactive compounds, including several plant essential oils (Calmasur et al., 2006; El Gengaihi et al., 1996; Pontes et al., 2007 a; b), plant extracts (Shi et al., 2006) and microbial secondary metabolites (Villanueva & Walgenbach, 2006) have been determined to have acaricidal effects (Cavalcanti et al., 2010) on two-spotted spider mites.

Essential oils have a broad spectrum of activity against insects and mites due to the presence of several modes of action, including repellency and antifeedant activities, contact activity, inhibition of molting and respiration, reduction in growth and fecundity, cuticle disruption, and an effect on the invertebrate octopamine pathway (Arnason et al., 1993; Prates et al., 1998; Isman, 2000; Enan, 2001; Akhtar & Isman, 2004; Isman et al., 2011). Plant essential oils and their constituents have been suggested as alternative sources for mite control products, largely because they constitute a potential source of bioactive chemicals that have been perceived by the general public as relatively safe, pose fewer risks to the environment, and have minimal impacts on human and animal health. Additionally, these compounds often act on multiple and novel target sites, thereby reducing the potential for resistance (Ahn et al., 2006; Isman, 2006; Han et al., 2010). Furthermore, essential oils derived from plants have minimal direct and/or indirect effects on natural enemies (Tunc & Sahinkaya, 1998; Bostanian et al., 2005; Isman, 2006).

The most important production centers of *Rosa damascena* Mill. (Damask rose) are Turkey and Bulgaria. *R. damascena* flowers are handpicked annually in May and June (approximately 35 to 45 days) and the essential oil content of its flower is 0.030-0.050% (Baydar et al., 2008). Citronellol and geraniol are the major compounds of *R. damascena* oil. These compounds can be obtained from different plant species (*Cymbopogon nardus*, *Pelargonium geranium*, etc.) and can also synthetically produced (Jain et al., 2001; Mahalwal & Ali, 2002). *R. damascena* is an ornamental species and, in addition to its perfuming effect, several pharmacological properties including anti-HIV, antibacterial, antioxidant, antitussive, hypnotic, antidiabetic, and a relaxant effect on tracheal chains have been reported for this plant (Boskabady et al., 2011). Rose oil has a wide range of uses in industry; however, there are currently no reports of its insecticidal or acaricidal effects on pests.

The aim of this study was to investigate the contact, repellency and ovicidal effects of *Rosa damascena* essential oil and its two major constituents, geraniol and citronellol, on different life stages of *T. urticae* under laboratory conditions.

## Materials and Methods

### Plant and chemicals materials

Fresh flowers of *R. damascena* were handpicked in the early morning hours (from 8:00 to 10:00 a.m.) of the flowering season (May and June, 2013) from the Rose and Rose Products Research and Implementation Center (GULAR) at Suleyman Demirel University in Isparta province of Turkey (latitude 37°45' N, longitude 30°33'E, altitude 997 m). The fresh flowers were distilled by using a hydro-Clevenger apparatus to obtain the rose oil. Citronellol ($C_{10}H_{20}O$, Sigma-Aldrich) and geraniol ($C_{10}H_{18}O$, Sigma-Aldrich) were used as synthetic forms.

### Origin and rearing of *Tetranychus urticae*

An insecticide-susceptible population of *T. urticae* (German Susceptible Strain, GSS) was obtained from the Rothamstad Experimental Station (England) in 2001 and reared under laboratory conditions to the present without any exposure to pesticides. *T. urticae* was reared on bean plants in climate chambers with a temperature of 26±2 ºC, 50-60% humidity and a 16:8 photoperiod.

### Isolation of essential oil

Fresh rose flowers (1 kg) and tap water (3 L) were placed inside in a flask (6 L) connected to the condenser of a hydro-Clevenger apparatus according to standard procedure described in European Pharmacopoeia (1975). The essential oil and water mixture were separated by decantation. The essential oil was dried with anhydrous sodium sulfate and stored at 4 °C until used for analysis.

