Acaricidal efficacy of aqueous extracts from different plants on Tetranychus urticae Koch, 1836 (Acari: Tetranychidae)

Yasin Nazım Alpkent

yalpkent@hotmail.com

https://orcid.org/0000-0003-1501-1223

Selçuk Ulusoy

Sait ERTÜRK

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Abstract

The two-spotted spider mite (*Tetranychus urticae* Koch,1836, Acari: Tetranychidae) causes significant yield losses in cultivated plants, especially depending on the season. Chemical methods are the main methods of controlling this pest. However, besides the environmental, human, and animal health problems arising from chemical control, adverse effects such as resistance and residues limit the success and sustainability of chemical control. In this study, the potential acaricidal effect of aqueous extracts of *Morus rubra, Daphne odora, Ficus carica, Matricaria chamomilla*, and *Mentha pulegium* collected from Ankara and Adana provinces on *T. urticae* was investigated. For this purpose, the leaves of the plants were dried and ground and 1%, 3%, 6%, and 12% concentrations (v/v) of the extracts obtained were tested in three different application periods (1-6-day). Dipping and spraying methods were used to determine mortality and toxicity. The highest effect was 94.4% at the end of the 6th day at a 12% concentration of *M. pulegium* in the dipping method. On the other hand, the lowest mortality effect was 18.2% at the same time and concentration of *M. chamomilla*. In lethal toxicity studies, the highest toxicity was obtained from *F. carica* with an LC_{50} value of 4756 mg L^{-1}, and the lowest toxicity was obtained from *D. odora* with an LC_{50} value of 12417 mg L^{-1}. We believe that plant extracts provide a valid alternative in effectively controlling two-spotted spider mites, reducing the need for pesticide use and minimizing residues.

1. Introduction

Two-spotted spider mite, *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae), is a cosmopolite organism that harms culture plants under suitable climate conditions (Assouguem et al. 2022). *T. urticae's*, parthenogenetic reproduction, rapid generation completion (4–14 days), and polyphagous feeding have contributed to its extensive distribution in agricultural areas around the world (Cullen 2013; Assouguem et al. 2022). This important polyphagous pest has been reported to infest over 1200 host plants, including vegetables, fruits, maize, cotton, ornamentals, and weeds (Zhang 2003; Sedaratian et al. 2011).

There are many pests and pathogens in culture plants besides two-spotted spider mites; when the control methods are insufficient or improper, important economic loss is inevitable (Ojeda-Martinez et al. 2020; Inak et al. 2022). Spider mites cause 20–45% yield loss in culture plants especially according to the season (Kumaran et al. 2007). The most frequently used method used in controlling this yield loss in Türkiye and around the world is the application of chemicals. Approximately 53,672 tons of pesticides were used in Türkiye in 2020; 4.1% of these pesticides were acaricides (GDFC 2023). There are 22 registered products and preparats that have been used to control in Türkiye (GDFC 2023). Although these applications that have been carried out to prevent economic and quantity loss decrease the impact of mites, they have various negative effects on human health, environment, and resistance. Improper applications that have not been carried out according to rules and recommended doses extend the time of decomposition of molecules and cause residue. Because of such failures in using improper methods and doses, humans are exposed to pesticides left on the products (Akdoğan et al. 2012). This causes

various adverse health problems (Infante-Rivard et al. 1999; Waddell et al. 2001; Ma et al. 2002; Provost et al. 2007; Humphries et al. 2021; Poh et al. 2022)

It has reported that the intense use of pesticides caused different levels of resistance in 586 pest and mite species and 325 pesticide active ingredients (Sparks et al. 2015). Besides, the use of these chemicals affects the environment, human beings, and non-target organisms. Because of these factors, there has been an increasing demand for natural bio-actives and bio-pesticides which can quickly decompose in the last few years. They are useful alternatives that can be used in integrated pest managment programs. The plant-based pesticides are environment-friendly, and proper agents for integrated control approaches; they are generally regarded as safe (GRAS) for human health. Plant based bio-pesticides are more specific in terms of controlling pests when compared to chemical pesticides. They are also advantageous as they do not leave residue (Essiedu et al. 2020). The use of bio-pesticides in agricultural pest control processes might prevent many quality and quantity losses caused by pests (Chang et al. 2003).

