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RESEARCH ARTICLE

Toxicological effects of compounds from the leaves of *schinus terebinthifolius* against two tetranychid mites (Acari: tetranychidae)

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ABSTRACT

The use of synthetic acaricides is becoming increasingly hazardous to non-target organisms. Plant-derived natural products are alternative candidates with general low toxicity to human and environment. Two extracts and nine phenolic compounds (**1**-**9**) from the leaves of the Brazilian pepper tree (*Schinus terebinthifolius* Raddi) were investigated for their acaricidal effects against two tetranychid mites, *Tetranychus cinnabarinus* (carmine spider mite) and *Tetranychus urticae* (two-spotted spider mite). After 7-days of treatment, the ethyl acetate fraction demonstrated remarkable mortality against *T. cinnabarinus* (86.67%) while, the *n*-hexane fraction showed the highest mortality against *T. urticae* (96.67%) at 200 µg/mL. Among the tested compounds, afzelin (**2**) and protocatechuic acid (**7**) exhibited remarkable activity. Regarding *T. cinnabarinus,* afzelin showed the highest mortality (86.67%), toxic activity (LC_{50} 22.32 µg/mL and toxicity index, Ti 0.972), and the fastest lethality by time. Whereas, protocatechuic acid achieved the highest mortality (96.67%), the lowest LC_{50} (0.232 µg/mL, Ti 73.71), and fastest lethality by time against *T. urticae*. This study reported a promising *in vitro* acaricidal activity of the phytoconstituents of the Brazilian pepper tree and suggested its isolated compounds, such as afzelin and chatechuic acid, as alternative leads for the development of echo-friendly acaricides.

Keywords: Tetranychus cinnabarinus; Tetranychus urticae; Brazilian pepper; Acaricide.

INTRODUCTION

A well-known fact is that the carmine spider mite, *T. cinnabarinus* (Boisduval) and the two-spotted spider mites, *T. urtica*e (Koch) became the most important agricultural pests through the last decades. Their threat is due to large diversity of host plants, including fruits, vegetables, and ornamentals (Le Goff et al. 2009); 3877 host species recorded by (Migeon et al. 2010) around the world for *T. urtica*e. The most effective method to control the acarid pests till now is to use the synthetic acaricides, but random uses of these acaricides lead to adverse effects on many other non-target organisms and human health. Also, extravagance in the use of acaricide are responsible for the increase of acaricide-resistance mites and this may be because of their short developmental period, high fecundity which increase their ability to produce several successive generations. In addition, the tetranychid mites' pest management became difficult, due to this resistance to acaricide (Beers et al. 1998).

So, the Integrated Pest Management (IPM) aims to decrease using acaricides to prevent damage of ecological system (Van Pottelberge et al. 2009). Therefore, it was necessary to develop new methods to control tetranychid mites' damage. One of the excellent alternatives to the synthetic acaricides is to use natural products because of their low toxicity to human, low environmental pollution levels, and their compatibility to the environmental components more than synthetic pesticides (Liu et al. 2000). For instance, the essential oils of chamomile, marjoram and *Eucalyptus* showed varied acaricidal effects against *T. urticae* (Koch) (Abd El-Moneim et al. 2012). Also, the different extracts of *Kochia*

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scoparia (L.) Schrad displayed both contact and systemic toxicity to three species of spider mites, *T. urticae*, *T. cinnabarinus*, and *T. viennensis* (Shi et al. 2006).

Brazilian pepper, *Schinus terebinthifolius* Raddi commonly known as Felfel Aareed, is a member of Anacardiaceae family. It is native to South America especially to Brazil; however, it is cultivated in many countries of the world as an ornamental plant (El-Massry et al. 2009). Brazilian pepper has many pharmacological activities and the different plant's parts are used to treat several ailments in traditional medicine. The leaves showed antimicrobial activity against wide array of microorganisms, including bacteria and fungi, and used for treatment skin, mucous membrane, genitourinary and respiratory infections. They also were reported to have analgesic, antipyretic, anti-inflammatory, immunomodulatory, chemo-preventive, antihypertensive, antiarrhythmic, antidepressant, antioxidant, and wound healing and detoxification properties (Bernardes et al. 2014; de Lima et al. 2006; Fedel-Miyasato et al. 2014; Silva-junior et al. 2015). The barks have antirheumatic, anti-inflammatory, hemostatic, antimicrobial, antiviral, urinary antiseptic, and in respiratory tract infections (Johann et al. 2010; Silva-junior et al. 2015). The fruits showed antioxidant ability due to nitrous oxide (NO) synthase inhibition, antimicrobial activity, (Bernardes et al. 2014; Silva-junior et al. 2015).

The leaves and fruits of Brazilian pepper were reported to contain volatile (essential) oil which mainly composed of mono- and sesquiterpenes (Santos et al. 2009). The monoterpene component (e.g., myrcene, limonene, and α-pinene) in leaves and fruits represents about 36.4% and 57.1%, respectively. While the sesquiterpene component (e.g., germacrene D, δ-cadinene, and α-copaene) represents 39.1% and 23.2%, respectively (Santos et al. 2009). Also, the presence of an additional characteristic group of spirocyclopropane sesquiterpene was reported (Richter et al. 2010).