### GS-MS analysis of essential oils

Gas Chromatography/Mass Spectrometry (GC-MS) analysis of the rose samples was performed on a Shimadzu 2010 Plus GC-MS equipped with a Quadrupole (QP-5050) detector. The analysis was performed under the following conditions: capillary column, CP-Wax 52 CB (50 m x 0.32 mm, film thickness 0.25 µm); injector temperature, 240 °C; detector temperature, 250 °C; oven temperature program, from 60 °C (10 min. hold) to 220 °C rising at 2 °C/min. and increasing to 220 °C (11.5 min. hold) rising at 20 °C/min.; flow speed, 10 psi; detector: 70 eV; ionization type, EI; carrier gas, helium (20 ml/min.); and sample injected 1 µl. Identification of constituents was determined by comparison of the retention times of standard substances (by composition of mass spectra) with data from the Wiley, Nist, and Tutor libraries (Stein, 1990).

### Contact assay

The method reported in Miresmailli et al. (2006) was adapted and used to determine the contact effect of essential oils on *T. urticae* adults and nymphs. To ensure homogeneity of the contact effect, *T. urticae* nymphs and the adults of the same age were used. To obtain same-aged nymphs for the experiment, fifteen female individuals were transferred to bean leaf disks prepared in ten Petri dishes (nine-centimeter-diameters) for oviposition. As a result of eggs hatching within twenty four hours of one another, nymphs were the same age for the trials. Eggs left on the leaves became adults of the same age for use in the adult trials. Adult trials were conducted with *T urticae* adults of the same age. Four concentrations of the essential oils were used to determine the contact effect in either nymphs or adults of *T urticae*. In trials, 1, 5, 10 and 20 ml/l concentrations of santolina, sage, rosemary and hyssop were used. Essential oils were diluted to these doses by dissolving them in water containing 0.3% Tween 20. Trials were conducted with three repetitions with fifteen individuals in each repetition for each essential oil concentration. Water containing 0.3%-Tween 20 was used as the control in these trials. Either nymphs or adults of *T. urticae* were transferred to bean leaf disks (three-cm diameter) using a thin brush. There was wet cotton under the bean leaf discs to ensure a nine-centimeter radius of moisture. In the trials, Petri dishes containing nymphs or adults of *T. urticae* were treated with pesticides using spraying towers. Essential oil concentrations were administered directly on the nymphs and adults of *T urticae*. Essential oil solutions were applied on the leaf surface using a spraying tower at one-bar pressure and a rate of 1.2 to 1.6 mg/cm$^2$ (Mansour et al., 1986). Counts of mortality and survival were conducted at 24, 48 and 96 h after treatment.

**Ovicidal effects of essential oils on eggs**

The method of Badawy et al. (2010) was adapted and used to determine the ovicidal effects of essential oils on *T. urticae* eggs. To obtain eggs of the same age for use in the trials, twenty adult females were transferred to bean leaf disks that were prepared in ten Petri plates (nine-centimeter-diameter). After twenty-four hours, adult females were removed and eggs were used in the trials. In trials, 1, 5, 10 and 20 ml/l concentrations of santolina, sage, rosemary and hyssop were used and essential oils were diluted to these doses by dissolving in water containing 0.3% Tween 20. Trials were conducted with three repetitions using fifteen individuals in each repetition for each essential oil concentration. Water containing 0.3% Tween 20 was used as a control. Eggs of *T urticae* were transferred to bean leaf disks (three-centimeter diameter) using a thin brush. There was wet cotton under the bean leaf discs to ensure a nine-centimeter radius of moisture. To measure the contact effect, Petri dishes containing eggs of *T urticae* were sprayed with pesticides using spraying towers. Essential oil concentrations were administered directly on eggs of *T urticae*. Essential oil solutions were placed on the leaf surface using a spraying tower at one-bar pressure and a rate of 1.2 to 1.6 mg/cm$^2$ (Mansour et al., 1986). For the ovicidal effect trials, observations continued until last egg in the control group hatched.