These and similar methods have led people to develop more natural chemicals that can degrade in nature in a shorter period and do not harm the environment or it has led to the investigation of phytochemicals derived from existing plants. Today, it is highly important to use products that are specific to a pest, with low toxicity on humans, and have minimum side effects to beneficial organisms; additionally, products that are proper for integrated management systems have been researched by professionals in the field. One of these products is plant-extracts. Extracts derived from plants are among the most promising options for potential utilization in chemical control methods (Kasap and Kök 2019). Numerous scientific studies have been conducted to explore the potential of utilizing naturally occurring compounds in plants as an alternative to synthetic pesticides (Shi et al. 2006; Villanueva and Walgenbach 2006; Cavalcanti et al. 2010; Wei et al. 2011; Salman and Bayram 2017). Most of these studies focus on eggs, larvae-nymph, or adults (Erdogan et al. 2012; Geng et al. 2014; Salman and Bayram 2017). There are studies in the literature such as toxicity, ovicidal, repellent, and oviposition deterrent impacts (Mansour et al. 2004; El-Sharabasy 2010; Ghaderi et al. 2013), contact and fumigant toxicity (Ebadollahi et al. 2017; Salman and Bayram 2017). As such products are regarded as safe, most of them have still been researched by researcher as insecticides or acaricides (Tomczy and Szymanska 1995).

In this study, the acaricidal effect of water extracts obtained from *Morus rubra* L. (Moraceae), *Daphne odora* (Thymelaeaceae), *Ficus carica* (Moraceae), *Matricaria chamomilla* L. (Asteraceae) and *Mentha pulegium* L. (Lamiaceae) collected from Adana and Ankara provinces on *T. urticae* adults was investigated. The acaricidal activity of *D. odora*, *M. rubra*, and *F. carica* was investigated for the first time. It was also attempted to reveal the current activities of local plant species and their potential for use as acaricides.

2. Materials and Methods

2.1. Collection and rearing of *Tetranychus urticae*

Populations of *T. urticae* were collected from gardens in which fresh peppers were grown in Kapaklı Village, Çubuk, Ankara in 2022. *T. urticae* were brought to the rearing room and kept in the incubators at 26±1 °C temperature, 60±5% humidity, and 16 L: 8 D h photoperiod conditions on bean (*Phaseolus vulgaris*).

2.2. Collection of plants

Morus rubra was collected from Directorate of Plant Protection Central Research Institute, (Ankara, Türkiye) garden, while the other plant species were collected from Biological Control Research Institute, (Adana, Türkiye) garden (Table 1, Fig. 1).

2.3.Preparation of aqueous extracts

Branches and leaves of plants were collected together to obtain aqueous extracts. After that, they were brought to the laboratory and washed with tap water. Then, they were dried on filtering papers at room temperature (Whatman No: 1). Dried leaves were separated from branches and re-dried in a drying oven (Memmert UM200, Germany) at 30 °C temperature for 72 hours. The leaves were ground in a mill (Ultra-turrax t8 IKA, Germany). The grounded leaves were placed in 50 ml falcon tubes (Spinwin PP) in 200 g L^{-1} pure sterile water. They were mixed in a vibratory magnetic mixer (ThermoMixer F1.5, Germany) for 24 hours at 350 rpm speed. The obtained aqueous solution was first rotated in the centrifuge (NF 1200 R) at 10000 *g* speed for 5 minutes. After that, it was drained from the filtering paper (Whatman no 41). Finally, it was filtrated through cellulose membrane filters (0.22 µm Millex). The obtained filtrates were placed in 50 mL dark glass bottles and kept at +4 °C in the refrigerator.

2.4. Bioassays

2.4.1. Dipping method

For this purpose, the bean leaves bred in Plant Protection Central Research Institute, Physiology, and Toxicology laboratory, at 26±1 °C temperature and 65±5% relative humidity at 8h D: 16h L conditions were cut in the shape of 3 cm diameter disks. 0.01% Triton X-100 solution was prepared with pure water and plant extracts were dipped inside this solution in the shape of 1, 3, 6, and 12% (v/v). The cut bean leaves were dipped into this solution for 15 seconds. After that, they were placed into glass Petri plates (9 cm diameters) containing 1.5% agars; they were shaped in a way that the leaves' upper surfaces were upside. Leaves were kept in laminar-flow (Telstar, Japan) cabins for half an hour. The adult female individual was left on these dry leaves. Petri plates were placed in incubators that were kept at 25±1 °C temperature, 50 – 65% humidity, and 16 L: 8 D h photoperiod conditions (Sanyo MIR552, Japan). Dead and living individuals were counted on the 1^{st}, 3^{rd}, and 6^{th} days for evaluation. Orange oil (Prev-am 60 g L^{-1}) and pyridaben (Sanmite 200 g L^{-1}) were the positive control group elements. Distilled water was used in the negative control group. IRAC 004 method was modified and studies were conducted in all contact

