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Sustainable control of *Tetranychus urticae* (Trombidiformes: Tetranychidae) using plant-derived oils on squash and eggplant under field conditions

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ABSTRACT

As global agriculture intensifies, the need for effective and environmentally sustainable pest control strategies has become critical. Synthetic pesticides, while effective, pose considerable risks to human health, ecological balance, and contribute to the development of pest resistance. This study investigates the dual acaricidal and antioxidant potential of essential oils (EOs) derived from *Annona* Abdel Razik hybrid, *Citrullus colocynthis*, and *Eucalyptus citriodora* against the two-spotted spider mite (*Tetranychus urticae*), a major pest of vegetable crops. The oils were extracted and chemically characterized using GC-MS and analyzed for their fixed oil fatty acid profiles. In addition to their pest control efficacy, the antioxidant activities of the oils were assessed using DPPH• and ABTS^{+}• radical scavenging assays. *Annona* oil demonstrated the strongest antioxidant effect in the DPPH assay with an IC_{50} of (1.318 µg/mL), surpassing both vitamin C (1.664 µg/mL) and Trolox (2.073 µg/mL). In the ABTS assay, the mixed oil formulation (1:1:1) exhibited the highest scavenging capacity (IC_{50} = 1.337 µg/mL), indicating a potential synergistic effect among the three oils. These results reflect the oils' capacity to neutralize free radicals and suggest additional health and shelf-life benefits if incorporated into crop protection strategies. Laboratory acaricidal bioassays showed significant mite mortality with increasing EO concentrations, with *Annona* oil being the most potent (LC_{50} = 0.17%). Field trials conducted on eggplant and squash in Qalubia and Fayoum Governorates confirmed the efficacy of the EO mixture, which achieved the highest and most consistent reduction in *T. urticae* populations up to 76.37% on squash and 70.47% on eggplant. These findings underscore the potential of EO-based formulations, particularly in combination, as eco-friendly alternatives to synthetic acaricides. Their added antioxidant properties further enhance their value in sustainable pest management and crop protection systems.

KEYWORDS

Acaricidal activity, Antioxidant activity, Eco-friendly alternatives, GC–MS characterization, Natural oils, two-spotted spider mite

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INTRODUCTION

Pest management techniques that successfully strike a balance between efficacy and environmental sustainability are becoming increasingly important as global agriculture gets more intensive. The demand for pest management techniques that successfully implement a balance between efficacy and environmental sustainability has increased due to the expansion of global agriculture. Concerns regarding the long-term effects of synthetic pesticides on ecosystems and human health have led to a change in focus toward investigating alternative strategies (Ali *et al.* 2021). There are many difficulties due to the high reliance on chemical pesticides. On the one hand, it seriously endangers both the environment and human health (Basit 2019). However, the rate at which pests are becoming resistant is concerning. Highly resistant strains have emerged as a result of their quick reproduction, short life cycles, and repeated exposure to pesticides (Wu *et al.* 2016). The two-spotted spider mite (*Tetranychus urticae* Koch) is a widespread and economically damaging pest that infects a variety of crops, including vegetables, fruit trees, ornamentals, and field crops. A key issue in treating *T. urticae* is its outstanding adaptability and capacity to develop resistance to practically every class of acaricides introduced, including organophosphates, pyrethroids, carbamates, avermectins, and ketoenols (Kim *et al.* 2021). The two-spotted spider mite, a polyphagous pest, causes significant harm to economically important crops in protected farming systems and irrigated areas worldwide (Jakubowska *et al.* 2022).

Essential oils (EOs) are unique active components that are prized for their biological characteristics. These oils have drawn a lot of attention from a variety of industries, including pharmaceuticals and cosmetics, because of their widespread acceptability and natural and safe status. By customers, and adaptable functional characteristics (Jugreet *et al.* 2020). Growing interest has been seen in using essential oils (EOs) as biopesticides to manage pests in recent years (Chaudhari *et al.* 2021). Concerns about environmental contamination, pesticide resistance, and human health can all be addressed with EOs through their use in pest control (Katopodi and Detsi 2021). *Tetranychus urticae*, the spider mite, is a pest that is found all over the world and seriously damages agricultural crops economically. According to Farazmand and Amir-Maafi (2018), essential oils are volatile and odorous phytochemical products of plants' secondary metabolism. They have been widely utilized as pesticides and repellents (Nikolaou *et al.* 2021). The objective of the present study was to evaluate the efficacy of three Natural oils in controlling *T. urticae* under both laboratory and open-field conditions.

MATERIAL AND METHODS

Natural oils (plant material)

Three plant species, soursop Egyptian hybrid, *Annona cherimola* × *Annona squamosa*, known as Abdel Razek (AARH) (Annonaceae), bitter apple *Citrullus colocynthis* (Cucurbitaceae) and leaves of lemon-scented gum *Eucalyptus citriodora* (Myrtaceae) were sourced from the National Research Centre (NRC), Egypt and included for essential oil extraction and analysis.

Essential oil extraction

Fresh plant materials were washed, shade-dried, and coarsely ground before undergoing hydro-distillation using a Clevenger-type apparatus for 4.5 hours. The collected essential oils were dried over anhydrous sodium sulfate, stored at 4 °C in airtight amber vials, and prepared in triplicate for further analysis. The average oil yield from the triplicate extractions was documented (Guenther 1952; Mohammed *et al.* 2022).

Fixed oil extraction and fatty acid methyl ester (FAME) preparation

Fixed oils were extracted from powdered fruits of *Annona* Abdel Razik hybrid and *C. colocynthis*. Two grams of each sample were subjected to Soxhlet extraction using 100 mL of petroleum ether for four hours. The solvent was evaporated, and the residue was weighed to determine the total lipid content.

