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Effects of Aqueous Plant Extracts on Adult Females *Tetranychus urticae* Koch (Acari: Tetranychidae)

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ABSTRACT

The Two-spotted spider mite, *Tetranychus urticae* Koch,1836 (Acari: Tetranychidae), reduces quality and yield in cultivated plants. The most widely employed method for controlling this mite is the application of chemical acaricides. However, over time, researchers have focused on the adverse effects such as resistance, side effects on beneficial organisms, and environmental issues. In this laboratory study, the acaricidal effects of aqueous extracts of *Juglans regia*, *Dieffenbachia amoena*, *Lantana camara*, *Eucalyptus globulus*, and *Nerium oleander* against *T. urticae* were investigated. Plant extracts at concentrations of 1%, 3%, 6%, and 12% (v/v) were applied to *T. urticae* adult females using leaf dipping and spraying methods. The numbers of dead and live individuals were evaluated 24, 72, and 144 hours after the applications. In the dipping method, the highest contact effect was obtained in *J. regia* and D. amoena at 12% concentration at 99.6 and 94.5%, respectively, 144 hours after treatments. Conversely, at the same time and at 1% concentration, *L. camara* extract exhibited a low contact effect of 20.68%. *Juglans regia* extract at 12% concentration showed the highest effect and reached a 71% mortality rate at the end of 24 hours in the dipping method, The lowest effect (10.8% mortality rate) was obtained with *L. camara* at 1% concentration, and the same duration. *Dieffenbachia amoena* showed the highest toxicity with an LC_{50} value of 2561 mg L^{-1} in the spraying method, while the lowest toxicity was found in the *E. globulus* application (4388 mg L^{-1}). This study revealed that aqueous plant extracts showed toxic effects on *T. urticae.*

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Sulu Bitkisel Ekstraktların *Tetranychus urticae* Koch (Acari: Tetranychidae) Ergin Dişileri Üzerine Etkileri

ÖZET

İki noktalı Kırmızıörümcek, *Tetranychus urticae* (Koch,1836) (Acari: Tetranychidae) kültür bitkilerinde kalite ve verimi düşürmektedir. Bu akarla mücadelede yaygın olarak kullanılan yöntem kimyasal akarisitlerin uygulanmasıdır. Ancak zaman içerisinde araştırıcılar direnç, faydalı organizmalar üzerindeki yan etkiler ve çevresel sorunlar gibi olumsuz etkiler üzerine odaklanmıştır. Bu laboratuvar çalışmasında *Juglans regia*, *Dieffenbachia amoena*, *Lantana camara*, *Eucalyptus globulus* ve *Nerium oleander* sulu ekstraktlarının *T. urticae*'ye karşı akarisit etkileri araştırılmıştır. %1, 3, 6 ve 12 (v/v) konsantrasyonlardaki bitki ekstraktları yaprak daldırma ve püskürtme yöntemleri ile *T. urticae* ergin dişilerine uygulanmıştır. Uygulamalardan, 24, 72 ve 144 saat sonra elde edilen ölü-canlı birey sayıları değerlendirilmiştir. Daldırma yönteminde en yüksek değme etkisi, %12 konsantrasyonda *J. regia* ve *D. amoena*'da uygulamalardan 144 saat sonra sırasıyla %99.6 ve 94.5 olarak elde edilmiştir. Buna karşılık, aynı süre ve %1'lik konsantrasyonda *L. camara* ekstraktında %20.68'lik düşük değme etkinliği belirlenmiştir. Daldırma yönteminde %12 konsantrasyonda *J. regia* ekstraktı en

Pestisitler ve Toksikoloji

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yüksek etkiyi göstermiş ve 24 saatlik sürenin sonunda %71 ölüm oranına ulaşmıştır. En düşük etki (%10.8'lik ölüm oranı) ise %1 konsantrasyonda ve aynı sürede *L. camara* ile elde edilmiştir. Püskürtme metodunda en yüksek toksisiteyi 2561 mg L^{-1} LC_{50} değeri ile *Dieffenbachia amoena* gösterirken , en düşük toksisite *E. globulus* uygulamasında (4388 mg L^{-1}) saptanmıştır. Bu çalışma, sulu bitki ekstraktların *T. urticae*'ye toksik etki gösterdiğini ortaya koymuştur.