Regarding the non-volatile phytocomponents of *S. terebinthifolius*, two main groups have been reported from leaves, barks, and fruits, including triterpenoids (e.g., masticadienoic acid, bauerenone, α-amyrene, α-amyrenone, ursolic acid, and simiarenol) and phenolics (e.g., tannins, gallates, free phenolic acids, and flavonoids) (Abdel Bar et al. 2018; Campello and Marsaioli 1975; Lloyd et al. 1977). Also, a characteristic biphenyl group represented by schinol has been also reported from the leaves of *S. terebinthifolius* and was revealed to have antifungal properties (Johann et al. 2010).

As a continuation to our effort to investigate the potential pesticidal effects of the different extracts and compounds of *Schinus terebinthifolius* Raddi, the current study aimed at *in vitro* evaluation of the toxic activity of the *n*-hexane and EtOAc fractions, in addition to nine isolated compounds from the EtOAc and aqueous fractions of the leaf extract of this plant, against two tetranychid mites, *T. cinnabarinus* (carmine spider mite) and *T. urticae* (two-spotted spider mite) from the spider mite family, Acari: Tetranychidae.

MATERIAL AND METHODS

Plant material

Branches from the tree of *Schinus terebinthifolius* Raddi were collected from Damietta, Egypt, in June 2018. The leaves were separated from the stems, dried, and powdered. The plant was authenticated by Dr. Ibrahim A. Mashaly, Professor of Plant Ecology- Faculty of Science- Mansoura University. A voucher sample was preserved in the herbarium of Pharmacognosy Dep.- Faculty of Pharmacy- Mansoura University (ID: 018-Mansoura-1).

Extraction

Ground leaves of *S. terebinthifolius* (4000 g) were soaked in MeOH (6 L.) for four-times, successively. The combined MeOH extracts were evaporated by a rotary, then allowed to further dry to afford a total extract (~ 817 g). The total extract was dissolved in MeOH, diluted with distilled water, and successively partitioned with *n*-hexane, methylene chloride, and EtOAc. After evaporation of solvent, three main fractions were obtained: 1) the *n*-hexane fraction (~ 125 g), 2) the methylene chloride fraction (~ 35 g) and 3) the EtOAc fraction (~ 372 g). The fourth fraction was the aqueous fraction, which was further purified by diaion by washing with water, followed by elution with MeOH and evaporation at 45 C° using rotary evaporator. The obtained residue (~ 86 g) was further purified to afford compounds **1**-**6**, whereas purification of the EtOAc fraction afforded compounds **4**, **6** and **7**-**9** (Fig. 1) according to the previously published procedure by our research group (Abdel Bar et al. 2018).

Evaluation of the acaricidal activity of extracts and isolated compounds against two Tetranychid Mites

Experimental mite cultures

T. cinnabarinus and *T. urticae* were collected from infected castor (*Ricinus communis* L.). The mites were transferred to the Acarology Laboratory, Plant Protection Research Institute, ARC, Egypt, and identified by following the detailed descriptions recorded by (Zhang and Jacobson 2000; Zhang 2003). Collected mites were maintained on castor leaves upside down on dampening cotton pads in Petri-dishes (12 cm in diameter), cotton pads were moistened daily, and all the ends of the leaves were covered with wet cotton to prevent mite escape and to avoid leaves dryness. Petri-dishes were placed in a closed box and kept in controlled conditions at 25±2°C, and 16:8 h (L: D) in

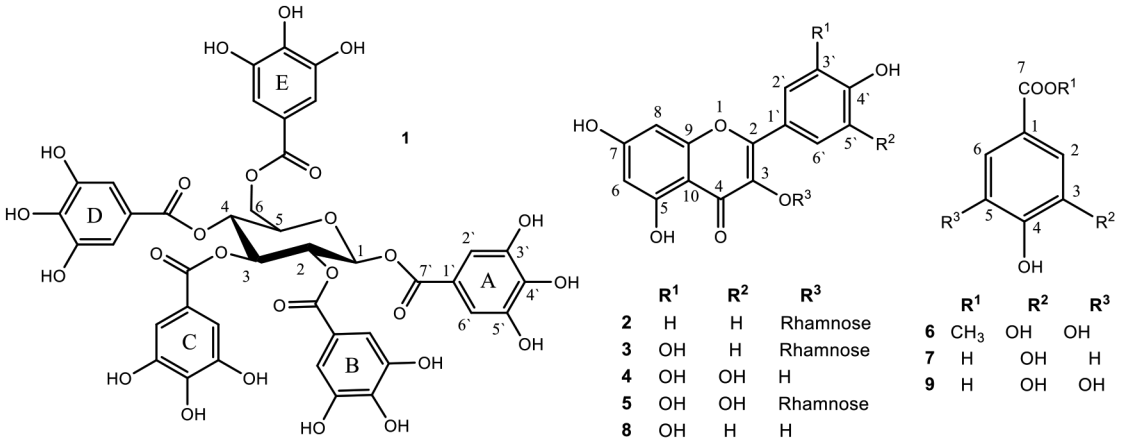

Fig 1. Compounds (1-9) isolated from *Schinus terebinthifolius*.

the laboratory. The humidity in the box was kept at 85±5% RH using saturated solution of KCl. Mites were transferred on fresh castor leaves every 5 days.

