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Original article (Orijinal araştırma)

Contact toxicities of some plant extracts in Apiaceae family on different developmental stages of *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae)

Apiaceae familyasındaki bazı bitki ekstraktlarının *Tetranychus urticae* Koch (Acari: Tetranychidae)'nin farklı gelişme dönemleri üzerine kontakt toksisiteleri

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Summary

The contact toxicities of extracts of anise (*Pimpinella anisum* L.), coriander (*Coriandrum sativum* L.), cumin (*Cuminum cyminum* L.), dill (*Anethum graveolens*) and fennel (*Foeniculum vulgare* Mill.) plants, included in the Apiaceae, prepared with water on different stages of *Tetranychus urticae* Koch, 1836*,* were studied. The study was conducted in a laboratory and insect breeding rooms between in 2015-2016. Leaf disc - spray tower method was used for the purpose of determining toxic effects of the plant extracts. The effect of plant extracts on adult and protonymphs stages of *T. urticae* were determined. Also, the effects of plant extracts were determined on the hatching of *T. urticae* eggs. Four different concentration of extracts were tested;1, 3, 6 and 12%. The experiments consisted of one control and four replicates for each concentration. Fifteen individuals were included in each repeat. Dead-live counts were made 1, 3 and 6 days after treatment to determine contact effects of the extracts. It was found that the contact effects of plant extracts on *T. urticae* adult and protonymphs increased with rising concentration, caused 100% death on *T. urticae* adults and nymphs at 12% after 6 days. Also, the greatest effect on *T. urticae* egg hatching was in dill extracts with mortality of over 91%. It is considered that these plant extracts may constitute an alternative acaricides for control *T. urticae*.

Keywords: Apiaceae, plant extracts, *Tetranychus urticae*, toxicity

Özet

Apiaceae familyası içerisinde yer alan anason (*Pimpinella anisum* L.), dere otu (*Anethum graveolens*), kimyon (*Cuminum cyminum* L.), kişniş (*Coriandrum sativum* L.) ve rezene (*Foeniculum vulgare* Mill.) bitkilerinin su ile hazırlanan ekstraktlarının *Tetranychus urticae* Koch, 1836' nin farklı dönemleri üzerinde değme yoluyla toksisiteleri araştırılmıştır. Çalışma 2015-2016 yılları arasında laboratuvar ve böcek üretim odalarında yürütülmüştür. Bitki ekstraktlarının zehir etkilerini belirlemek amacıyla, yaprak disk-ilaçlama kulesi yöntemi kullanılmıştır. Çalışmalarda bitki ekstraktlarının *T. urticae*' nin ergin ve protonimf dönemlerine etkisi belirlenmiştir. Ayrıca bitki ekstraktlarının *T. urticae*' nin yumurta açılımı üzerine olan etkileri de belirlenmiştir. Bitki ekstraktlarının %1, 3, 6, 12 olmak üzere dört farklı konsantrasyonu kullanılmıştır. Denemeler bir kontrol ve her konsantrasyon için dört tekerrür olarak yürütülmüştür. Her tekerrürde 15 birey kullanılmıştır. Bitki ekstraktlarının kontakt etkilerini belirlemek amacıyla ölü-canlı sayımları 1, 3 ve 6. günlerde yapılmıştır. Çalışmada bitki ekstraktlarının *T. urticae* ergin ve nimfleri üzerindeki kontakt etkilerinin konsantrasyon miktarı artışına bağlı olarak yükseldiği, altıncı gün sayım sonuçlarına göre bitki ekstraktlarının %12 konsantrasyonunda *T. urticae* ergin ve protonimflerinde %100 ölüme neden olduğu belirlenmiştir. Ayrıca *T. urticae* yumurta açılımı üzerinde en yüksek etki %91 ile dere otu ekstraktında belirlenmiştir. Çalışma sonucunda, kullanılan bitki ekstraktlarının *T. urticae* ile mücadelede sentetik akarisitlere alternatif oluşturabileceği düşünülmektedir.