activity experiments (IRAC 2022). Minimum 6 concentrations were used and 8 repetitions were completed for each concentration. For each repetition 10 adult female individuals were used. Experiments were established on completely random plots design.

2.4.2. Spraying method

For this purpose, cotton was placed at the bottom of the Petri plate (9 cm) and it was wetted. Bean leaves cut in the shape of a 3 cm circle were placed on wet cotton. The sides of the leaves were defined with paper towels to prevent spider mites from getting out. 0-72 hours old 25-30 adult individuals were placed on each leaf. Petri plates that were prepared were placed in Spray Tower (Spray Tower, Burkard Scientific-BS00281, England) and 2 ml (1.83 ± 0.05 mg cm^{-2}) aqueous extract solutions were sprayed into Petri plates under 1 bar pressure. Orange oil (Prev-am 60 g L^{-1}) and pyridaben (Sanmite 200 g L^{-1}) were the positive control group elements. Pure water was used in the negative control group. The Petri plates were dried for half an hour in a laminar flow cabin (Telstar, Japan). After that, they were placed in incubators that were kept at 25±1 °C temperature, 50 – 65% humidity, and at 16 L: 8 D h photoperiod conditions (Sanyo MIR552, Japan). Dead and living individuals were counted at the end of the 72^{nd} hour to determine the effect. A minimum of six concentrations were used in each extract to determine lethal concentrations, and three repetitions were carried out for each concentration. Experiments were established according to completely random plots experimental design. In both trials, mites that could not walk the length of the individuals when touched with a brush were considered as dead.

2.4.3.Data analysis

Data obtained in contact activity experiments were first transformed by arcsine transformation. One-way ANOVA was used and the Tukey test was conducted MINITAB 18 (Release 18.1) package program (α= 0.05) was used for statistical analysis. These results were compared with one another in terms of concentration and day and the efficiency of each extract was evaluated. The data obtained from each dose-response bioassay were analyzed using probit analysis according to Finney (1971), and the LC_{50} and LC_{90} values were estimated utilizing the Polo-Plus (LeOra software 1994).

Results

Effect of dipping method on mortality of *Tetranychus urticae* adults

It was observed that the dose and time increase resulting from the application of plant extracts of *T. urticae* with the dipping method increased the mortality of adult individuals (Table 2). Among the studied extracts, the highest impact was in *M. pulegium* aqueous extract with 12% concentration at the end of the 6^{th} day. The mortality was 94.4% (F=23.71; df= 4, 19; P<0.05). In the same plant extract with 12% concentration, the mortality was 62.9% on the first day and 90.5% on the third day. The lowest effect was recorded in 1% concentration after one-day exposure was 15.4% (F=44.36; df= 4, 19; P<0.05). According to the trials, the highest mortality was in *F. carica* extract with 12% at the end of 6-day of application led

to 82.2% mortality (F=8.67; df= 4, 19; P<0.05). At the same concentration the morality after the first and third-day application were 46.2% and 75.0% respectively.

Among the *M. rubra* extracts collected from Ankara, the highest mortality was observed in 12% concentration, 6-day application; the mortality was 70.1% (F=37.22; df= 4, 19; P<0.05). The lowest mortality was in 1% concentration with 1-day application; the mortality recorded as 28.3% (F=59.34; df= 4, 19; P<0.05). The highest dose and exposure time in *D. odora* and *M. chamomilla* extract applications were caused 27.2% and 18.2% mortality, respectively. According to all of the studies, the extract that had the lowest impact was *M. chamomilla* with 1% concentration and 1-day application; the mortality was 4.7% (F=2.76; df= 4, 19; P>0.05). In the positive control group, 3-12% concentrations of pyridaben caused 100.0% mortality in all application periods. The lowest concentration of pyridaben application on *T. urticae* adults varied between 76.4% and 85.2% in all three application times. In the lowest concentration (1%) of Prev-am, which is formulised of orange oil, the mortality was 100.0% at the end of one day application. This showed that it had the highest impact of all the products studied (Table 2).

