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Original article (Orijinal araştırma)

A laboratory study of the acaricidal, repellent and oviposition deterrent effects of three botanical oils on *Tetranychus urticae* (Koch, 1836) (Acari: Tetranychidae)[1]

Tetranychus urticae (Koch, 1836) (Acari: Tetranychidae)'ye karşı üç bitkisel yağın akarisit, uzaklaştırıcı ve yumurta bırakma engelleyici özellikleri üzerine laboratuvar çalışması

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Abstract

The biological activities of essential oil obtained from water distillation process of basil leaves [*Ocimum basilicum* L. (Lamiales: Lamiaceae)] cv Round Midnight and crude oil obtained from the cold-pressed process of chinaberry tree seeds [*Melia azedarach* L. (Sapindales: Meliaceae)] and a commercial neem oil product (Nimbecidine) [*Azadirachta indica* (A. Juss, 1830) (Sapindales: Meliaceae)] were assessed against two-spotted spider mites, *Tetranychus urticae* (Koch, 1836) (Acari: Tetranychidae) using a residual method on leaf disc under laboratory conditions at Bursa Uludağ University during 2018-2019. The lethal concentrations (LC_{50} and LC_{90}) of Nimbecidine, chinaberry and basil oils were estimated as 0.8 and 1.8 mg/L, 4.0 and 6.9%, 5.4 and 11.7%, respectively, 72 h after treatment. The lethal times (LT_{50} and LT_{90}) of Nimbecidine (1 mg/L), chinaberry (6%) and the basil (8.4%) were 64 and 107 h, 41 and 73 h, 65 and 110 h, respectively. The females had a strong aversion to bean leaf surfaces sprayed with the sublethal concentrations of Nimbecidine (0.125-0.75 mg/L), chinaberry (0.75-3%) and basil (0.7-1.4%) oils. Significant decreases were recorded in the number of eggs laid on bean leaves sprayed with the sublethal concentrations for Nimbecidine (0.031-0.5 mg/L), chinaberry (0.75-3%) and the basil (1.4-5.6%) oils compared with unsprayed bean leaves. The study showed that the assessed concentrations of the oils obtained from the basil and chinaberry compared to the commercial botanical product (Nimbecidine) have similar biological effects on *T. urticae.*

Keywords: Acaricide, biological effects, chinaberry, basil, neem, spider mite

Öz

Mor reyhan [*Ocimum basilicum* L. 1753 'Round Midnight' (Lamiales: Lamiaceae)] yapraklarından su distilasyonuyla ve tesbih ağacı [*Melia azedarach* L., 1753 (Sapindales: Meliaceae)] tohumlarından soğuk presleme yöntemi ile elde edilen yağların ve karşılaştırma materyali olarak ticari bir neem yağı formülasyonun (Nimbecidine) [*Azadirachta indica* (A. Juss, 1830) (Sapindales: Meliaceae)], İkinoktalı kırmızıörümcek *Tetranychus urticae* (Koch, 1836) (Acari: Tetranychidae) üzerindeki biyolojik etkileri, yaprak diski üzerinde kuru kalıntı yöntemi kullanılarak Bursa Uludağ Üniversitesi'nin laboratuvar koşullarında 2018-2019 yıllarında değerlendirilmiştir. Uygulamadan 72 saat sonra, Nimbecidine, tesbih ağacı ve mor reyhanın öldürücü konsantrasyonları (LC_{50} ve LC_{90}) sırasıyla 0.8 ve 1.8 mg/L, %4.0 ve 6.9, %5.4 ve 11.7 olarak tespit edilmiştir. Nimbecidine (1 mg/L), tesbih ağacı (%6) ve mor reyhanın (%8.4) belirlenen konsantrasyonlarının öldürücü zamanları (LT_{50} ve LT_{90}) 64 ve 107 s, 41 ve 73 s, 65 ve 110 s olarak belirlenmiştir. Nimbecidine (0.125-0.75 mg/L), tesbih ağacı (%0.75-3) ve mor reyhanın (%0.7-1.4) öldürücü altı dozlarıyla ilaçlanan fasulye yaprak yüzeylerinden *T. urticae* dişilerinin güçlü bir şekilde kaçtıklarını göstermiştir. Nimbecidine (0.031-0.5 mg/L), tesbih ağacı (%0.75-3) ve mor reyhanın (%1.4-5.6) sublethal dozlarının uygulandığı yapraklarda akarların yumurta bırakma sayıları kontrole göre önemli şekilde azalmıştır. Bu çalışma tesbih ağacı ve mor reyhandan elde edilen yağların belirlenen konsantrasyonlarının neemin ticari formülasyonu olan Nimbecidine gibi biyolojik etkiler gösterebileceğini ortaya koymuştur.

Anahtar sözcükler: Akarisit, biyolojik etkiler, tesbih ağacı, mor reyhan, neem, kırmızıörümcek

[1] This study is a part of Gizem Keskin's MSc research at the University of Bursa Uludağ, Graduate School of Natural and Applied Science, Entomology Department.