Anahtar sözcükler: Apiaceae, bitki ekstraktı, *Tetranychus urticae*, toksisite

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Introduction

Tetranychus urticae Koch, 1836 (Acari: Tetranychidae), two-spotted spider mite, causes losses in many agricultural crops of economic importance (Zhang, 2003; Hoy, 2011; Vacante, 2016). *Tetranychus urticae* causes necrotic stain formation as the result of leaf damage, and in high populations it causes drying and leaf fall (Brandenburg & Kennedy, 1987). *Tetranychus urticae* damages more than 1100 plants in 126 families (Migeon & Dorkeld, 2017). To control *T. urticae,* various acaricides and insecticides with extensive effects are used to reduce losses in cultivated plants (Van Leeuwen et al., 2006). *Tetranychus urticae* can develop resistance against insecticides and acaricides as the result of frequent application at short intervals because of its rapid haplodiploid sexual reproduction and short life cycle (Song et al., 1995; Van Leeuwen et al., 2009, 2015).

In recent years, natural pesticides have gained importance as alternatives to synthetic insecticides because of disadvantages of chemical control (Feng & Isman, 1995; Laborda et al., 2013). There have been many studies on the possibility of using compounds found naturally in the plants as an alternative to synthetic pesticides (Shi et al., 2006; Villanueva & Walgenbach, 2006; Cavalcanti et al., 2010; Wei et al., 2011). The studies put emphasis on the compounds obtained from the plants that do not put additional toxic materials into the environment, breakdown in a short span of time, and do not cause soil and water contamination (Liang et al., 2003; Isman & Akhtar, 2007). There have been many studies on the effects of extracts from plants obtained by various methods on *T. urticae* (Choi et al., 2004; Matinez-Villar et al., 2005; Rasikari et al., 2005; Chermenskaya et al., 2010).

An extract of *Satureja hortensis* L. (Lamiaceae) was toxic to *T. urticae* (Aslan et al., 2004). Similarly, extracts of neem (Meliaceae) (Matinez-Villar et al., 2005), some species of Solanaceae (Rasikari et al., 2005), *Capparis aegyptia* Lam. (Capparaceae) (Hussein et al., 2006) and *Nerium oleander* L. (Apocynaceae) (Islam et al., 2008) were found to be effective against *T. urticae*. Kumral et al. (2010) reported that ethanol extracts of *Datura stramonium* L. (Solanaceae) leaves and seeds exhibited acaricidal effects against *T. urticae*. However, there have been no studies in Turkey and only a few elsewhere to determine toxic effects of extracts obtained from the plants in the Apiaceae. It is known that some compounds in plants from the Apiaceae have insecticidal and repellent effects on insects (Regnault-Roger et al., 2005; Isman, 2006). Chermenskaya et al. (2010), determined that extracts of *Angelica tschimganica* (Korov.), *Conium maculatum* L., *Dorema microcarpum* Korov., *Ferula foetidissima* Regel. & Schmalh., *Heracleum dissectum* Ledeb., *Mediasia macrophylla* (Regel. & Schmalh.) plants within the Apiaceae have an average toxic effect on *T. urticae*. However, in this study, the extracts were prepared with an organic solvent. However, we postulate that the water based extracts cold have different effects on *T. urticae*. Therefore, in this study, contact toxicities of water-based leaf extracts of five plants within the Apiaceae on different developmental stages of *T. urticae* has been investigated in laboratory conditions to provide an alternative to synthetic insecticide and acaricide.

Material and Methods

Tetranychus urticae culture

The study was conducted in a laboratory and insect breeding rooms at Suleyman Demirel University, Faculty of Agriculture, Plant Protection Department between in 2015-2016. *Tetranychus urticae* susceptible population (German Susceptible Strain, GSS) was brought from Rothamsted Experimental Station (UK) to insect cultivating cabins in Süleyman Demirel University in 2001 and has been cultivated since without exposure to any pesticides. The GSS population was cultured on bean (*Phaseolus vulgaris* L. cv. Barbunia) in climate chambers at 26±2ºC, 60±5% RH and 16 h photoperiod.

Plant species investigated

Leaves from five species in the Apiaceae, anise (*Pimpinella anisum* L.), coriander (*Coriandrum sativum* L.), cumin (*Cuminum cyminum* L.), dill (*Anethum graveolens*) and fennel (*Foeniculum vulgare* Mill.) were used to make extracts investigated in this study.