Determination of lethal doses by spray application

There were different sensitivity values in terms of the response of two-spotted spider mites to five different spraying applications (Table 3). Among the plant extracts, the aqueous extract of *F. carica*'s LC_{50} value on *T. urticae* was calculated as 4756 mg L^{-1}; this indicated that it had the lowest LC_{50} value among the other aqueous extracts. This value was followed by *M. pulegium with* 5211 ppm*, M. rubra with* 6767.8 mg L^{-1}, *M. chamomilla with* 8271 mg L^{-1}, and *D. odora with* 12417 mg L^{-1} respectively. The lowest LC_{50} and LC_{90} value was in Prev-am preparate with 556.9 and 1811.3 mg L^{-1} doses.

Discussion

In this study, the toxicity of water extracts from five plants collected from Ankara and Adana provinces, as well as two synthetic acaricides (Sanmite and Prev-am), was assessed against adult *T. urticae*. Plant extracts are considered as an ideal option for controlling pests in agricultural production as they contain secondary metabolites that have anti-feeding, fumigant, larvicidal, ovicidal, adulticidal, oviposition deterrence, repellent, and egg-laying inhibitory effects to prevent economic losses in the control of pests, as well as low toxicity to mammals, fish, and humans (Liu et al. 2006; Tomczyk and Suszko 2011; Mozaffari et al. 2013; Kök et al. 2016; Tavassoli et al. 2018; Hassan et al. 2021). The results of our study demonstrate that the extracts of *M. pulegium* exhibited notable toxic effects after 6-day exposure, adult stages of *T. urticae* (Table 2). In addition, the LC_{50} value of *M. pulegium* against *T. urticae* was determined as 5211 ppm after a 72-hour application and presented in Table 3. Due to limited reports on the effectiveness of Mentha extract in comparison to Mentha essential oil, some studies have referenced both to address this gap. Hassan et al. (2021) reported that the leaf extract of *M. longifolia* exhibited adulticidal properties against *T. urticae*, with a mean lethal concentration (LC_{50}) value of 5114 ppm after a 48-hour application. In addition, the LC_{50} values of *M. longifolia* against females of *T. urticae* by

fumigant application, was 3.74 µl/L, while the LC_{90} values was 11.01 µL/L (Hassan et al. 2021). Reddy and Dolma (2018) found that *M. longifolia* and *M. piperita* essential oils were more toxic to *T. urticae* among the eleven essential oils tested under laboratory conditions and their LC_{50} values were 11.08 and 15.86 mg/L air, respectively. Mozaffari et al. (2013) demonstrated that the essential oils of *M. pulegium* exhibited significant toxicity against both eggs and adults of the two-spotted spider mite, with LC_{50} values of 2.57 and 2.25 µL L^{-1} for eggs and adults, respectively. Cetin et al. (2006) determined that the ethanolic extracts of *M. pulegium* exhibited a toxicity level of LC_{50} 81 mg L^{-1} against the third and fourth instar larvae of the house mosquito *Culex pipiens* L. (Diptera: Culicidae). Mozaffari et al. (2012) revealed that ethanolic extracts of *M. pulegium* was toxic to values of LC_{50} 59.149 mg/L after 24 h application against *T. urticae*. Sayeda Farghaly et al. (2009) employed formulated extracts of *M. microphylla* to target nymphs of the whitefly, *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae), and *Aphis craccivora* Koch. (Hemiptera: Aphididae). Their findings revealed that the formulated extract exhibited enhanced efficacy compared to the crude extract, indicating its superior effectiveness in controlling the targeted pests. As stated by Kouninki et al. (2005), the difference in activity between plant species was considered to be related to differences in the chemical composition and proportions of the main components present in the extracts. In addition, the presence of secondary metabolites in the plant, the type of extraction, and the plant parts from which essential oils are obtained are thought to play an important role in biological activity.