Experimental design

Laboratory Experiments

Impact against two Tetranychid Mites

To evaluate the acaricidal properties of the extracts and isolated compounds against the two tetranychid mites, *T. cinnabarinus* and *T. urticae*, the treatments were tested in Petri-dishes, as triplicates at concentrations of 200, 100, 50 and 25 µg/mL. Water and mites alone were used as negative control. Abamectin 1.8% EC was used as a standard acaricidal agent, at concentrations of 200, 100, 50, 25, 10, 1 and 0.1 µg/mL, for comparative evaluation. Uninfected castor leaves were transferred to the laboratory then cleaned carefully with distilled water. Experiments were carried out using castor leaf discs 1.2 cm in diameter at room temperature (25±2°C, 60±5% RH). Disks were placed upside down on dampening cotton pads in Petri-dishes (9 cm in diameter) and each disk was infested with ten adult newly emerged females aged 0-48 hours. Mites were treated by spraying the prepared concentrations mentioned before using hand sprayer. Mites' mortality was checked after one, three, five and seven days of treatment and compared to control using a binocular stereomicroscope.

Statistical analysis

The effect of isolated compounds on mortality was evaluated as percentages of daily and total mortality, corrected for mortality in control variant according to Abbott's formula (Benelli et al. 2018). Lethal effect of the compounds was determined based on median lethal time (LT_{50}) and median lethal concentration (LC_{50}) after seven days of treatment calculated using Probit analysis according to (Finney, 1971). A computerized software program (LDP line) a copyright by Ehab, M. Bakr, Plant Protection Research Institute, ARC, Giza, Egypt, was used

$$Ti = \frac{\text{LC50 of the most toxic insecticide}}{\text{LC50 of less toxic insecticide}} x100$$

to calculate both of LT_{50} and LC_{50} at p-level < 0.05 (Bakr 2005). The same program was used to calculate Toxicity index (Ti) according to Sun (1950) equation as follow:

Data concerning mortality were analyzed by one-way analysis of variance (ANOVA) (Snedecor and Cochran 1980), least significant difference (LSD) was used to evaluate differences between means at $p < 0.05$ using CoStat (version 6.204) statistical software package, CoHort Software (CoStat-Software 2004).

RESULTS AND DISCUSSION

Characterization of the isolated compounds

The structures of isolated compounds were identified by our research group using different spectral analyses, including nuclear magnetic resonance (NMR), infrared (IR), and mass (MS) spectroscopy. They were identified as 1,2,3,4,6-pentagalloyl glucose (1), kaempferol-3-O-α-L-rhamnoside (afzelin) (2), quercetin-3-O-α-L-rhamnoside (quercetrin) (3), myricetin (4), myricetin-3-O-α-L-rhamnoside (myricetrin) (5), methyl gallate (6), protocatechuic acid (7), quercetin (8), and gallic acid (9) according to the previously reported methods (Agrawal 1989; Ceruks et al. 2007; da Silva et al. 2017; Santana et al. 2012). It is worth to note that the identified compounds were of either phenolic or flavonoidal nature, which are allochemicals or more specifically are phytoalexins produced by plants in response to biotic and abiotic stressors as a defence mechanism (Jeandet et al. 2014). Pathogens, such as bacteria, fungi, nematodes, and insects, including mites are examples of these biotic stressors (Santamaria et al. 2020). This motivated us to investigate the in vitro acaricidal effect of these compounds (1-9) against two selected tetranychid mites.

Impact against two Tetranychid Mites

The influence of four concentrations of the eleven examined materials (compounds **1**-**9**, EtOAc and *n*-hexane extracts) to control two tetranychid mites *T. cinnabarinus* and *T. urticae*, was *in vitro* investigated. The results revealed that mite population were significantly decreased by all screened materials nevertheless abamectin was the most toxic compound, as using concentrations 200, 100 µg/mL result in 100% mortality 24-hours post treatment (Fig. 2). Out of the evaluated materials, afzelin (**2**), myricetrin (**5**) and EtOAc extract achieved the highest percentage mortality (86.67%) in *T. cinnabarinus* population seven days post treatment at the concentration of 200 µg/mL followed by protocatechuic acid (**7**) (83.33%) come after methyl gallate (**6**) (76.67%) then 1,2,3,4,6-pentagalloyl glucose (**1**) and quercetin (**8**) (73.33% each) at the same conditions mentioned before, Fig. 2. Meanwhile protocatechuic acid (**7**) and *n*-hexane extract at the concentration of 200 µg/mL achieved the highest toxicity effect for *T. urticae* with mortality rate (96.67%), followed by afzelin (**2**) (86.67%), come next methyl gallate (**6**) (76.67%) then 1,2,3,4,6-pentagalloyl glucose (**1**), quercetin (**8**), gallic acid (**9**), and EtOAc extract with mortality rate (73.33%) at the same concentration after seven days of treatment (Fig. 2). Similar trend was detected at the concentration of 100 and 50 µg/mL for the same treatments. Control variants have no mortality through the experiment.