For FAME preparation, the extracted lipids underwent alkali-catalyzed transesterification by reacting with 2M potassium hydroxide (KOH) in methanol. The methyl esters were extracted with hexane and stored at −20 °C until gas chromatographic analysis (Olubomehin *et al.* 2020).

GC-MS analysis of essential oils and GC analysis of FAMEs from fixed oils

The volatile components of the essential oils were analyzed using an Agilent 7890B gas chromatograph coupled with a 5977A mass selective detector (MSD) equipped with a DB-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Helium was employed as the carrier gas at a constant flow rate. The oven temperature program was initiated at 40 °C (held for 2 min.), then increased to 320 °C at a rate of 5 °C/min. Mass spectra were obtained by electron ionization (EI) at 70 eV. Constituents were identified by comparison with the Wiley and NIST Mass Spectral Library databases (Mohammed *et al.* 2022).

FAME profiling was conducted using an Agilent 7890B GC system equipped with a flame ionization detector (FID) and a Zebron ZB-FAME column (30 m × 0.25 mm i.d., 0.20 µm film thickness). Hydrogen was used as the carrier gas. The oven temperature was programmed from 100 °C to 240 °C at a rate of 4 °C/min. The injector and detector temperatures were set to 250 °C and 285 °C, respectively (Hassan *et al.* 2020)

In vitro *antioxidant assays of tested essential oils*

The antioxidant activity of the oil samples was assessed in vitro using two standard assays: 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging methods (Taleb *et al.* 2024). Samples were tested at concentrations of 1.25, 5, 10, 15, and 25 µg/mL. Ascorbic acid and Trolox were used as positive controls (Dinkova-Kostova *et al.* 2007). ABTS + radical scavenging activity was evaluated following the method described in, while DPPH radical scavenging was performed according to the percentage inhibition of radicals was calculated using the formula:

Inhibition (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$ (Shimada et al. 1992; Ellaithy *et al.* 2022).

Acaricides

Tetranychus urticae *colony*

Tetranychus urticae colony was prepared at the Plant Protection Research Institute Giza Egypt. *Tetranychus urticae* was grown Acalypha (*Acalypha marginata*) leaves placed on moist cotton and kept in incubator at 25 ± 1 °C and 65 ± 5% RH and 16/8 h (L/D). Acalypha leaves were replaced when necessary (Ellaithy *et al.* 2022).

Toxicity of tested essential oils against T. urticae *in the laboratory*

Leaf discs of *A. marginata* (2 cm diameter) were individually placed upside down on moist cotton inside Petri dishes. Fifteen adult females of *T. urticae* were transferred onto each disc. Treatments were applied using a hand-held precision glass atomizer (manual laboratory sprayer) to ensure uniform and fine droplet distribution. Each disc was sprayed evenly from a fixed distance of approximately 20 cm, delivering 2 mL of solution per replicate to achieve complete and homogeneous coverage of the leaf surface. Four concentrations (1, 0.5, 0.25, and 0.125%) of the essential oils (AARH), *C. colocynthis*, and *E. citriodora* were tested, with four replicates per treatment. Control discs were sprayed with 2 mL of distilled water containing 0.01% Tween 80. Mortality was recorded after 24 hours of treatment.

Toxicity of tested essential oils against T. urticae *in the field studies*

The field experiment took place in Qalubiya and Fayoum Governorates, Egypt, during the spring season of 2024. For this study, two different vegetable crops, eggplant (*Solanum melongena*) and zucchini (*Cucurbita pepo*), were grown under identical environmental conditions.

Experimental design

The experimental design comprised planting eggplant and zucchini on a 472.5 m² area divided into two equal plots (236.5 m²/Crop) with five repetitions each, using Randomized Complete Block Design. Standard agricultural practices were followed, with no pesticides utilized.

The growing plants were sprayed with ($LC_{90} \times 2$) of the essential oils tested (soursop at 7 ml/l, bitter apple at 11 ml/l, and lemon-scented gum at 14 ml/l), and a mixture of equal parts of these three essential oils was also evaluated. The control was treated with water with 0.01% Tween 80.

Application of essential oils

Essential oils were applied using a 25-L capacity plastic sprayer. All treatments were applied once per season, three weeks after planting (April 2024). Sampling was conducted randomly before treatment and at 1, 2, 3, 5, and 7 days after application. The sample size (40 leaves per replicate) was determined based on standard sampling procedures for estimating *T. urticae* population density in field experiments. The number of leaves required was calculated using the conventional formula for estimating population mean with a defined precision level: $n = (t^2 x\ S^2 / d^2)$. Where *n* is the required sample size, t^2 is the t-value at 95% confidence level, S^2 is the variance obtained from preliminary observations, and *d* is the desired level of precision (Henderson and Southwood 2016). Preliminary field counts indicated that 40 leaves per replicate provided an acceptable standard error and reliable estimation of mite population density. Accordingly, 40 leaves were randomly collected per replicate from both treated and control plots at each sampling date. The collected leaves were placed in labeled paper bags and examined individually in the laboratory under a stereoscopic binocular microscope. This sampling approach follows the methodology described by Abd El-Karim *et al.* (2024).

Statistical analysis

The average mortality rate (± S.E.) of mites was determined as a percentage of dead females. The concentration and mortality rates of adult females were determined using Abbott's formula. LC_{50}, LC_{90} and slope values were calculated according to Finney method (Gharedaashi *et al.* 2013) and using (Ldp line) software by Bakr (Abdelfattah and Zinhoum 2024). The percentage reduction in mite infestation for each treatment was calculated using Henderson and Tilton's formula (Abdallah 2021): as follows:

$$\text{Reduction (\%)} = \left(1 - \frac{\text{Treatment after} \times \text{Control before}}{\text{Treatment before} \times \text{Control after}}\right) \times 100$$

Collection, regression and ANOVA calculated in SAS were used to evaluate the collected data. The analysis of data was performed on each dependent by using LSD test (P = 0.005).