Ficus carica content consists of alkaloids, flavonoids, coumarins, saponins, sterols and terpenes (Mawa et al. 2013). Given the scarcity of literature on the efficacy of *F. carica* in controlling *T. urticae*, several species within the Ficus genus were explored as potential alternative options. In this study, the water extract of *F. carica* was found to have the second highest toxic effect with a mortality of 82.2% for *T. urticae* adults at a 6-day exposure and 12% concentration (Table 2). Kim et al. (2005) reported mortality of 11.8-17.2% and 24.4-28.7% for *T. urticae* adults after 24 and 48 hours of exposure to the methanolic extract obtained from the leaves and twigs of *F. erecta*, respectively. In a study conducted by Romeh (2013), the hydrodistillation extract obtained from the leaves of *F. sycomorus* L. demonstrated contact and fumigant LC_{50} values of 0.35 and 0.129%, respectively, against *T. urticae*. In terms of mode of action, different researchers have reported that fumigation is a more toxic method than the contact toxicity (Park et al. 2003; Soylu et al. 2006; Rajendran and Sriranjini 2008; Ma´rio et al. 2012). The high toxicity of bioassays in the fumigation method is attributed to the penetration of oil vapors into the respiratory system. Although this study provided experimental results for contact toxicity such as leaf dipping and spraying method, there is a need for research on the fumigant toxicity of *F. carica* extract. When the result obtained and other studies are evaluated, it is thought that the effect increases with the increase in concentration and duration. Ulusoy et al. (2019) reported that when the toxic effects of aqueous leaf extract of *F. carica* on *Aphis gossypii* Glover (Hemiptera: Aphididae) were observed, the fastest and greatest toxic effect after malathion was found in 20% *F. carica* extract, which gave 75.6% mortality. Although acetylcholinesterase (AChE) and carboxylesterase (CE) activity, the enzymes responsible for detoxification, were not examined in this study, Ulusoy et al. (2019) reported that high mortality in *A. gossypii* were due to the suppression of these two enzymes. According to Wink and Schimmer (1999),

the diverse range of toxicity levels and enzyme inhibition effects observed in response to plant extracts can be attributed to the presence of various substances such as flavonoids, terpenes, phenols, alkaloids, sterols, waxes, fats, tannins, sugars, gums, suberins, resin acids, and carotenoids. Furthermore, the concentrations of these components within an organism also play a significant role in determining the extent of their effects.

This study represents the preliminary investigation into the acaricidal activity of *M. rubra* against *T. urticae.* Memete et al. (2022) reported that although the phytochemical components of *M. rubra* were predominantly phenolic compounds, it consists of anthocyanins, flavanols, quercetin and kaempferols. Also, it is found that the nonpolar extract of *M. nigra* contains a variety of substances including steroids, terpenes, acetophenones, aglycone, waxes, sapogenins, iridoids, and sesquiterpenes, all of which are highly lipophilic in nature (Cechinel-Filho and Yunes 1998). Dantas et al. (2017) revealed that the acaricidal activity of the *M. nigra* extract was predominantly observed in the chloroform phase, exhibiting a 65.44% increase in efficacy at a concentration of 25 mg/ml against *Rhipicephalus microplus* (Ixodida: Ixodidae). It is hypothesized that the secondary metabolites of *M. rubra*, as previously demonstrated, could potentially impact *T. urticae*. However, to determine which component is responsible for this activity, further component analyses are needed, and it is suggested to test extracts obtained not only with water extraction but also with polar and non-polar extraction methods.

Matricaria chamomilla, commonly known as chamomile, contains over 120 chemical constituents as secondary metabolites. These include terpenoids, flavonoids, and trace amounts of monoterpenes, which have demonstrated significant biological effects. Chamomile has been well-studied and shown to possess antioxidant, antibacterial, antifungal, anti-parasitic, insecticidal, anti-diabetic, anti-cancer, and anti-inflammatory properties (Singh et al. 2011; El Mihyaoui et al. 2022). In the study conducted by Abd El-Moneim et al. (2012), the essential oil of *M. chamomilla* was extracted using the hydrodistillation method. After a 24-hour application period, a concentration of 4% resulted in 100.0% mortality against *T. urticae.* Elhalawany and Dewidar (2017) found that chamomile flower extract exhibited LC_{50} values of 0.63, 0.51, and 0.44 after 24, 48, and 72 hours of exposure, respectively, against *T. urticae* individuals. Similarly, Afify et al. (2009) demonstrated that a 4% concentration of chamomile essential oil extract resulted in 100% mortality of adult *T. urticae* after 24 hours of application. Kawka (2004) reported that acaricidal effect of chamomile extracts from fresh and dried flowers on *T. urticae*. However, the highest mortality obtained from the *M. chamomilla* extract in this study did not exceed 18.2% after 6-day exposure. These findings are inconsistent with the results of the aforementioned research, which could be attributed to the extraction of the extracts from different parts of the plant. Furthermore, in other studies, increasing the application duration and dosage has shown a positive effect on mortality (Al-Mekhlafi et al. 2020; Elfaki Ibrahim and Al-Khalifa 2021). Therefore, more comprehensive and detailed studies are needed regarding the high-dose and longer-exposure applications of *M. chamomilla* aqueous leaf extract against *T. urticae*.