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INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae) (TSSM) is a harmful organism that highly prevalent pest in agricultural fields in favorable climate conditions (Helle & Sabelis, 1985; Maniania et al., 2009; Alpkent & Ferizli, 2024). This polyphagous mite feeds on approximately 3877 host plants, encompassing more than 150 plant species (Zhang, 2003; Attia et al., 2013). Ineffective control strategies of crop plants often lead to inevitable economic losses (Gore et al., 2013; İnak et al., 2022; Alpkent & Çobanoğlu, 2024). Acaricides are commonly used for their rapid effectiveness in controlling this mite (Van Leeuwen et al., 2006). Among arthropods, TSSM is notable for developing resistance at a rapid rate exhibiting the highest resistance coefficients (Van Leeuwen et al., 2010; Sugimoto & Osakabe, 2014). Factors such as parthenogenesis reproduction type in mite species, short life cycle, and high reproductive capacity under optimal conditions contribute to the accelerated development of resistance to acaricides (Van Leeuwen et al., 2009, Döker & Kazak, 2012; Döker et al., 2021). Cross-resistance can between acaricides with different modes of action but similar detoxification mechanisms, leading to rapid resistance development to other pesticides with different modes of action. *Tetranychus urticae* has developed resistance to a total of 92 active ingredients including various organophosphates, carbamates, pyrethroids, pyrazoles, pyridazines, and ketones (Van Leeuwen et al., 2010). The increased application of multiple pesticides including acaricides, has accelerated this resistance development in *T. urticae*. Consequently, researchers have begun to explore the impacts of these pesticides on non-target organisms, humans, and ecosystems. Studies indicate that pesticides from various groups can affect even phylogenetically distant non-target organisms (Wan et al., 2023). The intensive use of chemical pesticides has led to significant resistance problems for various pests (Ulusoy, 2020; Ulusoy, 2024). As a result, researchers are investigating alternative control methods, including biological control, bio-pesticides, and pesticide rotation with environmentally friendly active ingredients (Kahya et al., 2021; Hazir et al., 2022; Ulusoy et al., 2022). The focus is increasingly on finding or producing natural, non-harmful alternative acaricides that do not negatively affect humans or the environment. Under the European Commission's Green Deal, sustainable practices have gained prominence. The European Commission's Environmental initiative aims to significantly reduce agricultural pesticide use. The Farm to Table strategy within the Green Deal promotes sustainable farming practices by reducing reliance on pesticides and fertilizers (Wang et al., 2022). These efforts align with the Green Deal's overarching goals of achieving a climate-neutral economy and ensuring a healthy environment (Orgiazzi et al., 2022). Current research shifting towards natural materials that harmonize with both nature and human health. Among them, phytochemicals are a major focus. Natural acaricides, such as plant extracts are non-permanent on the environment, environmentally benign, and rapidly degradable; they also tend to have lower mammalian toxicity compared to synthetic acaricides (Regnault-Roger et al., 2012; Sararit & Auamcharoen, 2020). Numerous studies investigate the efficacy of plant extract compounds as alternatives to synthetic pesticides, with some demonstrating insecticidal, acaricidal, repellent, and residual effects (Kruewong & Auamcharoen, 2023; Li et al., 2023; Piffer et al., 2023; De Souza et al., 2023). Consequently, *T. urticae* remains a significant pest in production areas due to several reasons explained earlier (Zhang, 2003).

In this study, aqueous extracts obtained from *Nerium oleander* Linnaeus,1753 (Gentianales: Apocynaceae), *Lantana camara* Linnaeus,1753 (Lamiales: Verbenaceae), *Dieffenbachia amoena* Bull. (Alismatales: Araceae), *Juglans regia* Linnaeus,1753 (Juglandales: Juglandaceae), and *Eucalyptus globulus* Labillardière, 1800 (Myrtales: Myrtaceae) plants collected from Ankara and Adana provinces. No damage caused by mites has been reported for these plant species included in the study. Hence, to evaluate their control potential, the acaricidal properties of aqueous plant extracts from *J. regia*, *D. amoena,* and *N. olean*der, were tested against this mite species for the first time. Additionally, the study supports the broader goals of sustainable agriculture by investigating natural, environmentally friendly alternatives to synthetic pesticides with a focus on plant-based acaricides.

MATERIAL and METHOD

Mass-rearing of Spidermites

A sensitive population of spider mite (German Susceptible Strain, GSS) *Tetranychus urticae* Koch (Acari: Tetranychidae) was obtained from Isparta University of Applied Sciences (Türkiye) in 2015 and reared under pesticide-free conditions. These mites were cultivated on uncontaminated bean plants (*Phaseolus vulgaris* L. (Fabaceae)) in a climate chamber at 26±1ºC, 60±5% humidity, and a 16h Light: 8 h Dark photoperiod.