RESULTS

Natural oils (volatile oil composition of **E. citriodora***)*

The volatile oil extracted from *E. citriodora* was analyzed using gas chromatography–mass spectrometry (GC-MS), and 18 major constituents were identified based on their retention times, molecular formulas, and relative abundances (Table 1). These compounds represent the principal components of the essential oil, contributing to both its chemical identity and biological activity. The oil was dominated by oxygenated monoterpenes, with citronellal comprising 72.32% of the total oil composition. Citronellol (8.11%) and citronellyl citronellate (5.93%) followed in abundance, together accounting for over 86% of the oil. This dominance underscores their central role in the oil's characteristic lemon-like aroma and biological function. Citronellal, the primary constituent, is widely recognized for its antimicrobial, antifungal, and insect-repellent properties. Its high concentration corroborates prior reports identifying citronellal as the major active agent in *E. citriodora* oil, particularly in pest control applications. Its known efficacy against a range of arthropods including mites highlights its relevance as a potential acaricidal agent in this study (Chaisri *et al.* 2019; Venancio *et al.* 2025). Minor constituents such as L-α-pinene (1.4%), eucalyptol (0.81%), and m-cymene (0.79%) were also detected. These monoterpenes possess anti-inflammatory and bronchodilatory properties and may contribute synergistically to the oil's bioactivity (de Cássia da Silveira e Sá *et al.* 2013). Notably, some of these components have been associated with neurotoxic effects on mite nervous systems (Danna *et al.* 2024), supporting their potential role in enhancing acaricidal effectiveness. Sesquiterpenes and their oxygenated derivatives including caryophyllene (1.14%), 14-hydroxycaryophyllene (0.14%), and ageratriol (0.06%) were present in lower concentrations but are known to exhibit antioxidant, cytoprotective, and anti-inflammatory properties. While not the primary acaricidal agents, they may interact with monoterpenes to produce enhanced bioactivity through complementary mechanisms. The presence of isopulegol (2.42%), a biosynthetic precursor of menthol, suggests a role in sensory modulation through cooling and irritant effects that may interfere with mite feeding and mobility. Additionally, long-chain esters and epoxides such as 1,2-15,16-diepoxyhexadecane (1.92%) and methyl 10,11-tetradecadienoate (1.06%) may influence the oil's physical properties, including its viscosity and oxidative stability, potentially prolonging its activity in applied settings.

Table 1. The primary components of the Essential oil from *Eucalyptus citriodora.*

Peak	RT	Name	Formula	Area Sum %
1	2.045	5,5-Dimethyl-cyclohex-3-en-1-ol	$C_8H_{14}O$	1.2
2	2.812	L-alpha-Pinene	$C_{10}H_{16}$	1.4
3	3.378	8-p-Menthadiene	$C_{10}H_{16}$	1.23
4	4.076	m-Cymene	$C_{10}H_{14}$	0.79
5	4.168	Eucalyptol	$C_{10}H_{18}O$	0.81
6	4.528	Methyl 10,11-tetradecadienoate	$C_{15}H_{26}O_2$	1.06
7	4.9	1,5,5-Trimethyl-6-methylene-cyclohexene	$C_{10}H_{16}$	0.11
8	6.039	***Citronellal**	$C_{10}H_{18}O$	**72.32**
9	6.228	Isopulegol	$C_{10}H_{18}O$	2.42
10	7.046	***Citronellol**	$C_{10}H_{20}O$	**8.11**
11	7.441	1,2-15,16-Diepoxyhexadecane	$C_{16}H_{30}O_2$	1.92
13	8.534	citronellyl citronellate	$C_{20}H_{36}O_2$	5.93
14	8.934	Isodihydrocarveol	$C_{10}H_{18}O$	0.37
15	9.283	Caryophyllene	$C_{15}H_{24}$	1.14
16	10.925	14-Hydroxycaryophyllene	$C_{15}H_{24}O$	0.14
17	11.578	Ageratriol	$C_{15}H_{24}O_3$	0.06
18	12.15	2-Naphthaleneethanol	$C_{15}H_{24}O$	0.15

Fatty acid composition of fixed oils

The fatty acid profiles of the fixed oils extracted from AARH and *C. colocynthis* were evaluated via GC analysis of their corresponding FAMEs. A total of 12 fatty acids were identified across both samples, with significant variation in their qualitative and quantitative distribution (Table 2).

In AARH oil, oleic acid (C18:1) was the predominant fatty acid, representing 47.00% of the total fatty acid content. This was followed by linoleic acid (C18:2, 22.97%) and palmitic acid (C16:0, 14.36%), highlighting a lipid profile rich in both monounsaturated and polyunsaturated fatty acids. Notably, stearic acid (C18:0) accounted for 10.82%, while minor contributions were observed from linolelaidic acid (1.31%), linolenic acid (0.91%), arachidic acid (0.88%), and others. These results align with previously reported data on the lipid profile of some *Annona* species, which are known for their high oleic acid content, supporting their use in nutraceutical and dermatological formulations (Mohammed *et al.* 2022). Conversely, *C. colocynthis* oil exhibited a markedly different fatty acid profile. Linoleic acid was the most abundant, comprising 67.76% of the total fatty acids, which is consistent with its classification as a linoleic-rich seed oil. Oleic acid was the second major component (14.56%), followed by palmitic acid (9.77%) and stearic acid (6.74%). Trace amounts of linolenic acid (0.15%), arachidic acid (0.36%), and other long-chain saturated fatty acids were also detected, though at considerably lower levels than in AARH. Interestingly, *C. colocynthis* lacked detectable levels of certain fatty acids present in AARH, such as palmitoleic, margaric, and linolelaidic acids. The significant variation in fatty acid profiles between the two oils underscores their potential for distinct biological applications. Oils rich in oleic acid, such as that of AARH, are associated with anti-inflammatory, cardioprotective, and skin-penetrating properties. This profile suggests suitability for cosmetic and therapeutic uses, particularly in wound healing and emollient formulations.