Results presented in Fig. 2 stated that, mortality of both tetranychid mites behaved in a dose-dependent manner. This is in consistent with several studies that reported increasing mortality with increasing the concentrations of the extract applied to phytophagous mites (Castagnoli et al. 2005; Habashy et al. 2016; Sivira et al. 2011).

The efficacy of the screened materials by time against *T. cinnabarinus* and *T. urticae*, was estimated based on the values of the median lethal time (LT_{50}) calculated for the replicates treated with examined extracts and compounds at a concentration 200 µg/mL at *p*-level < 0.05 (Table 1). Protocatechuic acid (**7**) was the most toxic compound to both tested mites where the values of the median lethal time equal 2.98 with overlapped confidence intervals from 2.09 to 4.05 days and 1.73 with overlapped confidence intervals from 1.25 to 2.20 days for *T. cinnabarinus* and *T.*

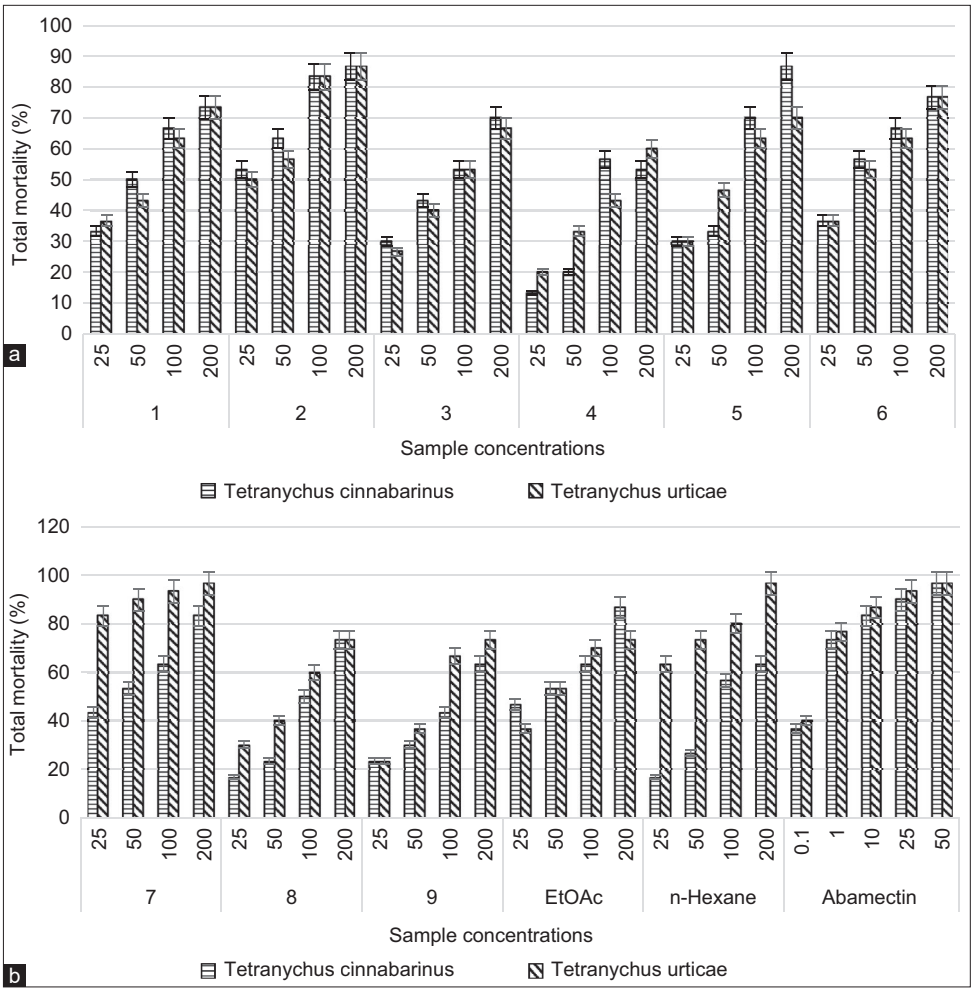

Fig 2. Total mortality percentage of *Schinus terebinthifolius*, after 7 days of treatment, against *Tetranychus cinnabarinus* and *Tetranychus urticae* adult females; a) For compounds (1-6), and b) For compounds (7-9), *n*-hexane and EtOAc extracts, and abamectin as a standard.

urticae, respectively. The calculated confidence intervals of concentration 200 µg/mL for protocatechuic acid (**7**) were narrow which indicates an equalized susceptibility to this concentration within the treated mite population. Followed by afzelin (**2**) and EtOAc extract where values of median lethal time equal 3.72 and 3.57 days, respectively, at the same concentration against *T. cinnabarinus*. The *n*-hexane extract was less toxic to *T. urticae*, as the calculated *p*-level < 0.05 values of LT_{50} was 2.86 days (overlapped confidence intervals from 2.30 to 3.41 days), Table 1. The values of the slope were less than four for all evaluated extracts and compounds except for *n*-hexane extract against *T. cinnabarinus*, and methyl gallate (**6**) against *T. urticae*. This showed that in former case, the tested compounds would result in optimally small increase in mortality of *T. cinnabarinus* and *T. urticae* by time, while in the latter cases; a slight increase in time would lead to higher mortality in treated mite population.