Source of Plants

Leaves of *Juglans regia* L. (Juglandales: Juglandaceae) were gained from the campus area of the Directorate of Plant Protection Central Research Institute, Ankara while other species were collected from the Adana province and garden of the Biological Control Research Institute, Adana, Türkiye (Table 1). Leaves and stems of the plants were collected. Plants were collected in the period after the fresh leaves had emerged.

Table 1. Plants collection sites and date
Çizelge 1. Bitkilerin toplandığı yerler ve tarihi

Heading	Locations (district/province)	Collection date
Juglans regia	39º 57' 17.6" N 32º 48' 19.6" E (Yenimahalle/ Ankara)	2022/6
Dieffenbachia amoena	37º 00' 38.0" N 35º 20' 23.5" E (Yüreğir/ Adana)	2022/6
Lantana camara	37º 00' 38.1" N 35º 20' 23.3" E (Yüreğir/ Adana)	2022/6
Eucalyptus globulus	37º 01' 37.5" N 35º 20' 02.7" E (Yüreğir/ Adana)	2022/6
Nerium oleander	37º 00' 38.0" N 35º 20' 23.8" E (Yüreğir/ Adana)	2022/6

Extraction of Aqueous Plant Extracts

Plant leaves and stems were gathered together and transferred to the laboratory in paper bags. The plant material was rinsed with tap water and then placed on filter paper (Wh 1) to dry. The foliage was separated from the stems and dried at 30ºC in an oven (Memmert UM200, Germany) for 72 hours. Dried leaves were crushed in a grinder until the particle size was below 1 mm (Ultra-turra t8 IKA, Germany) (Castillo et al., 2010). Obtained powdered foliage was transferred into 50 ml Falcon tubes (Spinwin PP) containing pure sterile water at a concentration of 200 g L^{-1}. The mixture was kept at 350 rpm speed for 24 hours in a magnetic shaker (Thermo Mixer F1.5, Germany). The resulting solution was centrifuged at 10000 *g* (NF 1200 R) for 5 minutes in a refrigerated centrifuge to allow particles to settle at the bottom. Subsequently, the solution was filtered with filter paper (Whatman no 41). The solution was passed via sterile cellulose filter membranes (0.22 µm Millex) using an injector. The filtrate was transferred into opaque (amber) 50 mL glass tubes and placed in the refrigerator at +4ºC. Each extract was prepared fresh before the experiment.

Biological Assays

Leaf-dip method

Clean bean leaves were grown in a plant growth chamber under conditions of 55±5% moisture and 26±1ºC temperature, with a 16-hour light-8-hour dark cycle. Plant leaves with a diameter of 3 cm were cut, and 0.01% triton x-100 purified water was used to prepare plant extract concentrations of 1, 3, 6, and 12% (v/v) (Mironova & Khorkhordin, 1997). The cut bean leaves were dipped into these solutions for fifteen seconds. Subsequently, they were placed with the upper sides of the leaves facing up on glass Petri dishes (9 cm in diameter) containing 1.5% agar gel. The leaves were left in laminar-flow cabinets for thirty minutes to dry. Adult female spider mites (2 to 3 days old) were then transferred to the bean leaves. This process was repeated four times for each concentration. A minimum of six concentrations were established, with 20 adult female individuals per replication. The Petri dishes were placed in a climate-controlled cabinet (Sanyo MIR552, Japan) set at 25±1ºC, with a humidity level ranging from 55 to 65%, and a 16/8-hours light/dark photoperiod. Alive and dead evaluations were conducted 24 hours after application to assess acute effects. Counts of dead and alive individuals continued after 72 and 144 hours to assess persistent toxicity. Preparations containing azadirachtin A (Econeem10 g active ingredient L^{-1}), and formulated pyridaben (Sanmite 200 g a.i L^{-1}) were used as positive controls. The negative control group was pure water. All contact activity experiments were set up following the modified IRAC 004 method (IRAC, 2022). Trials were arranged using a randomized block design.