The high linoleic acid content observed in *C. colocynthis* oil (67.76%) may play a significant role in its acaricidal activity against *T. urticae*. Linoleic acid, an unsaturated omega-6 fatty acid, has been previously reported to possess notable acaricidal and repellent properties. Barrozo *et al.* (2021) demonstrated that linoleic acid and its derivatives, derived from coconut oil, exhibited significant efficacy against *Amblyomma sculptum* (Acari: Ixodidae), suggesting a broader potential for this compound in mite and tick control strategies. In our study, the abundance of linoleic acid likely contributes to the observed bioactivity, possibly through membrane disruption or interference with the nervous system of the pest. These findings are consistent with earlier reports and further support the inclusion of linoleic acid–rich oils in natural acaricide formulations for integrated pest management.

Table 2. Fatty Acid Composition (%) of fixed oils extracted from AARH and *Citrullus colocynthis* based on GC/MS.

Peak	RT	Name	AARH (%)	*C. colocynthis* (%)
1	26.82	Palmitic acid	14.36	9.77
2	27.98	Palmitoleic acid	0.45	-
3	29.60	Margaric acid	0.21	-
4	32.24	Stearic acid	10.82	6.74
5	33.09	**Oleic acid**	47	14.56
6	34.01	Linolelaidic acid	1.31	-
7	34.81	Linoleic acid	22.97	**67.76**
8	36.96	Linolenic acid	0.91	0.15
9	37.32	Arachidic acid	0.88	0.36
10	38.04	*cis*-11-Eicosenoic acid	0.16	0.18
11	42.12	Behenic acid	0.14	0.16
12	46.54	Lignoceric acid	0.78	0.17

The *in vitro* antioxidant capacities of vitamin C, Trolox, and three natural oils AARH, *E. citriodora*, and *C. colocynthis* as well as a mixture of these oils, were evaluated using both DPPH• and $ABTS^{+}$• radical scavenging assays. The results were interpreted through nonlinear regression (log [inhibitor] vs. response, three parameters) to determine IC_{50} values and evaluate the dose-response behavior (Table 1, Fig. 1). In the DPPH assay, *Annona* oil showed the strongest radical scavenging activity among the oils, with the lowest IC_{50} value (1.318 µg/mL), outperforming both reference standards, vitamin C (1.664 µg/mL) and Trolox (2.073 µg/mL). This high efficacy may be due to its enriched phenolic and acetogenin contents. The R^2 value of 0.9319 confirmed a well-fitted model.

Eucalyptus citriodora and *C. colocynthis* oils had moderate antioxidant activities with IC_{50} values of 2.914 µg/mL and 3.860 µg/mL, respectively. These values suggest considerable but comparatively lower radical scavenging capacity, potentially due to the presence of monoterpenes and polyunsaturated fatty acids. The mixed oil formulation showed an IC_{50} of 3.318 µg/mL, indicating synergistic effects between its constituents but still falling behind *Annona* oil in efficacy. The R^2 values for all oils and standards ranged from 0.8686 to 0.9842, suggesting reliable fits across treatments.

In the ABTS assay, a shift in activity was observed. While vitamin C (IC_{50} = 1.827 µg/mL) and Trolox (IC_{50} = 3.180 µg/mL) maintained strong antioxidant activities, the *Annona* oil's IC50 increased to 18.66 µg/mL, suggesting a lower radical scavenging potential in this assay compared to DPPH. This could be due to differences in the interaction of $ABTS^{+}$• with the oil's phytochemical profile, particularly if the oil components are more reactive toward DPPH• radicals. On the contrary, *C. colocynthis* and *E. citriodora* oils showed improved performance in the ABTS assay with IC_{50} values of 5.472 µg/mL and 8.372 µg/mL, respectively. The mixed oil demonstrated the lowest IC_{50} (1.337 µg/mL) in the ABTS assay, indicating strong synergistic or additive effects among its components and enhanced compatibility with $ABTS^{+}$• radicals. Model fitting was robust, with R^2 values ranging from 0.9745 to 0.9999, and notably low Sy.x values (e.g., 0.1882 for Eucalyptus), indicating minimal residual error and high predictive confidence. The confidence intervals (CI) for IC_{50} were relatively broad in some cases, particularly for *Annona*, possibly due to experimental variability or narrow dose ranges. Nonetheless, these findings validate the utility of both assays in differentiating antioxidant profiles of complex natural matrices (Aboul Naser *et al.* 2024).

The results highlight that antioxidant efficacy is assay-dependent and can vary significantly based on the radical species used. *Annona* oil was the most potent in the DPPH assay but less effective in the ABTS test, whereas the mixed oil excelled in the ABTS assay. This underscores the importance of using multiple antioxidant models to comprehensively evaluate the radical-scavenging capabilities of natural extracts.

Together, these findings demonstrate that the tested oils particularly AARH oil and the mixed formulation exhibit promising antioxidant activities, which could be leveraged in pharmaceutical or nutraceutical applications targeting oxidative stress-related disorders (Mohammed *et al.* 2024).