**Repellency assay**

The methods of Nerio et al. (2009) and Araujo et al. (2012) were adapted and used to determine the repellency effect of essential oils on *T. urticae* nymphs and adults. Same-aged *T. urticae* nymphs and adults were used in the repellency effect assay. Nymphs and adults for this trial were obtained as described for the contact effect trials. The essential oils of *R. damascena* and its two major constituents, geraniol and citronellol were used the repellency effect assays at concentrations of 0.1, 1, 5 and 10 ml/l. Essential oils were dissolved in a solution of water and 0.3% Tween 20 and were diluted to the targeted doses. Half of each sample was placed in the water and 0.3% Tween 20 solution, and the other half was placed in a Petri dish (9 cm) with wet cotton and bean leaf discs, which were immersed in essential oil solution. A brush was used to place *T. urticae* nymphs or adults in the middle of the leaf surface on which the application was performed. The experiment was repeated 4 times with 10 individuals per trial. At 2, 6, 24 and 48 h, individuals on the essential oil treated and control portions of the leaf discs were counted.

**Data analysis**

Abbott’s formula (Abbott, 1925) was used to determine death % values in contact effect samples.

In the formula, Death value (%)=[ (A-B) / (A)] × 100, A refers to the number of live individuals in the control; B refers to the number of live individuals in the treatment dose. The Repellency effect index % developed by Obeng-Ofori et al. (1997) was used to calculate the results obtained from the repellency effect tests.

Repellency effect (%) = [(Nc-Nt) / (Nc+Nt)] × 100

In this formula, Nc refers to the number of individuals in the control area, and Nt refers to the number of individuals in the essential oil area. Contact and repellency effects were calculated using Abbott’s formula (without percentage) on data obtained from these experiments. The values were subjected to transformation, then, groupings were made by variance analysis (ANOVA) using a statistical analysis software (SAS_ v9.1.2 from SAS Institute, Inc., Cary, NC, USA). The significant differences were tested by Duncan’s Multiple Range Test ($P \leq 0.05$).

## Results

### Chemical compositions of essential oils

According to the GC-MS analysis of the hydrodistilled rose oil, a total of 22 volatile compounds were identified. A high percentage of identified compounds were non-cyclic monoterpene alcohols, represented by geraniol (34.91%), citronellol (23.43%), and nerol (15.43%), and long-chain hydrocarbons (alkanes) represented by nonadecane (3.01%), eicosane (2.52%), heneicosane (3.64%) and tricosane (1.92%) (Table 1). Although phenylethyl alcohol (or 2-phenylethanol) was the major scent compound of the fresh flower, it was found at only 0.98% in the hydrodistilled rose oil. It has been reported that over 100 components have been identified in rose oil by GC and GC-MS analysis. The most important compounds which constitute rose oil have been found to be monoterpene alcohols (citronellol, geraniol, nerol and linalool etc.), hydrocarbons (nonadecane, heneicosane, heptadecane, eicosane and tricosane etc.), sesquiterpenes (humulene and murolene etc.), oxides and ethers (methyl eugenol, etc.), ester and aldehydes (geranyl acetate and geranial, etc.), and phenols (eugenol, etc.) (Anaç, 1984; Kovats, 1987; Başer, 1992; Bayrak & Akgül, 1994). β-damascenone, β-damascene and β-ionene also contribute to the characteristic odor of rose oil; however these components were found in only trace amounts by GC and GC-MS analysis (David et al., 2006).

Table 1. Essential oil components and rates (%) of rose oil

| Components | RT | % | Components | RT | % |
|---|---|---|---|---|---|
| Alpha pinene | 8.1 | 0.24 | **Citronellol** | **51.2** | **23.43** |
| Beta myrcene | 14.0 | 0.23 | Nerol | 53.4 | 15.43 |
| Linalool | 37.7 | 0.81 | Aromadendrene | 54.9 | 0.46 |
| Trans-Caryophyllene | 41.0 | 1.15 | **Geraniol** | **56.0** | **34.91** |
| Terpinen-4-ol | 41.7 | 0.60 | Eicosane | 58.3 | 2.52 |
| Citronellyl acetate | 45.0 | 0.56 | Nonadecene | 59.3 | 3.01 |
| Alpha humulene | 46.1 | 0.61 | Phenethyl alcohol | 60.1 | 0.98 |
| Hexadecane | 46.5 | 1.20 | Methyl eugenol | 65.4 | 0.91 |
| Germacrene D | 48.5 | 1.96 | Heneicosane | 69.0 | 3.64 |
| E-Citral | 49.8 | 0.46 | Eugenol | 73.6 | 1.72 |
| Geranyl acetate | 50.7 | 2.17 | Tricosane | 78.9 | 1.92 |