Plant extract preparation

The method of Rezaei & Yarnia (2009) was used to the prepare the plant extracts. Plant material, leaves harvested in flowering phase, were dried for two weeks at room temperature. The dried leaves were pulverized in a blender. Milled material (20 g) from each plant was steeped in 100 mL distilled water for 24 h and then filtered with through cheesecloth. These extracts were transferred to falcon tubes and centrifuged for 5 min at 9,000 rpm then filtered through Whatman filter papers.

Contact toxicity bioassays

The method of Erdogan et al. (2012) was used for testing the contact toxicities of the plant extracts on eggs, protonymphs and adults of *T. urticae*. Concentrations of 1, 3, 6, 12% plant extracts were tested on protonymphs and adults by a leaf disc-spray tower method. In addition, the ovicidal effect of plant extracts on *T. urticae* eggs were evaluated. Fifteen adult females were put on bean leaf discs in 9-cm Petri dishes to obtain eggs, protonymphs and adults at the same stage of development eggs left in the dishes for 24 h. Bioassays with one control (water only) and the four concentrations of each extract were replicated four times. Fifteen individuals were included in each replicate. Triton X 100 at 0.01% was added to the pure water in which extracts were prepared and was also used in the water control as an extender and sticker. The plant extracts are applied as 2 mL on the leaf surface in 1 atm in a spray tower (Kumral et al., 2010). Contact effects of plant extracts were determined by dead-live counts of *T. urticae* nymphs and adults after 1, 3 and 6 days. For the egg hatching experiment, observations continued until all eggs in the control group had hatched.

Statistical analysis

Percentages of dead mites obtained from the contact experiments were calculated according to Abbott formula (Abbott, 1925) and arcsin transformed (Zar, 1999). Contact effects were analyzed by three-way repeated measures ANOVA. Ovicidal effects were analyzed by two-way ANOVA and Tukey's test was used to determine the difference between means ($p < 0.05$).

Results

Contact toxicities of plant extracts on adult period of *T. urticae* are shown in Table 1. The contact effects of the water extracts of all five plants was increased with concentration. The greatest effect occurred after 6 days. The greatest effect on adults after 1 day was with coriander extract at 12% and this was significantly different from other plant extracts ($p < 0.05$). The greatest effect after 3 days was with anise extract and 100% mortality was recorded. On that day, the effects of extracts of fennel, coriander, cumin and dill (in that order) were less than that of the anise extract. After 6 days, all plant extracts cause 100% adult mortality and therefore were not statistically different ($p < 0.05$).

Contact toxicities of plant extracts on *T. urticae* nymphs are shown in Table 2. After 1 day, the greatest effect was with fennel at 12%, which caused 100% mortality, and was significantly different from the other extracts ($p <0.05$). On that day, the effects of extracts of cumin, coriander, anise and dill (in that order) were less than that of fennel extract. After 3 days, 100% mortality of nymphs occurred with fennel extract at 6 and 12%. After fennel, the extracts of coriander, cumin and dill were also effective. All five extracts cause 100% mortality in nymphs after 6 days. Especially, all concentrations of fennel extract caused 100% mortality in *T. urticae* nymphs. After 6 days, contact effects of all plant extracts were therefore not statistically different ($p<0.05$).

Table 1. Contact effect of plant extracts at four concentrations on *Tetranychus urticae* adults (mean±SE)*