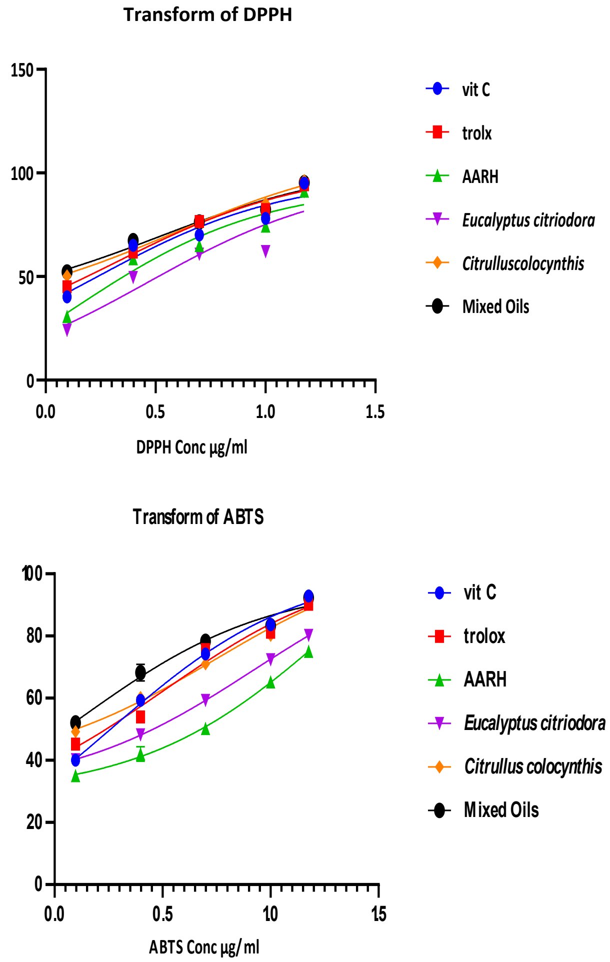

Figure 1. *In vitro* DPPH· and ABTS·+ antioxidant activity of standards with three natural oils plants.

Toxicity of tested essential oils against T. urticae *in laboratory*

Under controlled laboratory conditions, the acaricidal effects of three essential oils soursop (AARH), lemon-scented gum (*E. citriodora*), and bitter apple (*C. colocynthis*) were tested against *T. urticae* adult females. Four concentrations (1%, 0.5%, 0.25%, and 0.125%) were tested. Figure 2 shows that soursop oil is the most effective against *T. urticae*, with a mortality rate of 95 ± 1.7% at a concentration of 1% followed by bitter apple oil 87 ± 1.7% and lemon-scented gum oil 83 ± 1.9%, which had the same concentration, with no mortality observed in the control groups ($P < 0.000$). Mean mortality were 83 ± 1.9, 76 ± 1.9, and 73 ± 1.7% at a concentration of 0.5% with soursop oil, bitter apple oil, and lemon-scented gum oil, respectively. Mean mortality with 0.125% concentration was 43.3 ± 1.9, 35 ± 1.6, and 30.8 ± 0.8% with soursop oil, bitter apple oil, and lemon-scented gum oil, respectively, with no mortality observed in the control groups ($P < 0.000$).

The lethal concentration to achieve 50% mortality (LC50) was determined to be 0.17, 0.2, and 0.25% for soursop, bitter apple, and lemon-scented gum, respectively. LC_{90} was 0.7, 1.1, and 1.4%, with slope values of 2.08, 1.9, and 1.7 with soursop, bitter apple, and lemon-scented gum, respectively. AARH exhibited insecticidal activity from seed, leaves, bark, stems, roots, and flowers. Ethanolic extracts inhibited *Aedes aegypti* larvae (Hassan *et al.* 2020), indicate that aqueous leaf extract of *A. muricata* is used to manage many insects such as larvae of lepidopterans, aphids and thrips.

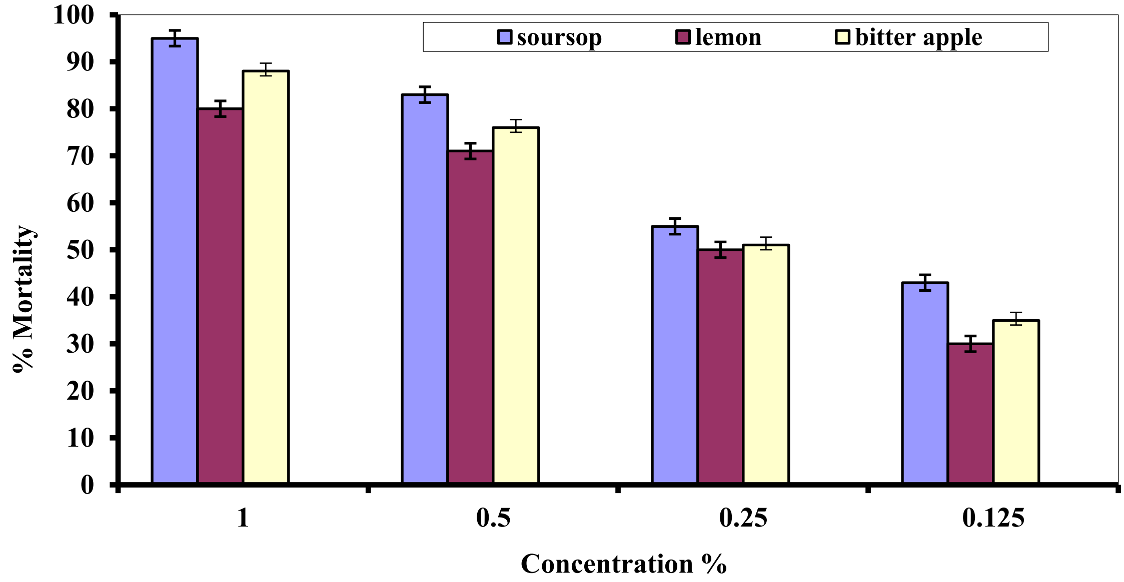

Figure 2. The average mortality rate (± SE) of *T. urticae* adult females treated with three natural oils.