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Introduction

The two-spotted spider mite, *Tetranychus urticae* (Koch, 1836) (Acari: Tetranychidae), is a common critical pest in several kinds of cultivated crops (Helle & Sabelis, 1985). The high population of *T. urticae* causes spotty yellowing, curling also drying in leaves and thus decreases the productivity. Various synthetic acaricides are used to control the mite in greenhouses and fields. The purpose of the application of these chemicals is to reduce the mite population, but this strategy is not sustainable for agriculture, because, the widespread use of acaricides can cause some ecological problems: (1) destroying non-target beneficial organisms, (2) threaten human health with their residues on food, and (3) developing resistance to these chemicals by pests (Nauen et al., 2001; Kim et al., 2004; Simon, 2014; Salman & Keskin, 2019; Kumral et al., 2020). Due to these negative impacts, researchers focused on searching for environmentally safe natural pesticides with low toxicity to non-target organisms (Nicoletti et al., 2012; Fernandes et al., 2019). For example, neem oil, derived from the seed of the neem tree [*Azadirachta indica* (A. Juss) (Sapindales: Meliaceae)], has specific biological effects, such as lethality, insect growth disrupter, repellency, feeding and oviposition deterrent, on mite and insect pests, because it contains various biologically active limonoids, such as azadirachtin, nimbin, salannin, azadirachtol, nimbidin and gedunin (Martinez-Villar et al., 2005; Isman, 2006; Nicoletti et al., 2012). Given that neem has multiple biological but low side effects, various formulations with different trademarks (e.g., Nemazal, Nimbecidine, Nimiks, Suhulet, Ozoneem) have been registered and used against spider mites, mouths, thrips, flies and aphids in Turkey and many countries (Immaraju, 1998; Anonymous, 2020).

In this study, as an alternative to neem oil, the biological activities of the essential oils of basil leaves [*Ocimum basilicum* L.t (Lamiales: Lamiaceae) cv. Round Midnight] and crude oil of chinaberry tree seeds [*Melia azedarach* L. (Sapindales: Meliaceae)] were investigated against *T. urticae*. Chinaberry tree is often confused with *A. indica*, also known as neem tree belonging to the same plant family. However, *A. indica* has some limitations being adapted to tropical and subtropical regions and altitudes not higher than 1000 m (Forim et al., 2010). Whereas, chinaberry trees can grow in subtropical regions in eastern parts of Australia and Asia, and are common in the Mediterranean basin of Turkey. This species is often planted in parks, public gardens, and along stream banks, footpaths and roadsides as an ornamental tree because of its dense canopy, fragrant lilac flowers and yellow fruits (Erdem, 2019). Like neem, limonoids, triterpenoids and steroids obtained from *M. azedarach*, have antifeedant and insecticidal effects on some arthropod pests (Nakatani et al., 1995; Castiglioni et al., 2002; Banchio et al., 2003; El-Sawi, 2008; Sharma et al., 2010; Attia et al., 2011; Yanar et al., 2011a, b; Akihisa et al., 2013; Elkertati et al., 2013). Although there have been some toxicological studies on *T. urticae* with extracts from different parts of *M. azedarach*, none were performed with the oil obtained from cold-pressed seeds or investigated the oils repellence and oviposition deterrent effects.

The genus *Ocimum* (Lamiaceae) has 65 species naturally distributed across Asia, Africa and Central America (Paton et al., 1999). The morphological features of *O. basilicum* and their chemical contents have wide variation (Marotti et al., 1996; Simon et al., 1999; Vieira & Simon, 2000; Labra et al., 2004). It is widely used in spices, pharmaceuticals, food, and perfumery industries due to its valuable essential oil and fragrance. Also, essential oil of basil has increasing importance due to the biological effects, such as antimicrobial, antifungal, insecticide, and antioxidant (Deshpande & Tipnis, 1977; Prasad et al., 1986; Bassiouny et al., 1990; Marotti et al., 1996; Zollo et al., 1998). Also, basil cv. Round Midnight is an essential source of anthocyanins for the food industry (Simon et al., 1999). Although a few toxicological studies of other basil cultivars on *T. urticae* and stored product pests, the toxic, oviposition deterrent and repellent effects on *T. urticae* have not been previously investigated (Keita et al., 2000; Aslan et al., 2004; Martins et al., 2016; Kasap & Kök, 2019). In this study, acaricidal and adverse biological effects of *M. azedarach* (chinaberry) and *O. basilicum* (basil cv. Round Midnight) on *T. urticae* were compared with a commercial neem oil product (Nimbecidine) under laboratory conditions.

Materials and Methods

Spider mite culture

A laboratory colony of *T. urticae* was used in this experiment. The colony was maintained without exposure to any chemicals for 10 years. Mites were reared on bean plants [*Phaseolus vulgaris* L. (Fabales: Fabaceae)] cv. Magnum from MayAgro Seed Corp., Bursa, Turkey, at 27±1ºC, 65% RH and 16:8 h L:D photoperiod in a controlled environment room.

Botanical test materials

Basil cv. Round Midnight used in this study was obtained from Medicinal and Aromatic Plants Research Area, Department of Field Crops, Agricultural Faculty, University of Bursa Uludağ during the growing season of 2018. Commercial seeds of this cultivar were obtained from a private company (Anadolu Seed Production and Marketing Inc., Turkey). The seeds were sown at the beginning of March in a greenhouse and when the seedlings had reached 10 cm high, they were transferred to the field (4 May 2018) on a 30 × 25 cm grid. During the vegetation period, plots were irrigated as needed. The dark violet leaves together stems were harvested in August at 50% flowering. The leaves were air-dried in the dark, then the leaves and stems were separated. The extraction of the essential oils from 50 g of dry leaves was done by hydro distillation with using a modified Clevenger-type apparatus for 2 h. The oil was stored in dark glass bottles at 4ºC (Marotti et al., 1996). The essential oil content was determined by the volumetric method (v/w) and expressed as a percentage (Wichtl, 1971). Mean essential oil content of dry leaf was 0.4-0.5%. Previous studies have shown that this genotype has a linalool chemotype (Kaçar et al., 2009). The fruits of chinaberry tree (*M. azedarach*) were collected by Greenza Company (Bursa, Turkey) from an ornamental planting of Adana (Turkey) in October 2018 during the ripening period. The fruits were dried indoors and seeds separated from the kernel with a knife. Paste from these seeds was obtained using a cold-pressed machine (under 40ºC) developed by Greenza Company and then pure oil was obtained from this paste by filtering. As a commercial neem oil (*A. indica*) product, Nimbecidine (0.3 g/L azadirachtin) was provided from Manufacturer Company (VitaLonga, AgroBest Grup, Izmir, Turkey).