Also, the acaricidial activity of these compounds estimated based on values of the median lethal concentration (LC_{50}) which calculated at *p*-level < 0.05 for different treatments. Results displayed in Table 2, indicate that abamectin was the most toxic compound for both of carmine spider mite (*T. cinnabarinus*) and two-spotted spider mite (*T. urticae*) with LC_{50} values 0.217 and 0.171 µg/mL respectively and toxicity index (Ti 100) at 7 days of exposure. Afzelin (**2**) comes after that as the calculated LC_{50} at 7 days exposure of *T. cinnabarinus* was 22.32 µg/mL with toxicity index (Ti 0.972). It was followed by EtOAc extract, protocatechuic acid (**7**) and methyl gallate (**6**) where the values of the median lethal concentration equal 36.47, 39.56, and 43.11 µg/mL and the toxicity index (Ti 0.595, 0.548 and 0.503), respectively at the same concentration against *T. cinnabarinus*. Meanwhile, protocatechuic acid (**7**) was the most toxic compound after abamectin against *T. urticae* as it has the lowest LC_{50} (0.232) with toxicity index (Ti 73.71). The *n*-hexane extract and afzelin (**2**) come next with median lethal concentrations 16.00 and 27.98 µg/mL and toxicity index (Ti 1.069 and 0.611), respectively at the same concentration against *T. urticae*.

Abamectin recorded the highest mortality numbers for *T. cinnabarinus* and *T. urticae*. However, afzelin (**2**) has the faster lethal effect after abamectin as the mean mortality values were 8.66, 8.33, 6.33, and 5.33 after 7 days for concentrations 200, 100, 50 and 25 µg/mL, respectively against *T. cinnabarinus*, followed by the EtOAc extract with mean mortality values 8.66, 6.33, 5.33, and 4.68 after 7 days for the aforementioned concentrations (Table 3). Regarding *T. urticae*, protocatechuic acid (**7**) achieved the highest mortality after abamectin as the mean mortality values were 9.66, 9.33, 9 and 8.33 followed by *n*-hexane extract with mean mortality values 9.66, 8, 7.33 and 6.33 after 7 days-exposure to the concentrations 200, 100, 50 and 25 µg/mL, respectively (Table 4).

Statistical analysis showed very highly statistically significant differences in mortality among all concentrations and tested materials for both *T. cinnabarinus* and *T. urticae* (Tables 3 & 4). In case of 7 days-exposure of *T. cinnabarinus* and *T. urticae* to concentration 200 µg/mL, there were not statistically significant or highly statistically significant changes observed, respectively, through all tested compounds.

Brazilian pepper tree (*Schinus terebinthifolius* Raddi) is a source of many bioactive compounds, such as terpenoids and flavonoids which have various biological properties. Essential oils obtained through extraction by solvents from berries and leaves are rich in monoterpenes and have strong *in vitro* antibacterial and antifungal actions against numerous

Table 1: Median lethal time (LT_{50}, days) of *n*-hexane, EtOAc extracts and compounds (1 - 9) of *Schinus terebinthifolius* against *Tetranychus urticae and Tetranychus cinnabarinus* at a concentration of 200 µg/mL and abamectin at a concentration of 50 µg/mL.

Test samples	Median lethal time (LT_{50}) (days)							
	Tetranychus cinnabarinus				*Tetranychus urticae*			
	LT_{50}	Confidence intervals*		Slope±S.E.	LT_{50}	Confidence intervals*		Slope±S.E.
		From	To			From	To	
1	5.05	-	-	1.60±0.42	4.27	2.78	8.36	1.29±0.39
2	3.72	2.79	4.41	3.86±0.97	3.93	3.23	4.70	3.54±0.64
3	5.23	3.72	9.51	1.61±0.42	8.11	-	-	1.21±0.41
4	7.45	6.12	12.80	3.88±1.15	6.12	4.37	12.08	1.70±0.445
5	4.55	-	-	2.59±0.52	5.43	3.96	9.38	1.77±0.44
6	4.76	3.90	5.66	3.12±0.62	5.09	4.32	6.15	4.15±0.99
7	2.98	2.09	4.05	1.86±0.40	1.73	1.25	2.20	2.89±0.50
8	4.93	4.13	5.96	3.96±0.97	3.92	2.88	5.62	1.86±0.42
9	6.78	5.04	12.46	2.08±0.52	3.48	2.56	4.74	1.97±0.417
EtOAc	3.75	-	-	2.14±0.44	4.13	3.88	5.86	1.96±0.431
n-Hexane	5.50	4.73	6.74	4.37±1.03	2.86	2.30	3.41	3.73±0.574
Abamectin50 µg/mL	0.70	0.08	1.30	1.34±0.41	0.50	-	-	1.07±0.40

*Confidence intervals were calculated at *p*-level < 0.05.
*Upper and lower limits calculated only for the data of which g is less than 0.4.