It has been reported that the chemical content of *D. odora* consists of flavonoids, coumarins, terpenoids, steroids, and lignan compounds (Tosun 2006). There is limited scientific research examining the

acaricidal activity of *D. odora* against *T. urticae* and this study is the first to demonstrate its potential efficacy in controlling or eliminating *T. urticae* infestations. However, Ulusoy et al. (2019) found that aqueous leaf extracts (%20) of *D. odora* was toxic to *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) with 62.2% mortality after 72 h exposure time. Inamori et al. (1987) isolated daphnodorin A, daphnodorin B and daphnodorin C from the roots and bark of *D. odora*. Although these isolated flavanoids had no significant insecticidal effects, compound B showed weak acaricidal activity against *Tetranychus urticae* with 45% mortality at a dose of 500 ppm. Similarly, this study found that the toxic effect of *D. odora* was even 27.2% mortality even at a concentration of 12% after 6 days of exposure (Table 2). When this study is evaluated together with the studies mentioned above, it is concluded that compounds or extracts obtained from *D. odora* leaves or other parts have weak lethal properties.

In conclusion, the findings of this study demonstrate the acaricidal effects of extracts obtained from *M. rubra, D. odora, F. carica, M. chamomilla*, and *M. pulegium* plants on *T. urticae*. These results highlight the potential of using plant-based pesticides as an alternative to conventional chemical control methods. Given the increasing concerns about the negative impacts of pesticides on human health and the environment, the utilization of herbal extracts presents a promising approach for sustainable pest management in agriculture. Further research and implementation of these natural compounds can contribute to the development of safer and more environmentally friendly strategies for controlling *T. urticae*, while ensuring the protection of human and environmental health.

Declarations

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Author contribution

YNA: Supervision, Conceptualization, Methodology, Investigation, Writing—original draft, SE: Conceptualization, Investigation, Writing—original draft, Supervision, Data curation, SU: Methodology, Writing—review and editing.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

The authors declare that they have no conflict of interest

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Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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Tables

Table 1. Data of sampled preparations and collection date

Plants	Locations (district/province)	Date of collection
Morus rubra	39°57'18.9"N 32°48'25.6"E (Yenimahalle/ Ankara)	2022/6
Mentha pulegium	37°00'38.0" N, 35°20'23.7" E (Yüreğir/ Adana)	2022/6
Daphne odora	37°00'38.0" N, 35°20'23.7" E (Yüreğir/ Adana)	2022/6
Ficus carica	37°00'38.0" N, 35°20'23.7" E (Yüreğir/ Adana)	2022/6
Matricaria chamomilla	37°00'38.0" N, 35°20'23.7" E (Yüreğir/ Adana)	2022/6

Table 2. Effect of different concentrations with dipping method on the mortality of adult *Tetranychus urticae*