Toxicity tests

To determine toxicity, bioassays were performed under laboratory conditions using a leaf disc in Petri dish (120 mm diameter) method following Keskin & Kumral (2015). Briefly, each bean leaf disc (90 mm diameter) was put on warm agar solutions (2%) poured into a Petri dish. The lid of Petri dish was pierced with a steel needle ensured the ventilation. A range of concentrations of the chinaberry and basil oils were prepared in 25% ethanol. Nimbecidine was diluted with distilled water. Two ml of different concentrations have applied the underside of the leaf disc with spray tower adjusted to 1.5 kg/cm^2 working pressure and 3-s delivery (Potter precision, Burkard Manufacturing Co. Ltd., Rickmansworth, UK). Leaf discs were then dried at room condition for 15-30 min (Potter, 1952). In each bioassay, six concentrations, three replicates and one control (25% ethanol for chinaberry and basil oils; distilled water for Nimbecidine) were used. The concentrations caused 10-90% mortality was used as an acaricide. Female *T. urticae* were collected from a synchronized colony reared from same-aged eggs. Twenty newly emerged females were transferred on sprayed leaf discs with a brush. To prevent the escape of the mites, the dishes were sealed with Parafilm. The Petri dishes were kept at 27±1ºC, 65% RH and 16:8 h L:D photoperiod in a controlled environment room. The Petri dish was checked after 24, 48, 72 and 96 h under a stereomicroscope. Mites unable to move when touched with a brush were considered dead. To determine the lethal times (LT_{50} and LT_{90}), nearly LC_{90} concentration (within confidence limits) at 96 h (1 mg/L of Nimbecidine, 8.4% of basil and 6% of chinaberry) were sprayed as described above, and mortality results were determined every 12 h.

Oviposition deterrent tests

The methodology using Petri dishes was similar to the toxicity test, except, based on toxicity test, sublethal and lethal concentrations were used in this test: Nimbecidine (0.031, 0.125 and 0.5 mg/L), basil (1.4, 2.8 and 5.6%) and chinaberry (0.75, 1.5 and 3%). After applying the oils, a teliochrysalid female and an adult male obtained from a synchronized colony were placed onto a leaf disc (90 mm diameter). For each concentration, 12 females were monitored until 20% of the females had died (Simon, 2014). The number of eggs laid by females was determined daily and removed. The females on leaves sprayed with distilled water for Nimbecidine and with 25% ethanol for others were used as control treatments. The Petri dishes were placed with the same conditions as above.

Repellency test

Repellency bioassays were performed using a two-choice leaf disc in the Petri dish method modified by van den Boom et al. (2003). Briefly, one bean leaf disc with the same diameter within plastic Petri dishes (120 mm diameter) was then sprayed with different concentrations of Nimbecidine (0.25, 0.5 and 0.75 mg/L), basil (0.7 and 1.4%) and chinaberry (0.75, 1.5 and 3%) under the Potter precision spray tower at same settings as the toxicity test. The other Petri dish was treated with distilled water for Nimbecidine and 25% ethanol for others. After spraying, the Petri dishes were dried for 15-30 min at room temperature. Then, the two Petri dishes were connected with a silicon tube (length of each side: 5 cm, width: 1 cm). The outlet of silicon tube was in contact with the leaves so the mites could walk on to the leaves easily. Twenty newly emerged females were individually transferred into an opening in the middle of the tube and the opening was sealed with Parafilm. If the mite moved from the tube to either leaf and started feeding, the chosen leaf was recorded as the preference of the mites, 24, 48 and 72 h after spraying. The experiment was repeated three times on different days.

Statistical Analysis

Mortality percentages and fecundity decreasing percentages were corrected using control percentages with Abbott's formula (Abbott, 1925). Lethal concentrations (LC_{50} and LC_{90} values), lethal times (LT_{50} and LT_{90}) and fecundity decreasing concentrations (EC_{50} and EC_{90}) of each test materials were estimated by Probit analysis (Finney, 1971) with SPPS software (version 23, IBM Corporation, Armonk, NY, USA). Then, the logistic regression analysis was performed for 72-h exposure data. The response variable for an individual mite was then either 1 if dead, or 0 if not (JMP, version 7.0.2, SAS Institute, NC, USA). For the oviposition deterrent test, differences in egg-laying per female were tested by repeated measured variance analysis (MANOVA) followed by a post hoc Tukey's HSD test (JMP, version 7.0.2). The normality of the means was tested with the Shapiro-Wilk test using a SPPS software. Non-normal data were log-transformed before applying the MANOVA. Pearson's X^2 test was used in repellency tests. In the test, it was expressed as the null hypothesis if the number of females exhibited a 50:50 distribution across the oil and solvent sprayed surfaces in each replicate (van den Boom et al., 2002).

Results

Acute toxicity effects

Basil, chinaberry oils and Nimbecidine killed *T. urticae* females at different concentrations and in different times (Table 1). Twenty-four h after applying chinaberry oil, LC_{50} and LC_{90} were 6.29 and 9.11%, respectively. The median and high lethal concentrations were decreased significantly in both 72 and 96 h after treatment. In Nimbecidine bioassays, the LC_{50} and LC_{90} were 4.26 and 7.79 mg/L, respectively, determined 24 h after spraying. Similarly, after 48 h and 96 h, a significant decrease was recorded in these lethal concentrations. After 96 h, the LC_{50} and LC_{90} were 0.41 and 0.97 mg/L, respectively. In the basil tests, the LC_{50} and LC_{90} were 8.16 and 13.8%, respectively, 24 h after applying the oil. This lethal

concentration significantly decreased by 72 h after treatment. Eventually, after 96 h, the LC_{50} and LC_{90} were 3.19 and 9.62%, respectively. Based on simple logistic regression analyses, all test materials significantly increased the death rate of female mites compared with the control (Figure 1). Moreover, these toxic effects depended on the concentration (chinaberry X^2=238, P<0.01; Nimbecidine X^2=123, P<0.01; the basil X^2=53.5, P<0.01).