Spraying method by Potter Tower

Cotton pads placed inside Petri dishes (9 cm) were moistened. Circular leaves (3 cm in diameter) were transferred

on the moistened cotton pads. The edges of bean leaves were covered with paper napkins to prevent the mites from escaping. Approximately 25-30 adult female *T. urticae* mites (2 to 3 days old) individuals were transferred to the leaves. The prepared Petri dishes were positioned within a Potter Tower (Burkard Scientific-BS00281, England), and a 2 ml extract solution (1.83 ± 0.05 mg cm^{-2}) was evenly sprayed (1 bar pressure) onto them. Preparations containing azadirachtin A (EcoNeem10 g a.i. L^{-1}) and pyridine (Sanmite 200 g a.i. L^{-1}) served as controls. The negative control group received only distilled water. Petri dishes were air-dried for thirty minutes in a fume hood (Telstar, Japan). The Petri dishes were then transferred to incubators set at 25±1ºC, with a humidity level between 55% to 65%, and a 16-h light/ 8-h dark photoperiod (Sanyo MIR552, Japan). The cotton pads in the Petri dishes were soaked daily to maintain moisture. At the end of the 72-hour application period, both dead and surviving individuals were counted to assess the effects of the application. Each extract was tested across a minimum of six concentrations to establish lethal concentrations, with each concentration tested in three replicates. Experiments were conducted using a randomized block design. Individuals who did not react to fine brush stimulation were considered dead. Experiments were performed by modifying according to the Irac 004 method.

Statistical Analyses Assessment of Data

Data obtained from biological assays were initially subjected to arcsine transformation. Subsequently, One-way ANOVA was performed, followed by Tukey's test ($\alpha = 0.05$). Statistical analyses were performed using the MINITAB 18 (Release 18.1) software. The efficacy of each extract was evaluated by comparing it with itself in terms of time (hours) and concentration. Additionally, accordingly, data from the bioassays underwent probit analysis according to Finney (1971), and LC_{50} and LC_{90} values were determined using Polo-Plus software (LeOra, 1994).

FINDINGS and DISCUSSION

Leaf-dip method

The leaf-dip method was used to assess the contact effects of various extracts on the adult females of the susceptible GSS population of *T. urticae*. Mortality rates increased proportionally with dosage escalation. These increases continued up to the 72nd hour in terms of contact toxicity. Mortality rates did not significantly increase after the 72nd hour (up to the 144th hour) and began to level off (Table 2). The highest contact effect was observed with *J. regia* collected from Ankara, where a 12% concentration resulted in 99.6% mortality after 144h of application ($F=58.02$; df= 4, 19; $P<0.05$). At a 12% concentration, the mortality rate was 71.5% at the end of 24 hours, and 99% at the end of 72 hours. Mortality rates continued to increase at a slower rate. The least pronounced effect was observed in a 1% concentration after 24 hours, with a mortality rate of 34.6% ($F=39.03$; df= 4, 19; $P<0.05$). The second-highest mortality rate was observed with *D. amoena*, reaching 94.5% after 144 hours of application ($F=53.97$; df= 4, 19; $P<0.05$). The contact effect after 72 hours resulted in a 93.4% mortality rate, while the 24-hour contact effect resulted in a 60.0% mortality rate.

For another efficient extract, *E. globulus*, the highest mortality rate was 88.5% at the end of 144 hours of application at a 12% concentration ($F=20.58$; df= 4, 19; $P<0.05$). The last contact effect was detected at a 1% concentration after 24 hours, with a mortality rate of 33.5% ($F=41.26$; df= 4, 19; $P<0.05$). The highest concentrations and residual effects in *L. camara* and *N. oleander* extracts were observed at 144 hours of application, with mortalities of 81.2 and 77.5%, respectively. Throughout the study, the lowest contact effect was in *L. camara* extract at a 1% concentration after 24-hours, with a mortality rate of 10.8% ($F=2.76$; df= 4, 19; $P>0.05$). In general, the impact of almost all extracts diminished after 72 hours. Results from the 144-hour trial showed slight changes compared to the 72-hour test results. This indicates that the efficiency of the extracts decreased after 72 hours. It is believed that the extracts were decomposed quickly in the incubator (25±1 ºC), resulting in a reduced residual effect. Azadirachtin A and the formulated synthetic acaricide pyridazine at a 1% concentration caused 100.0% mortality in all exposure times (Table 2).

Spraying method by Potter Tower

The susceptibility of *T. urticae* varied in response to the spray application of five different plant extracts (Table 3). The LC_{50} value for *D. amoena* on *T. urticae* was determined to be 2561 mg L^{-1}, representing the highest toxicity compared to the other aqueous extracts. This is followed by *N. oleander*, *J. regia*, *L. camara*, and *E. globulus*, respectively. The LC_{50} and LC_{90} for azadirachtin A were 73.2 and 250 mg L^{-1}, respectively. The toxicity levels of pyridazine, a synthetic chemical, were found to be 74.8 and 316 mg L^{-1}, respectively.