### Ovicidal effects of the rose essential oil, geraniol and citronellol on eggs

The effects of rose oil, geraniol and citronellol on *T. urticae* egg hatching are presented in Table 2. It was statistically determined that the effect of rose oil components (geraniol and citronellol) to prevent hatching of *T. urticae* eggs increased with an increase in concentration ($p \leq 0.05$). Geraniol had the highest ovicidal effect of all concentrations of rose oil and components, while citronellol had the lowest effect. At the 20 ml/l concentration, the highest effects were 80% for geraniol, 72.22% for rose oil and 53.10% for citronellol.

Table 2. The ovicidal effects of rosa essential oil, geraniol and citronellol on *Tetranychus urticae* eggs

| Concentration (ml/l) | Percentage effect (%) | | |
|---|---|---|---|
| | *Rosa damascena* (Rose oil) | Geraniol | Citronellol |
| 1 | 32.22 ±2.20 bD | 44.44 ±2.20 aD | 17.77 ±2.22 cD |
| 5 | 47.78 ±2.20 bC | 59.99 ±3.91 aC | 33.33 ±0.00 cC |
| 10 | 63.33 ±0.00 aB | 68.88 ±2.20 aB | 44.44 ±2.21 bB |
| 20 | 72.22 ±2.20 bA | 80.00 ±0.00 aA | 53.10 ±1.51 cA |

Different lower letters on the same row and different upper letters on the same column indicate that there is a significant difference by essential oils and application doses, respectively ($p \leq 0.05$).

**Contact effect of rose essential oil, geraniol and citronellol**

The contact effects of different concentrations of rose oil, geraniol and citronellol on *T. urticae* adults are presented in Table 3. Geraniol, which is the most important component of rose oil, had a higher contact effect on twospotted spider mite adults than did rose oil and citronellol at all concentrations and observation times. While the contact effects of the essential oil and its components used in the study were statistically similar to those of rose oil and geraniol at all concentrations at the end of 24 hours, the effect of citronellol was different ($p \leq 0.05$). At the end of 48 hours, the effect of geraniol was higher than that of rose oil and citronellol at all concentrations and was significantly different ($p \leq 0.05$). At the end of 96 hours, the contact effect of geraniol was higher than that of rose oil and citronellol at the 1 and 20 ml/l concentrations. At the 5 and 10 ml/l concentrations, the contact effects of rose oil and its two components on *T. urticae* were significantly different ($p \leq 0.05$).

Table 3. Contact effect of rosa essential oil, geraniol and citronellol on *Tetranychus urticae* adults

| Count time (hour) | Concentration (ml/l) | Contact effect (%) | | |
|---|---|---|---|---|
| | | *Rosa damascena* (Rose oil) | Geraniol | Citronellol |
| 24 | 1 | 17.92±0.30 aH | 23.17±1.75 aH | 7.81±0.67 bG |
| | 5 | 25.39±5.72 aG | 30.16±1.59 aGH | 15.28±2.37 bFG |
| | 10 | 33.33±0.00 aEF | 37.14±1.43 aG | 19.44±3.38 bEF |
| | 20 | 40.47±4.76 aDE | 45.39±1.27 aF | 30.14±5.00 bD |
| 48 | 1 | 15.87±2.08 bH | 35.71±0.00 aG | 14.28±0.00 bFG |
| | 5 | 30.14±1.93 bFG | 45.23±2.38 aF | 25.39±3.97 bDE |
| | 10 | 42.85±0.00 bCD | 57.14±4.12 aDE | 32.57±2.97 bD |
| | 20 | 48.16±4.97 bBC | 69.04±2.38 aBC | 41.43±2.97 bC |
| 96 | 1 | 30.03±0.73 bFG | 50.00±0.00 aEF | 23.80±0.95 bDE |
| | 5 | 52.56±1.28 bB | 64.28±0.00 aCD | 44.28±2.97 cC |
| | 10 | 63.36±0.92 bA | 76.19±2.38 aB | 56.19±0.95 cB |
| | 20 | 70.14±3.68 bA | 85.71±0.00 aA | 66.19±2.38 bA |

Different lower case letters on the same row and different upper case letters on the same column indicate that there is a significant difference by essential oils and application doses, respectively ($p \leq 0.05$).