Evaluation of essential oils against T. urticae *adult population on eggplant in the Governorates of Qalubia and Fayoum*

In the Governorates of Qalubia and Fayoum, four essential oils were tested against *T. urticae* adult populations on eggplant. The acute toxicity *of* AARH, *E. citriodora, C. colocynthis*, and a combination of three essential oils on *T. urticae.*

Table 3 shows that population reduction was examined in the effects of four distinct plant essential oils on *T. urticae* on eggplant at Qalubia Governorate was significantly different. After one to seven days of treatment, a mixture of three essential oils yielded was the highest 70.08% ± 1.88, followed by *Annona, Colocynthis, and Eucalptus* oils, 64.34 ± 1.91, 55.75 ± 2.0, and 53.00 ± 2.53 %, respectively.

Table 4 shows that there were significant changes when utilizing different four plant essential oils *on T. urticae* on eggplant at Fayoum Governorate. After one to seven days of treatment, the largest 70.47 ± 2.65% was attained when a mixture of three essential oils was employed, followed by *Annona, Colocynthis, and Eucalyptus oils* 68.02 ± 2.11, 60.37 ± 2.02, and 57.02 ± 3.35%, respectively. This demonstrates that the three oils were more effective in diminishing the population than either oil alone. *Tetranychus urticae* populations decreased at varied rates as a direct result of applications.

Table 3. Effect of four oils on *Tetranychus urticae* populations on Eggplant under field conditions at Qalubia Governorate.

Natural oils	Pre-count	Mean number (± SE) and reduction percentage of mites/leaf after										Mean reduction (%)
		One day		Two days		Three days		Five days		Seven days		
		No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	
AARH	21	15.5	32.52 ± 2.81b	13.5	44.40 ± 1.19b	6.75	74.91 ± 2.34ab	4.25	85.28 ± 1.66a	4.75	84.60 ± 1.55a	64.34 ± 1.91b
Eucalyptus citriodora	21	18	21.63 ± 1.78c	16.25	33.08 ± 2.59c	9.5	64.69 ± 3.22c	7.75	73.16 ± 2.96b	8.5	72.44 ± 2.09b	53.00 ± 2.53d
Citrullus colocynthis	20	17.25	21.14 ± 2.19c	14.75	36.22 ± 2.07c	8	68.78 ± 2.76bc	6.25	77.27 ± 1.74b	7.25	75.32 ± 1.63b	55.75 ± 2.08c
Mixed oils	21	13.75	40.14 ± 2.74a	12	50.58 ± 1.68a	5.25	80.49 ± 3.17a	2.5	91.34 ± 1.00a	3.75	87.84 ± 0.81a	70.08 ± 1.88a
Control	19	22.25	-	25.5	-	27.25	-	30.75	-	32.25	-	-
F-value			14.40		16.53		5.73		17.03		21.37	91.50
P-value			0.0003		0.0001		0.0114		0.0001		0.0001	0.0001
LSD (0.05)			7.44		6.01		8.91		6.06		4.89	2.52

No. = Mean number of mites, R (%) = reduction percentage; different letters in same column denote significant difference (P < 0.05).

Table 4. Effect of four oils on *Tetranychus urticae* populations on eggplant under field conditions at Fayoum Governorate.

Natural oils	Pre-count	Mean number (± SE) and reduction percentage of mites/leaf after										Mean reduction (%)
		One day		Two days		Three days		Five days		Seven days		
		No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	
AARH	26	17.5	38.46 ± 2.27a	13.75	54.26 ± 1.59a	7	78.99 ± 2.12ab	4.75	86.71 ± 1.34a	7	81.67 ± 3.21ab	68.02 ± 2.11a
Eucalyptus citriodora	25	20.25	25.94 ± 2.74b	16.5	42.92 ± 3.00b	9.75	69.56 ± 4.11b	8.5	75.27 ± 1.88c	10.5	71.40 ± 5.03b	57.02± 3.35 b
Citrullus colocynthis	25	19.25	29.60 ± 0.91b	16	44.65 ± 1.41b	9.25	71.12 ± 2.67ab	7	79.64 ± 1.19b	8.5	76.85 ± 3.93ab	60.37± 2.02b
Mixed oils	25	16.25	40.57 ± 2.74a	13	55.03 ± 3.16a	6.25	80.49 ± 4.30a	3.25	90.55 ± 0.73a	5.25	85.70 ± 2.3a	70.47± 2.65a
Control	26	27.25	-	29.75	-	33.25	-	37.75	-	40.5	-	-
F-value			9.31		6.80		2.57		26.01		2.70	13.10
P-value			0.0019		0.0063		0.1028		0.0001		0.0927	0.0004
LSD. (0.05)			7.06		7.46		10.55		4.15		11.57	5.37

No. = Mean number of mites, R (%) = reduction percentage; different letters in same column denote significant difference (P < 0.05).

Evaluation of essential oils against **T. urticae** *adult population on squash in the Governorates of Qalubia and Fayoum*

Table 5 shows that there were significant differences when utilizing different four plant essential oils on *T. urticae* and squash at Qalubia Governorate. After one to seven days of treatment, the highest percentage of 76.37 ± 1.35% was reached when a mixture of three essential oils was used, followed by *Annona*, *Colocynthis*, and *Eucalptus* oils at 72.70 ± 2.04, 68.36 ± 1.98, and 63.19 ± 1.97 %.

Table 5. Effect of four oils on *Tetranychus urticae* populations of squash under field conditions at Qalubia Governorate.