Water-based extracts obtained from Adana and Ankara, along with two formulated acaricides (pyridine and azadrahtin A), were evaluated for their potential acaricidal effects against adult females of *T. urticae*. Numerous studies have assessed the efficacy of various extracts in controlling insects and mites (Kim et al., 2005; Adenubi et

al., 2019; Piffer et al., 2023; Ellafi et al., 2023; Muhammad et al., 2023; Zhu et al., 2023). The lethal efficacy of plant extracts against various insects and mites has been well-documented. Studies indicate that many plants possess insecticidal and acaricidal properties, extracts demonstrating insecticidal, ovicidal, acute toxicity, larvicidal, reproductive-inhibitory, adulticidal, repellent, and fumigant effects (Kim et al., 2005; Cetin & Yanikoglu, 2006; Pavela et al., 2016; Fang et al., 2020; Assemie & Gemeda, 2023). Plant extracts are generally less toxic to parasitoids, predators, and biological agents, making them suitable for sustainable agricultural pest management. They are effective and harmless to non-target organisms (Kilani-Morakchi et al., 2021). Results of the current study indicate that *J. regia* extracts caused significant mortality in adult females of *T. urticae* after 6-days (Table 2). Moreover, the LC_{50} for *J. regia* (3257 mg L^{-1}) was found to be the second most toxic against *T. urticae*, following *D. amoena* (2561 mg L^{-1}) after a 3-day application (Table 3).

Table 2. Mortality rates of Tetranychus urticae adult females after exposure to aqueous plant extracts using the leaf-dip method, at 24, 72, and 144 hours of post-application

Çizelge 2. Sulu bitki ekstratlarının yaprak daldırma yöntemiyle uygulamadan 24, 72 and 144 saat sonra Tetranychus urticae ergin dişilerinde neden oldukları ölüm oranları

Plant extract	*Concentration (%)*	*Time (h)*		
		*Mortality (%) ± SE**		
		24	72	144
Juglans regia	1	34.61 ± 2.15A**b	56.53 ± 1.87Ab	59.24 ± 2.36Ab
	3	48.71 ± 1.75Bab	70.06 ± 0.20Ab	72.54 ± 0.10Ab
	6	49.99 ± 0.50Bab	71.42 ± 0.51Ab	76.92 ± 1.49Ab
	12	71.52 ± 0.88Ba	99.01 ± 3.90Aa	99.60 ± 2.57Aa
	Control	1.27 ± 0.32Ac	2.57 ± 0.65Ac	2.57 ± 0.65Ac
	F	39.03	55.75	58.02
Dieffenbachia amoena	1	25.72 ± 1.43Bb	55.06 ± 0.85Ab	58.80 ± 0.41Ab
	3	36.13 ± 0.61Bb	63.91 ± 0.65Ab	66.37 ± 0.46Ab
	6	58.85 ± 0.60Ba	92.40 ± 3.55Aa	93.38 ± 3.11Aa
	12	60.03 ± 0.17Ba	93.38 ± 3.11Aa	94.52 ± 2.95Aa
	Control	1.27 ± 0.32Ac	1.69 ± 1.27Ac	2.83 ± 1.27Ac
	F	56.05	47.64	53.97
Lantana camara	1	10.84 ± 0.66Ab	19.48 ± 1.10Ab	20.68 ± 1.22Ab
	3	43.73 ± 0.23Ba	62.72 ± 1.02Aa	65.21 ± 0.93Aa
	6	52.52 ± 0.42Ba	71.34 ± 0.28Aa	75.23 ± 0.64Aa
	12	55.01 ± 0.17Ba	75.30 ± 0.73Aa	81.23 ± 2.41Aa
	Control	1.69 ± 1.27Ac	1.69 ± 1.27Ac	1.69 ± 1.27Ac
	F	2.76	11.53	8.41
Eucalyptus globulus	1	33.56 ± 0.81Ab	46.24 ± 0.40Ac	50.04 ± 1.19Ab
	3	44.97 ± 0.50Aab	58.87 ± 0.77Abc	62.96 ± 2.00Aab
	6	48.73 ± 0.57Aab	69.48 ± 2.36Aab	76.10 ± 5.72Aab
	12	62.72 ± 1.02Aa	80.44 ± 0.90Aa	88.48 ± 5.49Aa
	Control	1.69 ± 1.27Ac	2.83 ± 1.27Ad	2.83 ± 1.27Ad
	F	41.26	46.77	20.58
Nerium oleander	1	32.37 ± 0.48Ba	56.27 ± 0.23Aa	58.76 ± 0.06Aa
	3	37.27 ± 1.18Ba	67.59 ± 0.30Aa	70.06 ± 0.20Aa
	6	46.24 ± 0.40Ba	67.69 ± 0.83Aa	71.34 ± 0.28Aa
	12	51.26 ± 0.57Ba	76.29 ± 0.09Aa	77.55 ± 0.12Aa
	Control	1.27 ± 0.32Ab	1.27 ± 0.32Ab	1.69 ± 1.27Ab
	F	26.32	68.96	90.61
Econeem (Azadirachtin A)	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
	F	-	-	-
Sanmite (Pyridaben)	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
	F	-	-	-