Table 1. Lethal concentrations and lethal times of chinaberry (*Melia azedarach),* Nimbecidine (*Azadirachta indica*) and basil cv. Round Midnight (*Ocimum bacillium*) on *Tetranychus urticae* females

Chinaberry n[8]	Time (hour) 24		48		72		96	
	% concentration (confidential limits)							
[1]LC_{50}	6.29 (5.65-7.24)	a[7]	5.20 (4.65-5.95)	a	4.04 (3.59-4.59)	b	2.34 (1.91-2.62)	c
[2]LC_{90}	9.11 (7.98-11.06)	a	8.31 (7.31-9.85)	a	6.94 (6.14-8.09)	a	4.65 (4.03-5.58)	b
[3]EC_{50}	1.90 (1.31-2.52)	a	0.96 (0.43-1.55)	ab	0.71 (0.01-1.28)	b	0.63 (0.43-0.83)	b
[4]EC_{90}	4.66 (3.77-6.35)	a	1.89 (1.38-3.83)	ab	1.97 (1.38-4.03)	ab	1.17 (0.95-1.57)	b
	hour (confidential limits)							
[5]LT_{50}	41.44 (37.47-45.68)							
[6]LT_{90}	72.68 (66.25-81.33)							
Nimbecidine n[8]	Time (hour) 24		48		72		96	
	mg/L (confidential limits)							
[1]LC_{50}	4.26 (2.49-27.44)	a	1.15 (0.99-1.35)	b	0.79 (0.57-1.21)	b	0.41 (0.32-0.52)	c
[2]LC_{90}	7.79 (4.40-2.71)	a	2.14 (1.84-2.59)	b	1.83 (1.35-3.05)	b	0.97 (0.79-1.28)	c
[3]EC_{50}	0.44 (0.17-0.70)	a	0.11 (0.06-0.16)	b	0.08 (0.06-0.11)	b	0.07 (0.02-0.16)	b
[4]EC_{90}	1.04 (0.76-1.77)	a	0.34 (0.26-0.51)	b	0.18 (0.14-0.27)	b	0.22 (0.14-1.35)	b
	hour (confidential limits)							
[5]LT_{50}	63.74 (57.48-70.33)							
[6]LT_{90}	107.37 (96.63-124.18)							
Midnight basil n[8]	Time (hour) 24		48		72		96	
	% concentration (confidential limits)							
[1]LC_{50}	8.16 (7.56-10.03)	a	7.61 (6.66-8.88)	a	5.40 (4.77-6.16)	b	3.19 (2.37-4.05)	c
[2]LC_{90}	13.82 (11.99-16.73)	a	13.7 (11.79-16.40)	a	11.7 (10.37-13.57)	a	9.62 (8.07-12.16)	a
[3]EC_{50}	1.77 (1.29-2.30)	a	1.28 (0.51-1.95)	ab	0.96 (0.21-1.74)	ab	0.97 (0.59-1.28)	b
[4]EC_{90}	3.93 (3.18-5.37)	a	3.56 (2.69-5.69)	ab	2.69 (1.88-5.21)	ab	2.15 (1.73-2.87)	b
	hour (confidential limits)							
[5]LT_{50}	64.53 (59.25-70.17)							
[6]LT_{90}	109.53 (100.94-120.73)							

[1] EC: Effective concentration; concentration that killed 50% of mite population; [2] Concentration that killed 90% of mite population;
[3] LC: Lethal concentration; concentration decreasing 50% of mite oviposition; [4] Concentration decreasing 90% of mite oviposition;
[5] LT: Lethal time; time in which 50% of mite population was killed with 6% of chinaberry, 1 mg/L of Nimbecidine and %8.4 of basil;
[6] Time in which 90% of mite population were killed with 6% of chinaberry, 1 mg/L of Nimbecidine and %8.4 of basil;
[7] The same letter within a row indicates no statistical differences based on confidence limits; [8] n (number of individuals)=360.