Time (day)	Concentration (%)	Mortality (%)				
		Anise	Coriander	Cumin	Dill	Fennel
1	1	4.47±0.25bF	10.44±0.16aF	9.09±0.18aF	5.97±0.65bF	5.88±0.35bF
	3	20.89±0.35aE	20.89±0.18aE	19.69±0.25aE	17.91±0.44aE	20.58±0.18aE
	6	32.83±0.27bD	37.31±0.16aD	28.78±0.33bE	31.34±0.27bD	32.35±0.16bE
	12	43.28±0.40bD	50.74±0.56aC	40.90±0.42bD	41,79±0.55bD	45.58±0.32bD
3	1	60.31±0.65aC	24.59±0.42bE	20.00±0.45bE	17.18±0.18cE	25.80±0.36bE
	3	75.01±0.55aB	42.62±0.23bD	35.38±0.75cD	34.37±0.68cD	48.38±0.45bD
	6	88.88±0.28aA	57.37±0.38bC	47.69±0.65cD	53.12±0.65bC	61.29±0.29bC
	12	100.00±0.25aA	80.32±0.45bB	67.69±0.54cC	55.75±0.22cC	83.87±0.47bB
6	1	79.03±0.55aB	57.37±0.55bC	57.37±0.36bC	58.66±0.45bC	63.93±0.32bC
	3	93.54±0.62aA	77.04±0.65bB	88.52±0.46aB	70.00±0.55bB	90.16±0.22aA
	6	100.00±0.25aA	88.52±0.75bA	100.00±0.25aA	85.00±0.38bA	100.00±0.25aA
	12	100.00±0.25aA	100.00±0.65aA	100.00±0.18aA	100.00±0.25aA	100.00±0.35aA

*Different lower case letters in the same line and different uppercase letters on the same column show that the means are significantly different for plant species and application concentration, respectively (p<0.05).

Table 2. Contact effect of plant extracts with different concentration on *Tetranychus urticae* protonymphs (mean±SE)*

Time (day)	Concentration (%)	Mortality (%)				
		Anise	Coriander	Cumin	Dill	Fennel
1	1	4.54±0.65cG	16.92±0.75bF	13.63±0.25b	11.11±0.26bG	25.75±0.33aD
	3	16.16±0.35cF	35.38±0.55bE	27.27±0.35b	25.39±0.45bG	50.06±0.45aC
	6	30.30±0.44cE	50.76±0.24bD	40.90±0.45c	36.50±0.55cF	78.78±0.55aB
	12	42.42±0.28cD	64.61±0.28bC	54.54±0.65b	49.20±0.38cE	100.00±0.65aA
3	1	25.80±0.25bE	31.14±0.34bE	24.19±0.23b	19.67±0.45cG	67.21±0.26aB
	3	46.77±0.55bD	49.18±0.54bD	46.77±0.38b	37.70±0.22cF	90.16±0.45aA
	6	62.90±0.16bC	68.85±0.45bC	69.35±0.42b	54.09±0.25cE	100.00±0.25aA
	12	84.64±0.18bB	88.52±0.65bB	88.70±0.65b	77.04±0.45cC	100.00±0.25aA
6	1	60.00±0.22bC	55.73±0.65bD	59.67±0.18b	39.34±0.65cF	100.00±0.25aA
	3	73.33±0.32cB	83.60±0.75bB	85.48±0.16b	65.57±0.75cD	100.00±0.25aA
	6	90.00±0.55bA	96.72±0.65aA	100.00±0.25	88.52±0.45bB	100.00±0.25aA
	12	100.00±0.25aA	100.00±0.25aA	100.00±0.25	100.00±0.25aA	100.00±0.25aA

*Different lower case letters in the same line and different uppercase letters on the same column show that the means are significantly different for plant species and application concentration, respectively (p<0.05).

The effects of plant extracts on *T. urticae* egg hatch are shown in Table 3. Inhibition of hatch rose with extract concentration. The greatest inhibition occurred with dill extract at 12% dill extract. The effects fennel, anise, coriander and cumin extracts (in that order) were less than the effect of dill extract.

Table 3. Egg hatching effect of plant extracts with different concentration on *Tetranychus urticae* eggs (mean±SE)*

Concentration (%)	Mortality (%)				
	Anise	Coriander	Cumin	Dill	Fennel
1	20.58±0.65bD	17.64±0.25bD	29.41±0.18aC	14.70±0.55bD	26.47±0.18aD
3	33.82±0.45bC	32.29±0.35bC	41.17±0.65aB	38.23±0.75aC	44.11±0165aC
6	51.47±0.35bB	47.05±0.44bB	58.82±0.25aA	63.23±0.42aB	60.29±0.25aB
12	69.11±0.26bA	64.70±0.55cA	63.23±0.45cA	91.17±0.25aA	73.52±0.25bA

*Different lower case letters in the same line and different uppercase letters on the same column show that the means are significantly different for plant species and application concentration, respectively (p<0.05).