Table 2: Toxicity of *n*-hexane, EtOAc extracts and compounds (1 - 9) of *Schinus terebinthifolius* against adult females of *Tetranychus cinnabarinus* and *Tetranychus urticae*, after 7 days of treatment.

Test samples	Median lethal Concentration (LC_{50}) (µg/mL)							
	Tetranychus cinnabarinus				*Tetranychus urticae*			
	LC_{50} Confidence intervals*	LC_{90}	Slope±S.E.	Toxicity index %	LC_{50} Confidence intervals*	LC_{90}	Slope±S.E.	Toxicity index %
1	52.22 (24.91- 82.66)	611.93	1.20±0.36	0.416	56.21 (26.35- 93.15)	765.13	1.13±0.36	0.304
2	22.32 (3.95- 38.27)	239.08	1.24±0.39	0.972	27.98 (9.08- 43.83)	237.89	1.38±0.39	0.611
3	73.97 (41.59- 138.30)	1018.75	1.13±0.36	0.293	84.56 (51.16- 167.60)	1075.99	1.16±0.36	0.202
4	132.96 (91.37- 270.66)	942.61	1.51±0.38	0.163	126.41 (79.80-363.20)	1558.11	1.17±0.36	0.135
5	58.54 (41.46-78.67)	269.30	1.93±0.39	0.371	62.13 (33.27-102.72)	745.15	1.19±0.36	0.275
6	43.11 (16.06- 68.55)	552.74	1.16±0.36	0.503	47.05 (19.02- 75.27)	615.26	1.15±0.36	0.363
7	39.56 (13.97- 62.51)	479.81	1.18±0.36	0.548	0.232 (--- - ---)	102.28	0.49±0.30	73.71
8	99.45 (73.52-148.88)	487.89	1.86±0.39	0.218	67.86 (41.23-108.49)	641.50	1.31±0.36	0.252
9	119.64 (76.21-312.42)	1417.05	1.19±0.36	0.181	70.86 (48.56-103.49)	441.9	1.61±0.37	0.241
EtOAc	36.47 (12.25- 57.52)	419.73	1.21±0.37	0.595	44.51 (15.94- 71.99)	627.54	1.12±0.36	0.384
n-Hexane	104.57 (73.65-177.38)	687.42	1.57±0.37	0.208	16.00 (1.86- 29.40)	145.04	1.34±0.42	1.069
Abamectin	0.217 (0.028- 0.642)	37.85	0.572±0.12	100.00	0.171 (0.027- 0.472)	16.09	0.65±0.13	100.00

*Confidence intervals were calculated at *p*-level < 0.05.
*Upper and lower limits calculated only for the data of which g is less than 0.4.
*Toxicity index was calculated with respect to abamectin as the most effective compound.

Table 3: Impact of treatments with *n*-hexane, EtOAc extracts and compounds (1 - 9), isolated from *Schinus terebinthifolius* leaves, against *Tetranychus cinnabarinus* under laboratory conditions.