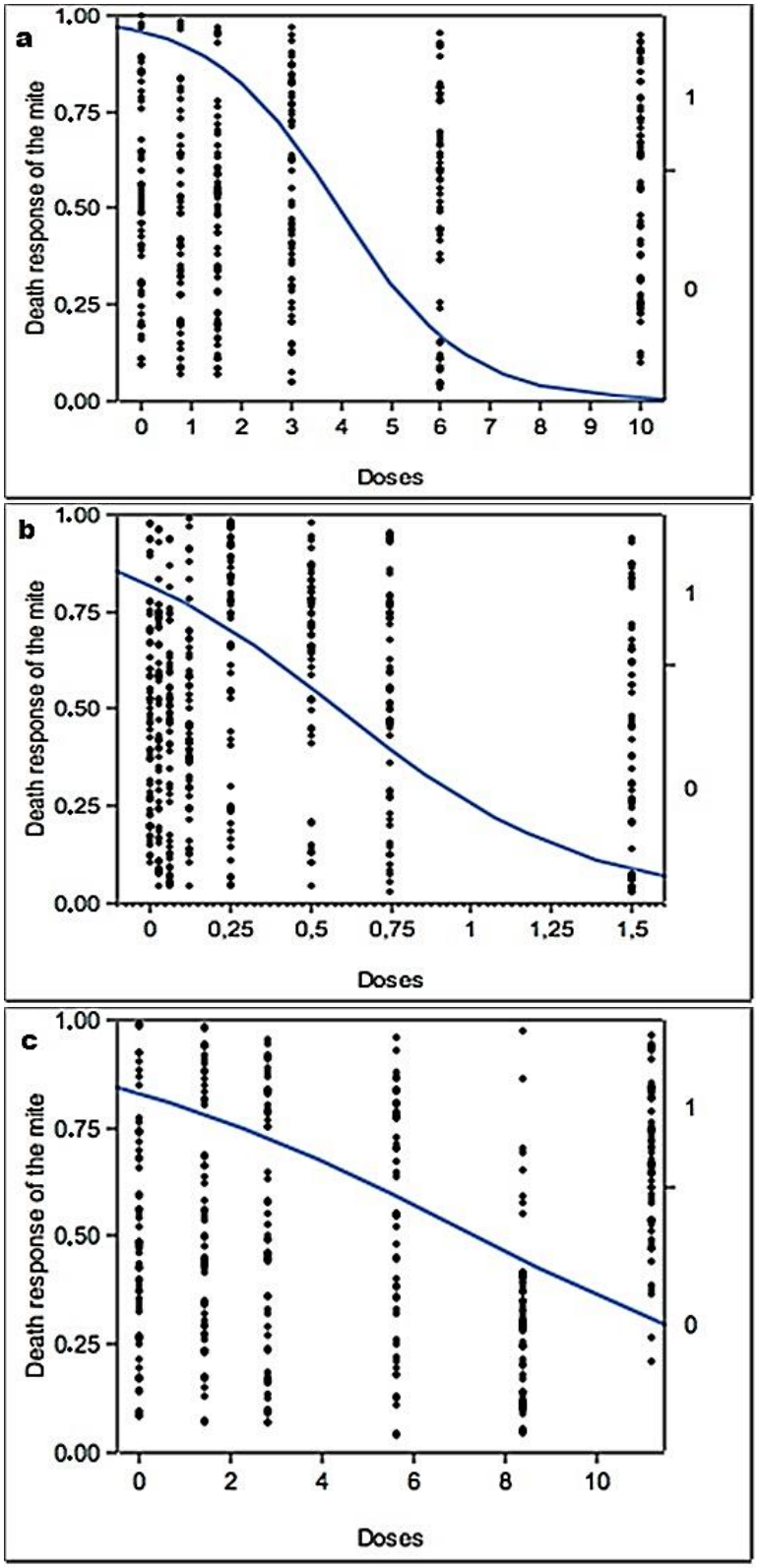

Figure 1. The logistic fit of the death response of *Tetranychus urticae* females by concentrations of a) chinaberry (*Melia azedarach*), b) Nimbecidine (*Azadirachta indica*) and c) basil cv. Round Midnight (*Ocimum bacillium*) at 72 h. "1" and "0" indicated alive and dead mites indicated, respectively.

The lethal times for determining concentrations of chinaberry, Nimbecidine and basil are summarized in Table 1. The median lethal times (LT_{50}) for chinaberry, Nimbecidine and the basil were 41.4, 63.7 and 64.5 h, respectively. The concentrations of chinaberry, Nimbecidine and basil killed 90% of the mite population after 72.7, 107 and 110 h, respectively.

Oviposition deterrent effects

The average numbers of eggs laid by females on leaves sprayed with different concentrations of the botanical oils and solvents are shown in Figure 2. After 24 h, a significant decrease was observed in the oviposition of females exposed to 3% chinaberry compared with ethanol (control). Similar significant reductions in egg-laying were recorded for females exposed to 1.5% of chinaberry, after 48 h ($F_{19,40}$=44.0, P<0.01) (Figure 2). Also, the exposure time was negatively related to the fecundity of the mite exposed to 0.75, 1.5 and 3% chinaberry (time $F_{7,7}$=40.7, P<0.01; concentration $F_{3,3}$=74.5, P<0.01). The effective concentrations (EC_{50} and EC_{90}) were estimated as 0.63 and 1.17%, respectively, 96 h after spraying chinaberry. Based on the confidence limits, the concentrations were decreased significantly after 48 h (Table 1). In Nimbecidine bioassays, significant differences between the water-treated control females and exposed to 0.125 and 0.5 mg/L Nimbecidine treated females after 24 h ($F_{24,50}$=28.3, P<0.01) (Figure 2). Additionally, exposure time for the concentrations (0.125 and 0.5 mg/L) was reduced significantly for egg-laying (time $F_{7,7}$=19.9, P<0.01; concentration $F_{3,3}$=80.4, P<0.01). The EC_{50} and EC_{90} at 96 h were 0.07 and 0.22%, respectively. Similarly, a significant decrease in the oviposition deterrent concentrations was determined after 48 h (Table 1). Similar significant differences between mean egg-laying of females on ethanol-treated and basil oil-treated leaves were found after 24 h ($F_{19,40}$=74.7, P<0.01) (Figure 2). Similar to other compounds, the exposure time for all tested concentrations was significant in terms of reducing the egg-laying (time $F_{7,7}$=16.3, P<0.01; concentration $F_{3,3}$=93.5, P<0.01). Also, EC_{50} and EC_{90} for basil at 96 h were 0.97 and 2.15%, respectively. Similarly, the oviposition deterrent concentrations were significantly lower after 48 h (Table 1).