The contact effects of different concentrations of rose oil, geraniol and citronellol on the nymphs of *T. urticae* are presented in Table 4. The contact effect of geraniol on nymphs of *T. urticae* was higher than that of rose oil and citronellol at all concentrations. Furthermore, as the concentration and observational time of all three components increased, the contact effect on the nymphs of twospotted spider mites increased as well. At 24 hours, the effect of geraniol was significantly different from the effects of rose oil and citronellol at the concentrations of 1, 5, 10 ml/l ($p \leq 0.05$). In contrast, there was no significant difference between the contact effects of all three components at the concentration of 20 ml/l ($p \leq 0.05$). At 48 hour, the contact effect of rose oil, geraniol and citronellol on the nymphs of *T. urticae* were statistically similar and these components were in the same group ($p \leq 0.05$). At the 96 hour observation, geraniol had the highest effect at the concentration of 1 ml/l, while the effects of all three components were statistically similar at the concentrations of 5, 10 and 20 ml/l ($p \leq 0.05$).

Table 4. Contact effect of rosa essential oil, geraniol and citronellol on *Tetranychus urticae* nymphs

| Count time (hour) | Concentration (ml/l) | Contact effect (%) | | |
|---|---|---|---|---|
| | | *Rosa damascena* (Rose oil) | Geraniol | Citronellol |
| 24 | 1 | 15.72±1.52 bH | 27.08±2.94 aE | 12.42±1.25 bG |
| | 5 | 34.94±12.13 bFG | 62.20±10.12 aBC | 36.35±1.95 bEF |
| | 10 | 61.65±2.70 bCD | 72.61±1.19 aAB | 54.44±2.94 bCD |
| | 20 | 65.47±5.20 aBC | 72.31±4.93 aAB | 60.15±3.26 aA-D |
| 48 | 1 | 32.27±8.95 aG | 41.07±6.64 aD | 28.14±4.12 aF |
| | 5 | 50.73±10.21 aDE | 53.34±7.97 aCD | 49.52±2.38 aDE |
| | 10 | 70.69±0.73 aA-C | 73.54±1.00 aAB | 65.04±0.38 aA-C |
| | 20 | 73.07±2.82 aA-C | 73.33±0.00 aAB | 62.85±2.98 aA-D |
| 96 | 1 | 47.20±1.42 bEF | 56.63±3.05 aC | 39.52±1.26 bEF |
| | 5 | 64.24±1.49 aB-D | 65.30±6.48 aBC | 59.04±2.38 aBC |
| | 10 | 77.75±3.46 aAB | 76.51±1.62 aAB | 70.23±2.81 aAB |
| | 20 | 83.25±0.81 aA | 89.10±2.06 aA | 74.42±1.19 aA |

Different lower case letters on the same row and different upper case letters on the same column indicate that there is a significant difference by essential oils and application doses, respectively ($p \leq 0.05$).

### Repellency effect of rose essential oil, geraniol and citronellol

The repellent effects of different concentrations of rose oil, geraniol and citronellol components on *T. urticae* adults are presented in Table 5. The repellent effects of rose oil, geraniol and citronellol on *T. urticae* adults in all concentrations decreased with time. While the repellent effects of rose oil and geraniol on *T. urticae* adults were similar at the 0.1 ml/l concentration at the end of 6, 24 and 48 hours, the effect of citronellol was significantly different ($p \leq 0.05$). Additionally, the repellent effect of all three components at the same concentration at the end of 2 hour significantly different ($p \leq 0.05$). In contrast, the repellent effects of rose oil and geraniol were statistically similar at the concentrations of 1, 5 and 10 ml/l for all observation times. In contrast, the repellent effect of citronellol was expressed in a different group ($p \leq 0.05$). The repellent effect of rose oil and geraniol on *T. urticae* adults was 100% at the 10 ml/l concentration at the end of 2 hours.