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

Table 3. Toxicity of aqueous plant extracts to Tetranychus urticae adult females at 72-h of post-application
Çizelge 3. Sulu bitki ekstraktlarının Tetranychus urticae ergin dişilerine uygulamadan 72 saat sonra toksisiteleri (72 s maruziyet)

Plants	*n*[a]	*Slope+SE*[b]	*LC_{50} (mg L^{-1}) (0.95 CL*[c]*)*	*LC_{90} (mg L^{-1}) (0.95 CL)*	*x^2(df)*[d]	*P-values*
Juglans regia	480	1.29 ± 0.18	3257(2393–4158)	32077 (20481–66962)	12.70(17)	0.76
Dieffenbachia amoena	480	1.50 ± 0.19	2561(1894–3223)	18352 (13096–30736)	14.10(17)	0.66
Nerium oleander	560	1.32 ± 0.13	3170(2517–3937)	29833 (19977–53100)	10.94(21)	0.96
Eucalyptus globulus	480	1.29 ± 0.17	4388(3367-5559)	42823 (26548–93249)	13.00(17)	0.73
Lantana camara	560	1.55 ± 0.14	3770(3110–4563)	25310 (18119–40019)	12.87(21)	0.91
Azadirachtin A	480	2.40 ± 0.19	73.2 (65.4–82.7)	249.9 (202.1–330.1	11.47(17)	0.83
Pyridaben	480	2.05 ± 0.24	74.8 (63.8–90.89)	316.3 (220.9–552.8)	10.53(17)	0.88

[a]n: Total number of mites; [b]SE: Standart error; [c]CL: Confidence limits; [d]Chi-square value and degrees of freedom

Few studies have documented the acaricidal efficacy of *J. regia*. Wang et al. (2007) investigated the contact and systemic effects of *J. regia* leaves on *Tetranychus viennensis* Zacher, 1920 (Acari: Tetranychidae) and *Tetranychus cinnabarinus* Boisduval, 1867 (Acari: Tetranychidae). They found that the mean lethal concentration (LC_{50}) for adult *Tetranychus* species ranged between 60.5 - 87.6 mg mL^{-1} after 24 hours, respectively, based on the three different bioassay methods. In addition, the LC_{50} for systemic activity ranged between 95 - 97 mg mL^{-1} after 48 hours, respectively. In another study, the authors reported that using various extraction methods on *J. regia* samples at a dosage of 1 mg mL^{-1} resulted in an 83.4% mortality rate for *T. cinnabarinus* after 24 hours (Wang et al., 2012). Erdogan and Yılmaz (2017) demonstrated that a 12% ethanolic leaf extract of *J. regia* was 83% toxic to adult females of *T. urticae* following a 24-hour using the leaf-dipping method. In the spraying method, at the same concentration, a 100% mortality rate was observed for adult females of *T. urticae*. Mahla et al. (2013) showed that an ethanolic leaf extract of *J. regia* affected the immature stages of TSSM, with mortality rates recorded between 13 and 30% after daily observations. Wang et al. (2009) revealed that using different extraction methods such as methanol, chloroform, and petroleum ether on *J. regia* and applying a concentration of 1 mg mL^{-1} resulted in mortality rates for *T. cinnabarinus* ranging from 23% to 80% after 24 hours. These variations among studies highlight that phytochemical content in the plants can vary. The choice of extraction method is crucial for determining biological activity because it affects the phytochemical composition and the ratio of main chemical components (Kouninki et al., 2005).