Natural Oils	Pre-count	Mean number (± SE) and reduction percentage of mites/leaf after										Mean reduction (%)
		One day		Two days		Three days		Five days		Seven days		
		No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	
AARH	26	17.5	38.46 ± 2.27a	13.75	54.26 ± 1.59a	7	78.99 ± 2.12ab	4.75	86.71 ± 1.34a	7	81.67 ± 3.21ab	68.02 ± 2.11a
Eucalyptus citriodora	25	20.25	25.94 ± 2.74b	16.5	42.92 ± 3.00b	9.75	69.56 ± 4.11b	8.5	75.27 ± 1.88c	10.5	71.40 ± 5.03b	57.02 ± 3.35b
Citrullus colocynthis	25	19.25	29.60 ± 0.91b	16	44.65 ± 1.41b	9.25	71.12 ± 2.67ab	7	79.64 ± 1.19b	8.5	76.85 ± 3.93ab	60.37 ± 2.02b
Mixed oils	25	16.25	40.57 ± 2.74a	13	55.03 ± 3.16a	6.25	80.49 ± 4.30a	3.25	90.55 ± 0.73a	5.25	85.70 ± 2.3a	70.47 ± 2.65a
Control	26	27.25	-	29.75	-	33.25	-	37.75	-	40.5	-	-
F-value			9.31		6.80		2.57		26.01		2.70	13.10
P-value			0.0019		0.0063		0.1028		0.0001		0.0927	0.0004
LSD. (0.05)			7.06		7.46		10.55		4.15		11.57	5.37

No. = Mean number of mites, R%= reduction percentage; different letters in same column denote significant difference (P < 0.05).

Table 6. Effect of four oils on *Tetranychus urticae* populations of squash under field conditions at Fayoum Governorate.

Natural Oils	Pre-count	Mean number (± SE) and reduction percentage of mites/leaf after										Mean reduction (%)
		One day		Two days		Three days		Five days		Seven days		
		No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	No.	R (%)	
AARH	22	14.25	40.78 ± 1.99b	11.5	54.79 ± 1.13b	4.75	83.15 ± 1.7ab	4	86.78 ± 1.35b	5	84.53 ± 2.53a	70.00 ± 1.74b
Eucalyptus citriodora	23	16.75	33.42 ± 1.90c	14.25	46.42 ± 0.94c	7.5	74.55 ± 1.70c	6.75	78.66 ± 0.79b	9.25	72.62 ± 2.53b	61.13 ± 1.57d
Citrullus colocynthis	22	16	33.51 ± 1.70c	13	48.89±1 6c	6.25	77.83 ± 0.89bc	5	83.47 ± 1.35c	5.75	82.21 ± 3.19a	65.18 ± 1.75c
Mixed oils	22	12.75	47.01± 1.99a	9.75	61.67±1 88a	3	89.36 ± 3.24a	2.5	91.74 ± 2.13a	3.5	89.17 ± 0.89a	75.79 ± 2.03a
Control	21	24	-	25	-	30.25	-	33.75	-	35.5	-	-
F-value			11.87		22.29		9.86		13.75		8.18	27.95
P-value			0.0007		0.0001		0.0015		0.0003		0.0031	0.0001
LSD. (0.05)			5.84		4.43		6.35		4.57		7.50	3.68

No. = Mean number of mites, R%= reduction percentage; different letters in same column denote significant difference (P < 0.05).

Table 6 shows that there were significant differences between applying various four plant essential oils on *T. urticae* on squash in Fayoum Governorate. After one to seven days of treatment, the maximum 75.79 ± 2.03% was attained when a mixture of three essential oils was employed, followed by *Annona, Colocynthis,* and *Eucalyptus* oils 70.00 ± 1.74, 65.18 ± 1.75, and 61.13 ± 1.57%, respectively. The same results were obtained against a *T. urticae* population on squash under field conditions in the Fayoum Governorate.

DISCUSSION

Our findings revealed that all tested natural oils exhibited acaricidal properties against *T. urticae*. The oil mixture demonstrated consistent results on eggplant in both Qalubia and Fayoum Governorates, while on squash it was more effective in Qalubia (76.37) than Fayoum (75.79) followed by *A. muricata*, *C. colocynthis*, and *E. citriodora*.

The use of plant-derived formulations, particularly essential oils (EOs), has gained attention due to their broad-spectrum activity against arthropods, eco-friendliness, biodegradability, and low toxicity to mammals (Tak and Isman 2017). These advantages support their potential as sustainable alternatives to synthetic pesticides. In agreement, Zubaidi *et al.* (2023) reported that the edible fruit of AARH (soursop) has therapeutic properties but may exert toxicological effects on pests. Mutakin *et al.* (2022) further highlighted the traditional uses of *Annona* species in tropical regions for treating infections, inflammation, parasites, and cancers, in addition to their insecticidal activities. Phytoconstituents such as flavonol triglycosides, alkaloids, phenolics, cyclopeptides, acetogenins, and essential oils contribute to the bioactivity of *A. muricata* (Prasad *et al.* 2019). These bioactive molecules act on the insect nervous system, disrupting neurotransmission and causing paralysis and death, making *Annona* extracts valuable for pest control (Khalequzzaman and Sultana 2006). Indeed, aqueous leaf extracts of *A. muricata* have been used successfully against lepidopteran larvae, aphids, and thrips (Hassan *et al.* 2020). Similarly, *C. colocynthis* demonstrates multiple pharmacological and pesticidal activities. Meybodi (2020) reported its antioxidant, antibacterial, anticancer, anti-inflammatory, and pesticidal potential. Urbaneja *et al.* (2020) emphasized its successful use in China for citrus pest management against aphids, scales, mites, and whiteflies, while Uysal *et al.* (2023) noted that its aromatic essential oils enhance its preference as a botanical pesticide.