Repellency effects

The repellency test results for different concentrations of chinaberry, Nimbecidine and basil are shown in Figures 3, 4 and 5. For each replicate, the two concentrations of chinaberry (1.5 and 3%) showed significant repellent activity at 24, 48 and 72 h (for 3% X^2=38.4, 56.6, 19.8, 33.6, 40.5, 27.4, 31.4, 42.5 and 44.4, P<0.01; for 1.5% X^2=39.1, 21.8, 16.0, 8.16, 18.4, 25.0 and 7.84; P<0.01). While two of the replicates at the 0.75% concentration displayed high avoidance of chinaberry (X^2=14.4, 23.4, 7.8, 25.0, 7.8 and 24.0; P<0.01), one showed no significant effect (Figure 3). Nimbecidine avoidance was significant for all replicates of all test concentrations (for 0.125 mg/L X^2=51.8, 70.6, 33.6, 43.6, 70.6, 36, 43.6, 70.6 and 29.2; P<0.01; for 0.5 mg/L X^2=40.9, 11.6, 21.2, 12.9, 4.8, 16.0 and 9.0, P<0.01; for 0.75 mg/L X^2=31.4, 60.8, 38.4, 21.2, 25.0, 31.4, 17.6, 25.0 and 25.0, P<0.01;), except one replicate in 0.5 mg/L concentration at 48 h (Figure 4). Avoidance rates for both concentrations of basil were significant for all replicates and times (for 0.7% X^2=100, 31.4, 11.6, 51.8, 21.2, 51.8, 11.6, 25.0 and 31.4, P<0.01; for 1.4% X^2=100, 54.8, 84.6, 100, 16.0, 60.8, 64.0, 64.0 and 81.0, P<0.01) (Figure 5).

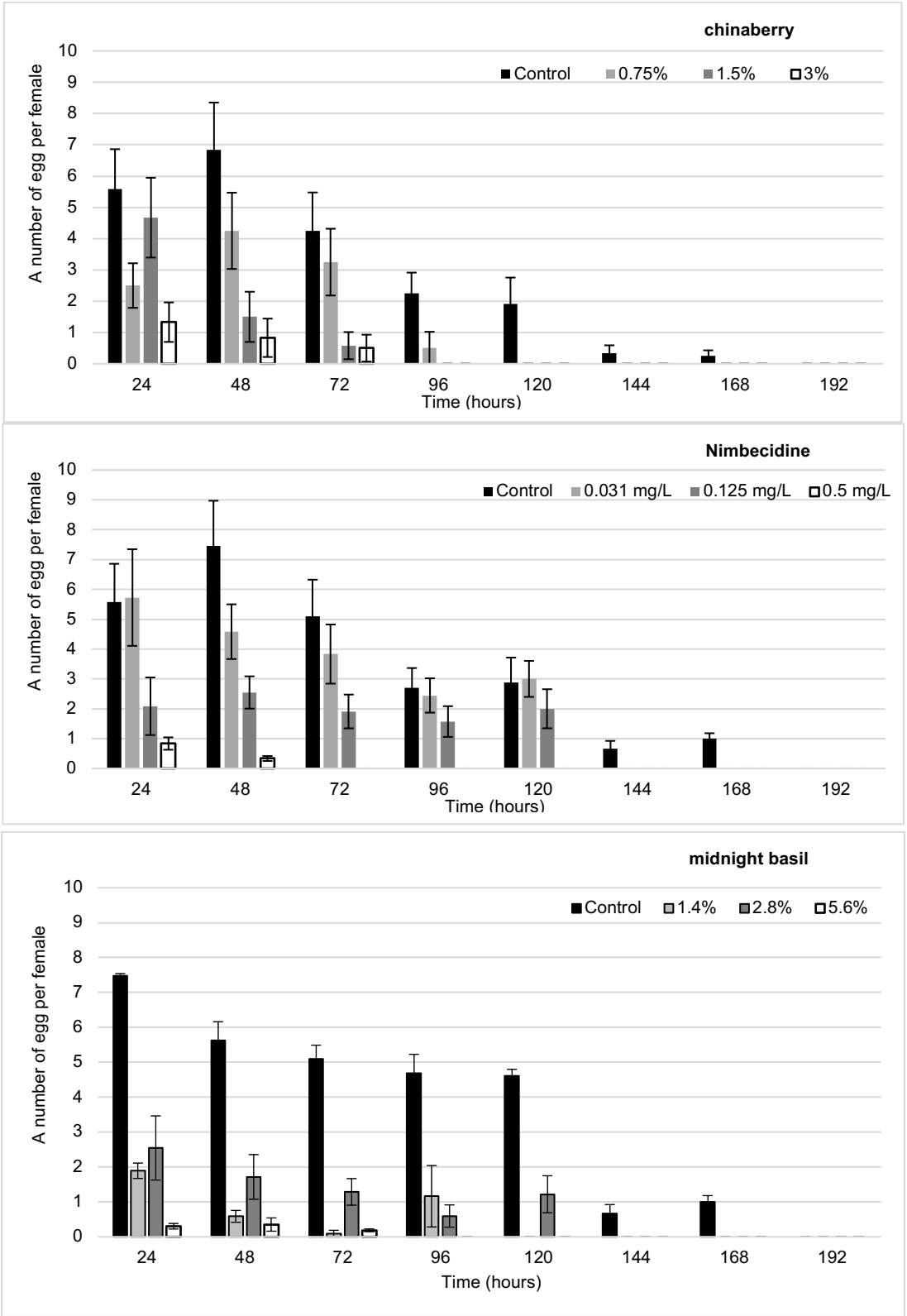

Figure 2. The egg-laying activity of *Tetranychus urticae* females at different concentrations of chinaberry (*Melia azedarach),* Nimbecidine (*Azadirachta indica*) and basil cv. Round Midnight (*Ocimum bacillium*).