Table 5. Repellency effect of rosa essential oil, geraniol and citronellol on *Tetranychus urticae* adults

| Concentration (ml/l) | Count time (hour) | Repellent effect (%) | | |
|---|---|---|---|---|
| | | *Rosa damascena* (Rose oil) | Geraniol | Citronellol |
| 0.1 | 2 | 45.00±2.90 bGH | 55.00±2.90 aGH | 27.5±2.50 cEG |
| | 6 | 40.00±0.00 aHI | 45.00±2.90 aI | 22.50±2.50 bFH |
| | 24 | 32.50±2.50 aIJ | 35.00±2.90 aJ | 20.00±0.00 bGH |
| | 48 | 25.00±2.90 aJ | 32.50±4.79 aJ | 15.00±2.90 bH |
| 1 | 2 | 67.50±2.50 aEF | 67.50±2.50 aEF | 42.50±2.50 bD |
| | 6 | 62.50±2.50 aF | 62.50±2.50 aFG | 32.50±2.50 bE |
| | 24 | 52.50±2.50 aG | 55.00±2.90 aGH | 30.00±4.10 bEF |
| | 48 | 45.00±2.90 aGH | 47.50±4.79 aHI | 25.00±2.90 bEG |
| 5 | 2 | 87.50±2.50 aB | 82.50±2.50 aBC | 67.50±2.50 bAB |
| | 6 | 80.00±0.00 aBD | 80.00±0.00 aCD | 62.50±2.50 bBC |
| | 24 | 75.00±2.90 aDE | 72.50±2.50 aDE | 60.00±0.00 bBC |
| | 48 | 65.00±2.90 aF | 60.00±0.00 aFG | 45.00±2.90 bD |
| 10 | 2 | 100.00±0.00 aA | 100.00±0.00 aA | 75.00±2.90 bA |
| | 6 | 97.50±2.50 aA | 90.00±5.65 aB | 72.50±2.50 bA |
| | 24 | 85.00±2.90 aBC | 80.00±0.00 aCD | 67.50±2.50 bAB |
| | 48 | 77.50±2.50 aCD | 75.00±2.90 aDE | 55.00±2.90 bC |

Different lower case letters on the same row and different upper case letters on the same column indicate that there is a significant difference by essential oils and application doses, respectively ($p<0.05$).

The repellent effects of different concentrations of rose oil, geraniol and citronellol on *T. urticae* nymphs are presented in Table 6. There was no statistically significant difference between the repellent effects of the 0.1 ml/l concentrations of rose oil, geraniol and citronellol on *T. urticae* nymphs at the end of 2 and 6 hours ($p \leq 0.05$). In contrast, rose oil and geraniol effect had a similar effect at the same concentration at the end of 24 hours, while the effect of citronellol was presented in a different statistical group ($p \leq 0.05$). At the end of 48 hours at the 0.1 ml/l concentration, the repellent effects of all three components on *T. urticae* nymphs were significantly different ($p \leq 0.05$). The effect of geraniol was higher than rose oil and citronellol at the 1 ml/l concentration at the end of 2 and 24 hour. The repellent effects of all three components at the end of 6 hours were statistically similar. At the end of 48 hours, the effect of rose oil and geraniol were similar, however the effect of citronellol was significantly different from the other two components. The repellent effect of geraniol on *T. urticae* nymphs at all observation times was 100% compared to the 5 and 10 ml/l concentrations. The repellency of rose oil and the two components, especially at the 10 ml/l concentration, were 100%, excluding citronellol at the 48 hour observation.

Table 6. Repellency effect of rosa essential oil, geraniol and citronellol on *Tetranychus urticae* nymphs