Discussion

Many volatile oil and plant extracts show acaricide and insecticide effects on economic plant pests, including mites (Prakash & Rao, 1997; Attia et al., 2013). It was determined that when the concentration and the exposure time of five plant extracts used in the study increased the contact effects on *T. urticae* protonymphs and adults. Complete mortality of *T. urticae* protonymphs and adults was observed, especially at 12% plant extracts after 6 days. Other studies have examined effects of plant extracts on *T. urticae*. Coelho et al. (2001) has found that *Petiveria alliacea* L. (Petiveriaceae) extract had acaricide effects. Likewise, Choi et al. (2004) reported that *Rosmarinus officinalis* L. (Lamiaceae) extract was fatal to *T. urticae* adults. Shi et al. (2006) determined that *Kochia scoparia* L. extract caused about 79% mortality of *T. urticae*. Saber (2004) found that *Artemisia monosperma* Delile (Asteraceae) extract has repellent, toxic and prevented egg-laying in *T. urticae*. El-Sharabasy (2010) determined the contact effects of *Artemisia judaica* L. (Asteraceae) leaf extract on *T. urticae* adults and nymphs. Jeon & Lee (2011) found that *Tabebuia impetiginosa* (Mart. ex DC.) (Bignoniaceae) extract had acaricide effects. This literature shows that extracts from many plants cause death in *T. urticae* at high levels.

However, the effects of extracts of *P. anisum*, *C. sativum*, *F. vulgare*, *A. graveolens* and *C. cyminum*, on *T. urticae* have not been previously studied. Chermenskaya et al. (2010) has indicated that the apiaceous species *A. tschimganica, C. maculatum, D. microcarpum, F. foetidissima, H. dissectum, M. macrophylla* plant extracts caused medium level of mortality in *T. urticae* adults. In the same study, it was found that *Prangos lipskyi* Korov. extracts caused more than 80% mortality in *T. urticae* adults. Similarly, in our study a 12% concentration of five plant extracts caused high rates of mortality of *T. urticae* adult and nymphs. This could indicate that high mortality rates in *T. urticae* are due to common active substance present in plants in the Apiaceae. However, it will be necessary to identify and study the active substances in these extracts in order to determine the validity of this suggestion.

The plant extracts studied also inhibited *T. urticae* egg hatch. There have been some studies in which ovicidal effects of some plant extracts on spider mite eggs were determined. Dimetry et al. (1993) determined that a commercial preparation of neem extracts reduced hatching of *T. urticae* eggs and also had ovicidal effect. Sarmah et al. (2009) reported 87% egg mortality at 10% concentration of aqueous extracts of *Xanthium strumarium* L. (Compositae) against *T. urticae*. Yanar et al. (2011) has reported that *Eucalyptus camaldulensis* (Myrtaceae) extract caused 63% death rate in *T. urticae* eggs. In another study, it was found that *Scaligeria meifolia* Boiss (Apiaceae), *Anisosciadium orientale* DC. (Apiaceae),

Trigonella elliptica Boiss (Leguminosae) and *Dodonaea viscosa* L. (Sapindaceae) extracts had ovicidal activity on *T. urticae* eggs; 46, 41, 40 and 38%, respectively (Ghaderi et al., 2013). Similarly, our results suggest that plant extracts, especially at 12%, strongly inhibit hatching of *T. urticae* eggs. Dill extract, in particular, was highly inhibitory to egg hatch. Therefore, it is recommended that the active substances in plants in Apiaceae family be determined and their activity against pest further investigated.

In conclusion, it is suggested that extracts of these five plants have the potential to be used as an alternative acaricides for control of *T. urticae*. It is an established fact that plant extracts can be effective on many pests and have advantages in respect of human health. However, considering there are many plants in the nature with unknown contents and efficiency, there is a need for laboratory and field studies to investigate the effects of plant extracts on pests. In addition, side effects of some plant extracts on natural enemies must be determined and their safety must be indicated with scientific studies. This study provides some useful new information and it is thought that it can provide a foundation for the future studies.

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