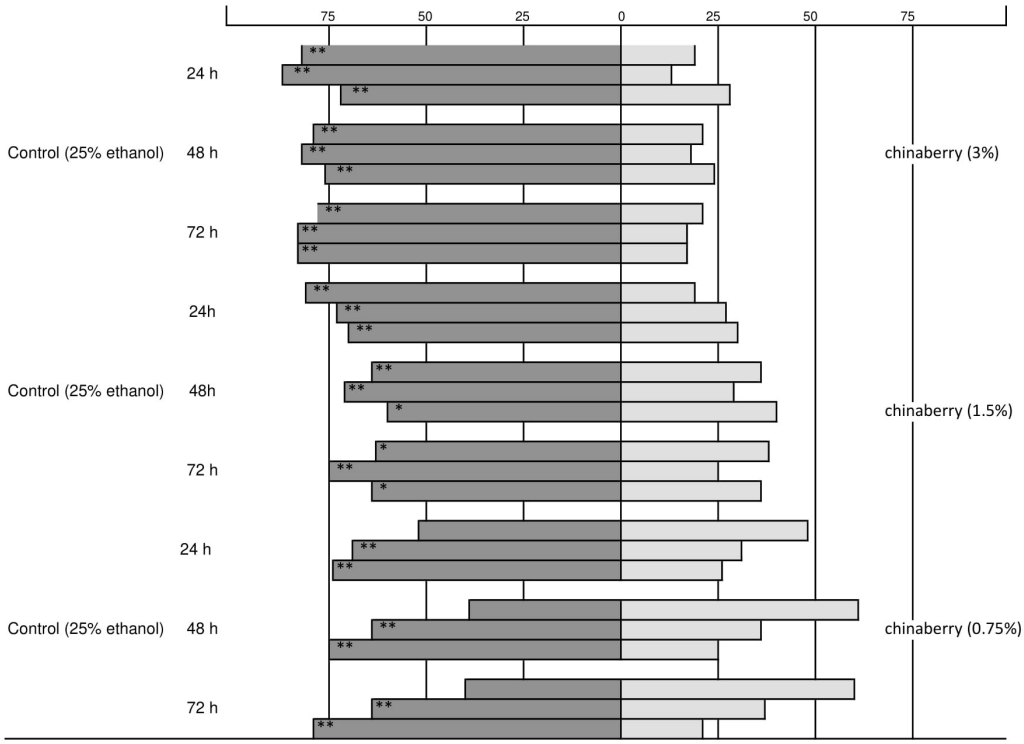

Figure 3. Percentage avoidance of the area sprayed with chinaberry (*Melia azedarach*) by *Tetranychus urticae* after 24, 48, 72 h (* $P<0.05$, ** $P<0.01$). Each column in each 24 h shows replicates for each concentration.

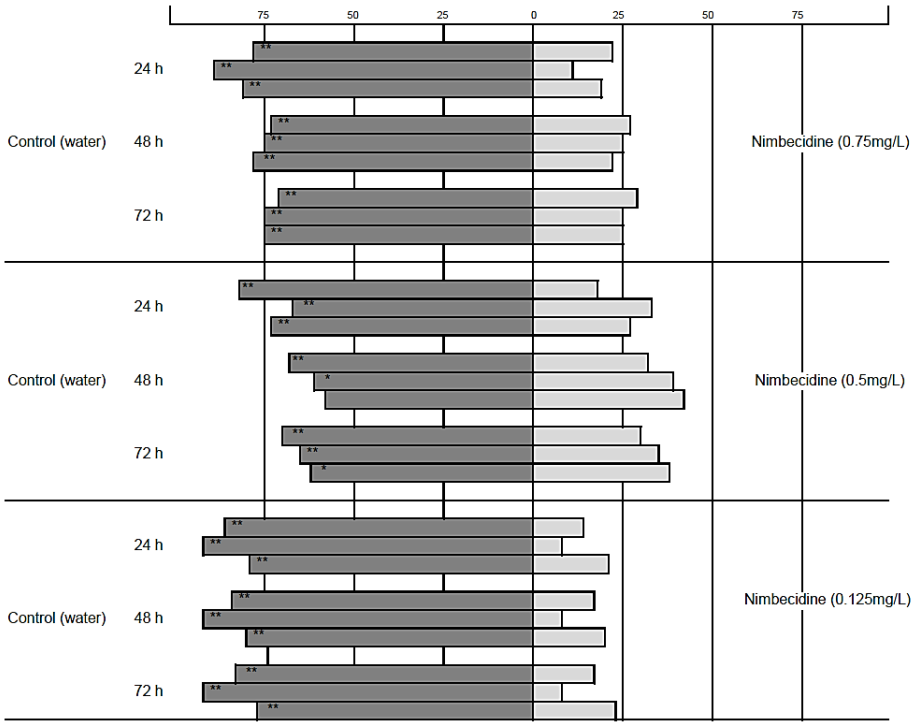

Figure 4. Percentage avoidance of the area sprayed with Nimbecidine (*Azadirachta indica*) by *Tetranychus urticae* after 24, 48, 72 h (* $P<0.05$, ** $P<0.01$). Each column in each 24 h shows replicates for each concentration.