Test samples	200 µg/mL				100 µg/mL				50 µg/mL				25 µg/mL			
	1 day	3 days	5 days	7 days	1 day	3 days	5 days	7 days	1 day	3 days	5 days	7 days	1 day	3 days	5 days	7 days
1	2^{bc}	2cd	4.33^{bc}	7.33^{bc}	1.66^{b}	2.33^{bc}	5c	6.66^{bcd}	1.33^{b}	2.66^{b}	4^{b}	$5b^{cd}$	0.66^{b}	1.33^{b}	3^{b}	3.33^{cde}
2	0e	3.66^{bc}	6.66^{b}	8.66^{ab}	$1b^{cd}$	2.66^{b}	7b	8.33^{ab}	0c	0.33^{d}	3.33^{bc}	6.33^{b}	0^{c}	0.33^{bc}	2.33^{bc}	5.33^{b}
3	1.66^{bcd}	2.66^{bcd}	$4b^{cd}$	$7b^{c}$	1.33^{bc}	2.33^{bc}	2.66^{d}	5.33^{cde}	0c	0.66^{cd}	2.66^{bcd}	4.33^{cde}	0^{c}	0.33^{bc}	1.66^{bcd}	3^{cdef}
4	0^{e}	1^{d}	1.33^{d}	5.33^{c}	0d	1.66^{bcd}	2.66^{d}	5.66^{cde}	0c	1^{cd}	1^{d}	2^{f}	0^{c}	0.33^{bc}	0.66^{d}	1.33^{f}
5	1^{cde}	2^{cd}	4^{bcd}	8.66^{ab}	0.66^{bcd}	2^{bcd}	4.33^{cd}	$7b^{c}$	0.33^{bc}	0.66^{cd}	2^{bcd}	3.33^{def}	0^{c}	0.33^{bc}	1.66^{bcd}	3^{cdef}
6	0.33^{e}	2.33^{bcd}	4.66^{bc}	7.66^{abc}	0.33^{Cd}	1.33^{bcd}	4.33^{cd}	6.66^{bcd}	1bc	1.66^{bc}	4^{b}	5.66^{bc}	0.33^{bc}	1^{bc}	3^{b}	3.66^{bcd}
7	2.33^{b}	4^{b}	6.33^{b}	8.33^{ab}	0^{d}	0.66^{cd}	2.66^{d}	6.66^{bcd}	0.66^{bc}	1.33^{cd}	2.33^{bcd}	5.33^{bc}	0^{c}	0.33^{bc}	1^{cd}	4.33^{bc}
8	0e	2^{cd}	5^{bc}	7.33^{bc}	0^{d}	1.66^{bcd}	3.33^{cd}	$5d^{e}$	0^{c}	1^{cd}	1.33^{cd}	2.33^{f}	0^{c}	0.33^{bc}	0.66^{d}	1.66^{ef}
9	0.66^{de}	2^{cd}	2.66^{cd}	6.33^{bc}	0.33^{cd}	1.33^{bcd}	3d	4.33^{e}	0.33^{bc}	0.66^{cd}	2.66^{bcd}	3^{ef}	0^{c}	0.33^{bc}	1.66^{bcd}	$2.33d^{ef}$
EtOAc	1.66^{bcd}	3^{bc}	5^{bc}	8.66^{ab}	0.33^{cd}	$2b^{cd}$	3.66^{cd}	6.33^{cd}	0.33^{bc}	0.66^{cd}	2.66^{bcd}	5.33^{bc}	0^{c}	0.33^{bc}	1.66^{bcd}	4.68^{bc}
n-Hexane	0^{e}	1^{d}	5^{bc}	6.33^{bc}	0^{d}	0.33^{d}	3^{d}	5.66^{cde}	0.33^{bc}	1^{cd}	1.33^{cd}	2.66^{ef}	0^{c}	0^{c}	0.33^{d}	1.66^{ef}
Abamectin	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}	6.33^{a}	8^{a}	8.66^{a}	9.66^{a}	6^{a}	6.66^{a}	8^{a}	9^{a}
LSD	1.256	1.945	2.918	2.589	1.087	1.753	1.904	1.753	1.012	1.256	2.157	1.753	0.628	1.012	1.588	1.945
F	41.39	12.88	4.62	2.14	56.21	17.31	11.31	6.45	25.90	23.72	7.58	12.81	63.76	27.76	14.0	9.952
P	0.0000 ***	0.0000 ***	0.0000 ***	0.575 ns	0.0000 ***	0.0000 ***	0.0000 ***	0.0001 ***	0.0000 ***	0.0000 ***	0.0000 ***	0.0000 ***	0.0000 ***	0.0000 ***	0.0000 ***	0.0000 ***

Each value presented the mean mortality number of three replicates.
Means in each column followed by the same letter (s) indicates that there is no significant difference while different letter(s) in same column indicates that there is a significant difference at $p \leq 0.05$ according to Duncan's multiple range test.

bacterial strains and fungi (as Candida) (Patocka and Diz de Almeida 2017). The present study showed for the first time, as far as we know, the acaricidal activity of compounds extracted from the medicinal plant *Schinus terebinthifolius*

Table 4: Impact of treatments with *n*-hexane, EtOAc extracts and compounds (1 - 9), from *Schinus terebinthifolius* leaves, against *Tetranychus urticae* under laboratory conditions.