| Concentration (ml/l) | Count time (hour) | Repellent effect (%) | | |
|---|---|---|---|---|
| | | *Rosa damascena* (Rose oil) | Geraniol | Citronellol |
| 0.1 | 2 | 75.00±5.00 aBC | 80.00±5.60 aBC | 70.00±8.16 aB |
| | 6 | 70.00±5.60 aC | 65.00±5.00 aC | 60.00±0.00 aCD |
| | 24 | 45.00±5.00 aD | 55.00±5.00 aCD | 25.00±5.00 bEF |
| | 48 | 30.00±5.60 bE | 45.00±10.00 aD | 15.00±5.00 cF |
| 1 | 2 | 85.00±9.67 bAB | 100.00±0.00 aB | 80.00±0.00 bA |
| | 6 | 70.00±12.60 aC | 80.00±0.00 aB | 80.00±0.00 aB |
| | 24 | 55.00±9.80 abD | 65.00±5.00 aC | 50.00±5.60 bD |
| | 48 | 50.00±5.60 aD | 60.00±0.00 aC | 30.00±5.60 bE |
| 5 | 2 | 100.00±0.00 aA | 100.00±0.00 aA | 100.00±0.00 aA |
| | 6 | 100.00±0.00 aA | 100.00±0.00 aA | 100.00±0.00 aA |
| | 24 | 100.00±0.00 aA | 100.00±0.00 aA | 90.00±5.60 aAB |
| | 48 | 80.00±0.00 bBC | 100.00±0.00 aA | 65.00±5.00 cC |
| 10 | 2 | 100.00±0.00 aA | 100.00±0.00 aA | 100.00±0.00 aA |
| | 6 | 100.00±0.00 aA | 100.00±0.00 aA | 100.00±0.00 aA |
| | 24 | 100.00±0.00 aA | 100.00±0.00 aA | 100.00±0.00 aA |
| | 48 | 100.00±0.00 aA | 100.00±0.00 aA | 80.00±0.00 bB |

Different lower case letters on the same row and different upper case letters on the same column indicate that there is a significant difference by essential oils and application doses, respectively ($p<0.05$).

## Discussion

Many essential oils and their major components are emerging as pest control agents due to their insecticidal, acaricidal, repellent, contact and antifeedant properties (Saxena, 1989; Isman, 2000; Barnard & Xue, 2004; Papachristos et al., 2004). This study analyzed the contact, repellent and ovicidal effects of rose oil and its two important components, geraniol and citronellol, on different life stages of *T. urticae*.

Rose oil and its two important components, geraniol and citronellol, were found to have a contact effect on *T. urticae* adults and nymphs. Rose oil, geraniol and citronellol concentrations that were applied, especially after 96 hours, had high lethal effects on adults and nymphs of *T. urticae*. Geraniol had the highest contact effect on adults and nymphs of *T. urticae* at all concentrations, followed by rose oil and citronellol. According to the GS-MS analysis, geraniol and citronellol are the most prevalent components of rose oil (34.91% and 23.43%, respectively). Overall, geraniol, which is most abundant component of rose oil, shows the highest effect on *T. urticae*. Although citronellol, which is the second most important component of rose oil, has a significant contact effect on adults and nymphs of *T. urticae*, this effect was lower than for that of rose oil. This supports the assertion that citronellol has a role in the contact effect of rose oil on adults and nymphs of *T. urticae*; however, geraniol, which is present in rose oil at a higher percentage, has a more significant role in the contact effect on *T. urticae*. The effect of the nerol component, which is the third most abundant component in rose oil (15.43%), should also be analyzed. Similar results were found for the ovicidal effects of rose oil, geraniol and citronellol on *T. urticae* eggs. Attia et al. (2012) reported that major compounds in essential oils play a role in the acaricidal effects of those oils. Essential oils and monoterpenoids have been reported to have an acaricidal effect on twospotted spider mites (Sanchez-Ramos & Castanera, 2000; Rasikari et al., 2005; Miresmailli et al., 2006; Badawy et al., 2010; Cavalcanti et al., 2010; Sertkaya et al., 2010; Roh et al., 2011). Although the literature contains no reports on the insecticidal or acaricidal activity of rose oil, there is some research on geraniol, which is contained in some essential oils. In a study that used α-pinene, geraniol, limonene and ρ-cymen monoterpenoids, Traina et al. (2005) reported that geraniol had a higher acaricidal activity on *Otodectes cynotis* (Acari: Psoroptidae) than other monoterpenoids. Comparing the activities of geraniol monoterpenoid and benzyl benzoate acaricide on *T. putrescentiae*, Jeon et al. (2009) reported that geraniol had a higher acaricide effect. Similarly, in our study, geraniol was found to have a high acaricidal effect. Badawy et al. (2010) showed that limonene, carvone, linalool, fenchone, 1-8-cineole, myrcene, geraniol and camphor monoterpenoids showed a 70.6%-6.3% ovicidal effect on *T. urticae* eggs. In agreement with other reports, this study found that the ovicidal effect of geraniol on *T. urticae* eggs was higher than that of rose oil and citronellol.