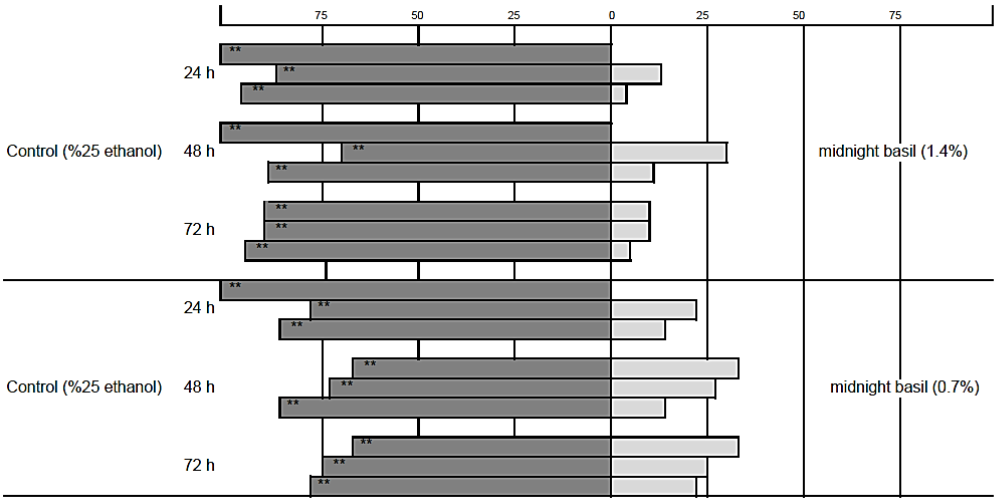

Figure 5. Percentage avoidance of the area sprayed with basil (*Ocimum bacillium*) cv. Round Midnight by *Tetranychus urticae* after 24, 48 and 72 h (* P<0.05, ** P<0.01). Each column in each 24 h shows replicates for each concentration.

Discussion

This showed that all test materials had lethal effects on *T. urticae* females depending on concentration and time. With the same conclusion, Elkertati et al. (2013) demonstrated that dichloromethane and ethanol extracts of oil obtained from chinaberry seeds were highly toxic to *T. urticae.* Similar acaricidal effects on *T. urticae* were reported by El-Sawi (2008) for an extract obtained from chinaberry leaves. An aqueous seed extract of chinaberry was found highly toxic to adults of tea red spider mite [*Oligonychus coffeae* (Nietner, 1861) (Acari: Tetranychidae)] with 56-96% mortality (Roy & Mukhopadhyay, 2012). The study demonstrated that the concentration (1.8 mg/L azadirachtin a.i.) of Nimbecidine killed 90% of *T. urticae* females 72 h after treatment. The recommended concentration (0.5 mg/L azadirachtin a.i.) of the formulation for other pests in Turkey killed 90% of *T. urticae* females at 107 h after application. With the similar results, the acaricidal effects of different formulations containing neem on *T. urticae* have also reported by numerous researchers (Sundaram & Sloane, 1995; Martínez-Villar et al., 2005; Pavela, 2009; Deka et al., 2011; Tehri & Gulati, 2014). In the present study, 9.6% basil oil killed 90% of the females within 100-121 h. Similarly, some researchers found that essential oils extracted from different cultivars of the basil had acute toxicity to *T. urticae* under greenhouse conditions (Refaat & Momen, 2002; Mateeva et al., 2003; Aslan et al., 2004; Pavela et al., 2016; Traka et al., 2018). Refaat & Momen (2002) reported that 0.5 and 2% basil oil effected *T. urticae* survival with rates of 80-100%. The main compounds contributing to significant mortality of *T. urticae* were determined to be linalool (66.5%), eugenol (18.9%) and eucalyptol (7.1%) (Traka et al., 2018).

The present study showed that all compounds had oviposition deterrent effects on *T. urticae* females depending on concentration and time. Moreover, the sublethal doses of all three compounds reduced the lifespan of *T. urticae* females compared to the control. Similarly, some other researchers have reported that seed extracts of chinaberry had deterrent effects on the fecundity of *T. urticae* (El-Sawi, 2008, Yanar et al., 2011a, b). In accordance with our results, commercial formulations of *A. indica* significantly reduced the oviposition of *T. urticae* (Sundaram & Sloane, 1995; Martínez-Villar, 2005). Our results are similar to those of some authors, who reported the negative impact of a sweet basil cultivar on the oviposition activity of the spider mite (Refaat & Momen, 2002; Pavela et al., 2016).

The two-choice experiments with *T. urticae* showed that females preferred non-oil treated surfaces over the solvent-only treated areas with sublethal concentrations of chinaberry, Nimbecidine and basil. Our results are in accordance with those of El-Sawi (2008), who showed that the extracts of the chinaberry leaves prepared with the various solvents had a repellent effect on *T. urticae*. Additionally, *A. indica* and/or its formulations were found to have repellent and antifeedant properties against some mite species, such as *T. urticae, Tetranychus cinnabarinus* (Boisd., 1867) (Acari: Tetranychidae) and *Euseius alatus* De Leon, 1966 (Acari: Phytoseiidae) (Sundaram & Sloane, 1995; Brito et al., 2006; Hummel et al., 2012). Similarly, Refaat & Momen (2002) showed that a sweet basil cultivar had repellency effects on *T. urticae*. Also, two studies showed minimal *T. urticae* preference to sweet basil volatiles, probably due to the dominant occurrence of the oxygenated hydrocarbon compounds camphor, caryophyllene oxide, cineole, methyl eugenol, limonene, myrcene, and thymol, all known insect repellents (Chokechaijaroenporn et al., 1994; Refaat & Momen, 2002).

The toxic, oviposition deterrent and repellency effects of all test materials were showed for *T. urticae* in this study. These effects could be due to the presence of certain alkaloids, terpenoids, flavonoids, other oxygenated hydrocarbon compounds that are responsible for many of the insecticidal and/or acaricidal properties of plants (Lee et al. 1991; Chokechaijaroenporn et al., 1994; Refaat & Momen, 2002; Maciel et al., 2006; López et al., 2008; Zheljazkov et al., 2008; Chiffelle et al., 2009; Elkertati et al., 2013; Martins et al., 2016; Pavela et al., 2016; Sharopov et al., 2016). Thus, the results of this study showed that the assessed concentrations of oils obtained from the basil and chinaberry compared to a commercial botanical product (Nimbecidine) have similar biological effects on *T. urticae*. The rapid degradation potential of botanical pesticides encourages the use of these acaricides for plant protection. In the future, field studies should be conducted to investigate their potential acaricidal effects under natural conditions.

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