Literature on *Dieffenbachia amoena* is limited. Hence, the present study represents the initial investigation into the acaricidal efficacy of *D. amoena* against adult females of *T. urticae*. *Dieffenbachia amoena* contains alkaloids, flavonoids, glycosides, phytic acid, tannins, oxalates, and saponin which is responsible for sudden and severe reactions (Dvorack et al., 1999; Eno & Ubi, 2021). It has been reported that extracts from leaves and stems of *D. amoena* are used as a poison in South America (Paris & Moyse, 1967; Altschul, 1973). Additionally, it is associated with inflammation, vomiting, nausea irritation of the tongue and throat. Plants from this family have been shown to possess high degrees of chronic and acute toxicity, with potential genotoxic, carcinogenic, and mutagenic effects (Eno & Ubi, 2021). Ganiyat et al. (2011) revealed that essential oils (EO's) from the leaves and stems of *Dieffenbachia picta* (Lodd.) Schott (1852) (Arecidae: Arales) exhibited antimicrobial and antioxidant activities. Ulusoy et al. (2019) assessed the impact of inhibiting acetylcholinesterase and carboxylesterase in *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) using *D. amoena*. The results indicated that *D. amoena* extracts at a concentration of 10% exhibited an inhibitory effect of 21% inhibitory effect on acetylcholinesterase (AChE) and a 36% inhibitory effect on carboxylesterase (CarE) with the lowest efficacy observed in the experiment. *Dieffenbachia amoena* extract was found to be the most effective in the current study through the spray method. Many studies report that some essential components of the plant extract being examined are not consistently found, even when sourced from the same plant (Djenane et al., 2011; Akolade et al., 2012; Shala & Gururani, 2021). Conversely, some researchers have noted significant variations in the content of the same product depending on the region where it is grown (Djenane et al., 2011; Hafsa et al., 2016). This variation may explain the differences in the acaricidal effectiveness among plants.

Various phytochemical components of *Nerium oleander* include amino acids, alkaloids, terpenoids, cholesterol, glycosides, flavonoids, proteins, tannins, tannins and floatiness (Kiran & Prasad, 2014; Çelebioğlu et al., 2020). According to the authors, *N. oleander* exhibits insecticidal, antibacterial, and cytotoxic properties (Mijatovic et al., 2007; Zaid et al., 2022; Saeed et al., 2023). Some researchers have mentioned its toxic effects on the nervous, reproductive, and digestive tracts of harmful organisms (Kumar et al., 2013). There is limited research on its acaricidal activity against *Tetranychus* species. The toxic activity of *N. oleander* has been studied by various researchers against different mites. In a study by Tawfik and Mahmoud (2009), ethanol and hexane extracts from

the leaves of *N. oleander* showed contact LC_{50} values of 32.8 mg mL^{-1} and 44.3 mg mL^{-1}, respectively, against *Rhizoglyphus echinus* Fumouze and Robin, 1868 (Acari: Acaridae). Salim et al. (2016) reported that at a 5% concentration, the mortality increased to 78%. When the concentration was raised to 10%, mortality ranged from 65 to 100% in the second stages of root-knot nematodes. Gholami et al. (2018) found that *N. oleander* hexane and alcohol extracts exhibited LC_{50} values of 3500 and 14000 ppm, respectively, after 24 hours of contact toxicity using the leaf-dip method against *T. urticae* individuals. Furthermore, another study observed a positive impact on mortality with increased application duration and concentration of the aqueous extract. Hasnawi and Shlash (2020) found that *Sarcoptes scabiei* Linnaeus, 1758 (Acariformes: Sarcoptidae) adults exhibited mortality rates of 50%, 80%, and 90% after 24, 48, and 72-h of treatment with EO obtained from *N. oleander* leaves at a concentration of 18 mg mL^{-1}, respectively. The median lethal concentration was (LC_{50}) 13.99 mg mL^{-1}.