Test samples	200 µg/mL				100 µg/mL				50 µg/mL				25 µg/mL			
	1 day	3 days	5 days	7 days	1 day	3 days	5 days	7 days	1 day	3 days	5 days	7 days	1 day	3 days	5 days	7 days
1	2.66^{b}	3^{bc}	4.66^{efg}	7.33^{bc}	0.66^{bc}	3.33^{bc}	5^{bc}	6.33^{de}	1^{bc}	2.66^{b}	3.33^{bcd}	4.33^{de}	0.33^{b}	1.66^{cd}	3^{bc}	3.66^{cd}
2	0.33^{d}	3^{bc}	6^{de}	8.66^{ab}	0.66^{bc}	1.66^{cd}	5.66^{b}	8.33^{abc}	0.33^{bc}	0.66^{c}	3.33^{bcd}	5.33^{cd}	0^{b}	0.66^{cd}	2.66^{bcd}	5^{bc}
3	2^{bcd}	2^{c}	2.33^{h}	6.66^{c}	0.66^{bc}	0.66^{d}	2^{d}	5.33^{ef}	0.33^{bc}	1.66^{bc}	3.33^{bcd}	4^{de}	0^{b}	0.33^{d}	1.66^{cd}	2.66^{de}
4	1bcd	3^{bc}	3.66^{gh}	6^{c}	0.33^{c}	1.66^{cd}	2.66^{d}	4.33^{f}	0^{c}	1.33^{bc}	2^{cd}	3.33^{e}	0^{b}	1^{cd}	1.66^{cd}	2^{e}
5	1.33^{bcd}	2.66^{bc}	3.66^{gh}	7^{bc}	0.66^{bc}	1.33^{d}	2.33^{d}	6.33^{de}	0.33^{bc}	2^{bc}	3^{bcd}	4.66^{de}	0.33^{b}	1.66^{cd}	2.66^{bcd}	3^{de}
6	0.33^{d}	2^{c}	$4f^{gh}$	7.66^{bc}	0c	2^{bcd}	5^{bc}	6.33^{de}	0.33^{bc}	1.66^{bc}	3.33^{bcd}	5.33^{cde}	0^{b}	1.33^{cd}	2.66^{bcd}	3.66^{cd}
7	2.33^{bc}	8^{a}	9.66^{ab}	9.66^{a}	0.66^{bc}	2^{bcd}	8.66^{a}	9.33^{ab}	1.33^{b}	7^{a}	8.33^{a}	9^{ab}	0.66^{b}	5^{b}	7.66^{a}	8.33^{a}
8	1.66^{bcd}	3.33^{bc}	5.66^{def}	7.33^{bc}	$1b^{c}$	3.33^{bc}	5.33^{bc}	6^{ef}	0.33^{bc}	1^{bc}	3.33^{bcd}	4^{de}	0^{b}	0.66^{cd}	2.66^{bcd}	3^{de}
9	1.66^{bcd}	3.66^{bc}	6.66^{cd}	7.33^{bc}	$1b^{c}$	3.33^{bc}	5.33^{bc}	6.66^{cde}	0.33^{bc}	1^{bc}	1.66^{d}	3.66^{de}	0^{b}	0.33^{d}	1^{d}	2.33^{de}
EtOAc	1.66^{bcd}	2.33^{bc}	6^{de}	7.33^{bc}	0.66^{bc}	2^{bcd}	5.33^{bc}	7^{cde}	0^{c}	0.66^{c}	4.33^{bc}	5.33^{cde}	0^{b}	0.33^{d}	3^{bc}	3.66^{cd}
n-Hexane	0.66^{cd}	4.66^{b}	8^{bc}	9.66^{a}	1.66^{b}	3.66^{b}	5.33^{bc}	8^{bcd}	$1b^{c}$	2.66^{b}	5.33^{b}	7.33^{bc}	0^{b}	2^{c}	4.33^{b}	6.33^{b}
Abamectin	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}	6.66^{a}	7.33^{a}	8^{a}	9.66^{a}	6.33^{a}	6.66^{a}	7.66^{a}	9.33^{a}
LSD	1.753	2.365	1.904	1.82	1.158	1.925	2.005	1.798	1.050	1.731	2.432	2.28	0.743	1.538	1.820	1.66
F	18.48	9.47	13.63	4.32	46.52	13.46	12.24	7.07	25.87	14.95	6.43	7.08	50.32	14.36	12.04	17.52
P	0.0***	0.0***	0.0***	0.0013**	0.0***	0.0***	0.0***	0.0***	0.0***	0.0***	0.0001***	0.0***	0.0***	0.0***	0.0***	0.0***

Each value presented the mean mortality number of three replicates.
Means in each column followed by the same letter (s) indicates that there is no significant difference while different letter(s) in same column indicates that there is a significant difference at $p \leq 0.05$ according to Duncan's multiple range test.

leaves against the carmine spider mite, *T. cinnabarinus* and two spotted spider mite, *T. urticae*. It has been shown that different concentrations of all tested compounds caused mortality for adult females. In this regard, Choi et al. (2004) recorded fumigant activity for oils of caraway seed, lemon eucalyptus and peppermint against *T. urticae* (Choi et al. 2004). Miresmailli et al. (2006) considered rosemary oil as an acaricide against the two-spotted spider mite (Miresmailli and Isman 2006).

do Nascimento et al. (2012) extracted the essential oils from unripe and ripe fruits of *S. terebinthifolius* and proved their toxicity against *T. urticae*; LC_{50} values for the unripe fruit oil in the fumigation and contact tests were 1.46 µL/L of air and 3.04 µL/cm^2, respectively (Nascimento et al. 2012). In their study, these essential oils strongly affect the behaviour of these mites, toxicity varied according to the type of oil and method used, and both oils showed a potent repellent effect.

CONCLUSIONS

The acaricidal properties demonstrated by the leaves extracts of *Schinus terebinthifolius* show promise towards the use of plant extracts as echo-friendly and effective alternative for green control of tetranychid mites. Additionally, the current study opens doors towards the use of simple phenolics (such as protocatechuic acid) and flavonoids (such as afzelin) as natural leads for future development of safe and efficient acaricides against tetranychid mites.

AUTHORS' CONTRIBUTIONS

Doaa A. Abou El Atta and Fatma M. Abdel Bar conceived the research. Mariam G. Habashy, Doaa A. Abou El Atta, Fatma M. Abdel Bar and Sahar R. Gedara conducted the experiments and analyzed the data obtained. Mariam G. Habashy and Fatma M. Abdel Bar wrote the manuscript. All authors read and approved the manuscript.

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