Plant	Concentration (%)	Exposure Time		
		1. day	3. day	6. day
Matricaria chamomilla	1	4.7±2.3Aa*	8.3±0.7Aa	8.3±0.7Aab
	3	4.7±2.3Aa	12.4±0.2Aa	13.7±0.14Aa
	6	7.3±0.3Ba	12.4±0.2ABa	13.7±0.1Aa
	12	8.6±0.2Aa	16.9±1.2Aa	18.2±1.2Aa
	control	1.3±0.3Aa	1.3±0.3Ab	1.7±1.3Ab
Mentha pulegium	1	15.4 ± 1.7Ac	30.6 ± 1.9Ab	31.8 ± 2.5Ab
	3	32.1 ± 1.7Abc	56.5 ± 1.7Ab	59.6 ± 3.7Ab
	6	41.2 ± 0.1Bab	62.7 ± 1.0Aab	66.9 ± 2.2Aab
	12	62.9 ± 2.0Aa	90.5 ± 7.0Aa	94.4 ± 8.9Aa
	control	0.0 ± 0.0Ad	1.3 ± 0.3Ac	1.3 ± 0.3Ac
Daphne odora	1	10.9±0.7Aa	13.5±0.5Aa	14.8±0.3Aa
	3	15.4±1.7Aa	18.6±0.4Aa	19.8±0.5Aa
	6	18.6±0.4Aa	22.3±0.4Aa	25.0±0.0Aa
	12	23.4±1.0Aa	27.2±0.9Aa	27.2±0.9Aa
	control	1.3±0.3Ab	1.3±0.3Ab	1.3±0.3Ab
Morus rubra	1	28.3±1.2Ab	41.0±1.4Ab	42.2±1.7Ab
	3	41.1±0.8Aab	52.5±0.8Aab	53.8±1.1Aab
	6	43.7±1.1Aab	56.3±0.7Aab	59.0±1.7Aab
	12	50.0±0.3Ba	68.8±0.1Aa	70.1±0.2Aa
	control	0.0±0.0Ac	1.7±1.3Ac	1.7±1.3Ac
Ficus carica	1	15.3 ± 4.3Abc	30.6 ± 8.8Ab	33.3 ± 12.0Aab
	3	25.9 ± 1.0Aab	46.2 ± 1.8Aab	49 ± 3.4Aa
	6	36 ± 1.3Bab	61.5 ± 1.0Aab	65.7 ± 2.5Aa
	12	46.2 ± 2.1Aa	75.0 ± 6.5Aa	82.2 ± 11.7Aa
	control	2.6 ± 0.7Ac	2.6 ±0.7Ac	2.6 ± 1.9Ab
Prev-am	1	100.0 ± 0.0Aa	100 ± 0.0Aa	100 ± 0.0Aa
	3	100.0 ± 0.0Aa	100.0 ± 0.0Aa	100.0 ± 0.0Aa

	6	100.0 ± 0.0Aa	100.0 ± 0.0Aa	100.0 ± 0.0Aa
	12	100.0 ± 0.0Aa	100.0 ± 0.0Aa	100.0 ± 0.0Aa
	control	0.0±0.0Bb	0.0±0.0Bb	0.0±0.0Bb
Sanmite	1	76.4 ± 0.3Ab	85.2±0.3Ab	85.2±0.3 Ab
	3	100.0±0.0Aa	100.0±0.0Aa	100.0±0.0Aa
	6	100.0±0.0Aa	100.0±0.0Aa	100.0±0.0Aa
	12	100.0±0.0Aa	100.0±0.0Aa	100.0±0.0Aa
	control	0.0±0.0Bc	1.7±1.3Bc	1.7±1.3Bc

* Different uppercase letters in the same row and different lowercase letters in the same column indicate a significant difference according to extracts and application doses, respectively ($p \leq 0.05$).

Table 3. Lethal concentration values of different extracts on *Tetranychus urticae* (72h)

Extracts	n[a]	Slope+SE[b]	LC_{50} (mg a.i./L) (0.95 CL[c])	LC_{90} (mg a.i./L) (0.95 CL)	x^2(df)[d]	*P* values
Matricaria chamomilla	480	1.1 ± 0.2	8271 (6055 – 12309)	>100000 (52624 – >100000)	19.3(17)	0.3
Ficus carica	480	1.5 ± 0.4	4756 (2809 – 19858)	32483 (10559 – >100000)	3.9(17)	0.9
Daphne odora	482	0.7 ± 0.2	12417 (4153.5 – >100000)	>100000 (71512- >100000)	7.4(17)	0.9
Morus rubra	480	0.9 ± 0.2	6767.8 (4902.8 – 9773.6)	>100000 (64659 – >100000)	11.6(17)	0.8
Mentha pulegium	480	1.5 ± 0.2	5211 (4060 – 6652)	36295 (23052 – 76438)	19.8(17)	0.2
Prev-am	480	2.5 ± 0.2	556.9 (486.9 – 634.8)	1811.3 (1457.7 – 2450.5)	14.8(17)	0.6
Sanmite	480	2.1 ± 0.2	2942 (2508 – 3517)	11798 (8747 - 17963)	6.3(17)	0.9

[a] n: Total number of mites

[b] SE: Standart error

[c] CL: Confidence limits

[d] Chi-square value and degrees of freedom