Eucalyptus globulus contains phenolic compounds, tannins, cardiac glycosides, saponins, steroids, terpenoids, glycosides, terpenes, alkaloids, resins, acidic compounds, and flavonoids (Obiorah et al., 2012; Ishnava et al., 2013; Jamil et al., 2017; Ajilore et al., 2021; Shala & Gururani, 2021). Numerous studies have previously shown the miticide activity of EOs or extracts of the *E. globulus* or *Eucalyptus* sp. against *T. urticae* and other mites. Afify et al. (2012) reported that 1% and 3% concentrations of *Eucalyptus* sp. essential oil resulted in 27.5% and 70% mortality of adult spider mites after 1-day application, respectively. Hamed et al. (2021) reported an LC_{50} value of 876.15 ppm after 24 hours of exposure against adult female *T. urticae* for *E. globulus* oil. A study conducted by Madreseh-Ghahfarokhi et al. (2019) showed that water-distilled leaf extract from *E. globulus* had the highest acaricidal activity with a mortality of 53.8% on *Rhipicephalus bursa* Canestrini and Fanzago, 1877 (Acari: Ixodidae) adults at 1 (pure) concentration and at the end of a 2-h monitoring time. Aissaoui et al. (2019) noted that the acaricidal impact of *E. globulus* EO, obtained from the stem by the distillation method, was 61%, 76%, and 85% mortality on adult *T. urticae* at concentrations of 1%, 4%, and 8%. Choi et al. (2004) state that they did not include the essential oil experiment they conducted on adult *T. urticae* in their evaluation because the mortality was less than 60%. In another study, the acaricidal activity of *E. globulus* ethanol plant extract against female *T. urticae* was found at an LC_{50} value of 4600 ppm using the dipping method (Eldoksch et al., 2009). Likewise, Monika and Rachna (2008) showed that the miticidal activity of aqueous *E. globulus* leaf extract on various developmental stages of *T. urticae* was 81-93% at a concentration of 10%.

Many constituents have been identified in *Lantana Camara*. Depending on the plant organ (stem, leaf, flower, fruit), there are between 52 and 71 ingredients. The plant is especially rich in terpenes. The main constituents are caryophyllene, germacrene-D, elemene, humulene, sabinene, bicyclogermacrene, palmitic acid, stearic acid, davanone, curcumene, caryophyllene (Ngassoum et al., 1999; Khan et al., 2002; Khan et al., 2003). Caryophyllene is the most common constituent of the compounds studied so far (Saikia & Sahoo, 2011). It has been reported to have insecticidal, nematicidal, larvicidal, attractant, anti-juvenile repellent, feeding deterrent, growth inhibitor, growth regulator, oviposition inhibitor, antibacterial, anticancer, antiproliferative, hemolytic, antifungal, antimutagenic, antiulcerogenic, antioxidant, antihyperglycaemic, anti-inflammatory, antimotility, anti-urolithiasis, anti filarial and antifertility activity (Saikia & Sahoo, 2011; Reddy, 2013; Thanavendanand & Kennedy, 2015; Murugesan et al., 2016). Premalatha et al. (2018) stated that the acaricidal impact of the *L. camara* water extract was the least effective among the plants tested, with 30% efficacy at a 10% concentration against adult female *T. urticae*. Radhakrishnan and Prabhakaran (2014) also reported almost similar results. According to their study, *L. camara* was extracted from leaves using the hydro distillation method. After an observation exposure time of 24-96 hours, a concentration of 2.5% and 5% resulted in 18% and 30% mortality against *Oligonychus coffee* Nietner, 1861 (Acari: Tetranychidae). Laya et al. (2022) found that 5%, 7.5%, and 10% concentrations of aqueous leaf extracts of *L. camara* were toxic to *Tetranychus truncatus* Ehara, 1956 (Acari: Tetranychidae) with 41%, 50% and 72% mortality after 120-h exposure, respectively. Mandal et al. (2013) indicated that the miticidal efficacy of chloroform and hexane extracts from mature *L. camara* leaves on *T. urticae* was low. However, the *L. camara* extract in their study yielded a maximum mortality rate of 8.72% after 3-days of exposure for both extracts. When the study activity was observed on the 2nd and 3rd days, it was noted that the residual and mortality effects diminished over time. This and other studies suggest that the residual and mortality effect diminished over time (Chowdhury et al., 2008; Mandal et al., 2013; Kaoutar et al., 2019).

CONCLUSION and RECOMMENDATIONS

The toxic effects of aqueous plant extracts on spider mites have been demonstrated. Further investigation is recommended to determine which components contribute to the efficacy of extracts, particularly when their phytochemical properties are identified and extracted using different distillation methods. Additionally, there is a need to assess the activity of these extracts against different insect species. The high degradation rate of herbal extracts and their rapid toxic effects on *T. urticae* indicate that they could serve as an alternative to synthetic miticides.

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Statement of Conflict of Interest

The author has declared no conflict of interest.

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