Repellence is another advantageous property of essential oils; some contain numerous secondary metabolites that can deter attacks from insects and general herbivores (Isman, 2000). Repellency of the rose oil, geraniol and citronellol on *T. urticae* adults and nymphs at all concentrations were observed to be high at 2 hours. However, this effect decreased with time. The repellent effects of rose oil, geraniol and citronellol on *T. urticae* nymphs were higher than their effects on adults. All three components had 100% repellency in nearly all of the 5 and 10 ml/l concentrations. Similar to the contact effect, geraniol had the highest repellent effect on twospotted spider mite adults and nymphs, followed by rose oil and citronellol. Similar to our findings, other reports have shown that some essential oils and components have repellent effects against pests. According to the results of Mansour et al. (1986), different concentrations of acetonic solutions of essential oils from 14 species of Labiatae caused mortality and induced repellency in adult females of the *Tetranychus cinnabarinus* Boisd. (Acari: Tetranychidae) and oviposition was also reduced. Hori & Komatsu (1997) found that *Rosmarinus officinalis* L. volatile oil and its principle component 1,8-cineole was repellent against *Neotoxoptera formosana* (Takahashi) (Homoptera: Aphididae). Jantan & Zaki (1998) reported that formulations made of *Cinnamomum camphora* Linnaeus (Lauraceae), *Mentha pulegium* Linnaeus (Labiatae) essential oils and camphor components had long-term repellent effects against pests, and some monoterpenoids such as α-pinene, limonene, terpinolene, citronellol, citronellal and camphor also had repellent effects. Araujo et al. (2012) reported that *Piper aduncum* essential oil and its principle components (nerolidol, α-humulene and β-caryophyllene) did not show a repellent effect in *T. urticae*. Conclusive research has shown geraniol to be an effective plant-based mosquito repellent and an insecticide for controlling pests with low mammalian toxicity and biodegradability (Omolo et al., 2004; Chen & Viljoen, 2010).

Different concentrations of rose oil and its two important components geraniol and citronellol were found to have contact and repellent effects on *T. urticae* adult and nymphs and ovicidal effects on eggs. Furthermore, in all experiments on contact, repellent and ovicidal effects, geraniol was found to have the highest effect, followed by rose oil and citronellol. Based on these findings, it is believed that geraniol and citronellol play a significant role in the contact and repellent effects of rose oil on *T. urticae*; however, the effect of geraniol, which is more abundant in rose oil, was higher than that of citronellol. In addition, none of the concentrations of rose oil were observed to have a phytotoxic effect on the leaves of beans used in Petri dishes. Many plant essential oils and their components have a broad spectrum of activity against mites and other pests (Attia et al., 2013). It has been recognized that essential oils and their constituents can be developed into products suitable for integrated pest management because some of them are selective for pests, have few harmful effects on non-target organisms, are environmentally non-persistent, and have behavioral (repellence and feeding deterrence) and physiological efficacy (acute toxicity and developmental disruption) against various types of pest complexes (Ahn et al., 2006; Isman, 2006). Furthermore, as essential oils are a mixture of terpenoid compounds, the rapid resistance development observed in two-spotted spider mites will be slower than for insecticides containing only one active ingredient. However, because large amounts of rose material are necessary for rose oil extraction, it is a very expensive essential oil. Therefore, the use of rose oil as an alternative to pesticides against *T. urticae* is not considered possible, as it will considerably increase agricultural inputs. In contrast, geraniol and citronellol, which are two important components of rose oil and have important contact and repellent effects on *T. urticae*, could be included in pest control programs because they can be synthetically produced. However, the number of studies in laboratory and field conditions should be increased to test the appropriateness of these compounds in pest control programs. In addition, this study is significant as it is the first study to analyze the potential use of rose oil to control *T. urticae*.

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