Eucalyptus oil also showed promising acaricidal effects. Natural-based pesticides from *Eucalyptus* extracts have been investigated as alternatives to reduce the adverse impacts of synthetic acaricides (An and Tak 2022) which confirmed the miticidal and repellent effects of its aqueous, ethanol, and essential oil extracts (Mdellel *et al.* 2023). Abdelkader (Abdelkader *et al.* 2020) also reported the insecticidal efficiency of *Eucalyptus globulus* essential oil against *T. urticae*. Several studies (e.g., Malaspina *et al.* 2022; Nguyen *et al.* 2023) highlighted its allelopathic and repellent properties, while Salehi *et al.* (2019) described its antioxidant, antimicrobial, insecticidal, herbicidal, and antiviral activities. More recently, Ashour *et al.* (2023) and da Silva *et al.* (2020) pointed out its considerable biomass and strong EO yield, making it a significant natural resource. Phytochemical investigations revealed that *Eucalyptus* species are rich in terpenoids, carotenoids, phenolic acids, flavonoids, and phloroglucinols (Malakar 2024). Their essential oils consist of complex volatile blends, including monoterpenes, sesquiterpenes, phenols, ethers, esters, and aldehydes, which act effectively as acaricides (Rodilla *et al.* 2024). While essential oils provide promising eco-friendly pest control options, concerns remain regarding possible ecological or human health risks associated with their long-term application (Haddi *et al.* 2020). Therefore, further studies on formulation safety, persistence, and large-scale field application are necessary to ensure their sustainable integration into pest management strategies.

Overall, our study supports the integration of plant-derived natural oils such as *Annona*, *Colocynthis*, and *Eucalyptus* into pest management programs as sustainable alternatives to chemical acaricides. Their bioactivity, safety profile, and environmental compatibility make them promising candidates for controlling *T. urticae* and other agricultural pests. Han *et al.* (2010) indicated that many essential oils are effective against females and eggs of *T. urticae* lacking direct contact but by fumigant, resulted mode of division of the oil was greatly caused by action in the vapor phase via the respiratory system. Amizadeh *et al.* (2013) showed that the biological activities of plant essential oils are regarded to the monoterpenes and phenols in their contents. According to Isman (2006) proved that essential oils contain different biochemical including acids, alcohols, ketones, aldehydes, esters, terpenes, phenols and sesquiterpenes. Essential oils are used as, antiviral, acaricidal, antimicrobial, antifeedant, antioxidant, contact antifungal, and repellent. Jeschke (2021) showed the action mechanisms of many acaricides and reported that the primary targets in mites are enzymes, receptors, or channel sites. Plant essential oils and monoterpenes exhibit neurotoxic effects against pests, inhibiting acetylcholinesterase and directly affecting the nervous system, channel sites, enzymes, and pest receptors. These compounds may have multiple target sites, making them suitable for pest management due to their minimal pest resistance (Ebadollahi *et al.* 2017; Jeschke 2021). Farahani *et al.* (2020) indicated that essential oils have a high potential for application in integrated management programs against *T. urticae*, which is a major pest in greenhouse.

This study demonstrated that the tested natural oils, particularly the mixture oil, exhibited strong acaricidal activity against *T. urticae*. Among the individual oils, AARH, *C. colocynthis*, and *E. citriodora* showed the highest effectiveness, confirming their potential as eco-friendly alternatives to synthetic acaricides. Their bioactivity is largely attributed to diverse phytochemicals, including acetogenins, terpenoids, and fatty acids, which act through insecticidal and repellent mechanisms. Given their biodegradability, low mammalian toxicity, and minimal environmental impact, these essential oils can be integrated into sustainable pest management strategies. Future studies focusing on formulation development, field application, and safety assessment will further support their practical use in agriculture.

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کنترل پایدار *Tetranychus urticae* (Acari: Tetranychidae) با استفاده از روغن‌های مشتق شده از گیاهان بر روی کدو و بادمجان در شرایط مزرعه‌ای

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چکیده

با افزایش میزان کشاورزی در سطح جهان، نیاز به راهبردهای مؤثر و سازگار با محیط زیست برای مهار آفات بیش از پیش اهمیت یافته است. آفت‌کش‌های شیمیایی سنتزی اگرچه مؤثر هستند، اما خطرهای زیادی برای سلامت انسان و تعادل بوم‌شناختی ایجاد می‌کنند و همچنین در ایجاد مقاومت در آفات نقش دارند. در این مطالعه، پتانسیل دوگانه کنه‌کشی و آنتی‌اکسیدانی روغن‌های اسانسی استخراج‌شده از هیبرید عبدالرازق *Annona*، *Citrullus colocynthis* و *Eucalyptus citriodora* علیه هرنای تارتن دو لکه‌ای (*Tetranychus urticae*)، به عنوان یکی از آفات مهم محصولات سبزی و صیفی، مورد بررسی قرار گرفت. روغن‌های اسانسی استخراج شده و ترکیبات شیمیایی آن‌ها با استفاده از روش GC–MS شناسایی شد و همچنین ترکیب اسیدهای چرب روغن‌های ثابت آن‌ها مورد تجزیه و تحلیل قرار گرفت. افزون بر ارزیابی کارایی در مهار آفت، فعالیت آنتی‌اکسیدانی این روغن‌ها با استفاده از آزمون‌های مهار رادیکال‌های آزاد $DPPH^{\bullet}$ و $ABTS^{\bullet+}$ بررسی شد. روغن *Annona* در آزمون DPPH قوی‌ترین اثر آنتی‌اکسیدانی را با مقدار IC_{50} برابر با ۱/۳۱۸ میکروگرم بر میلی‌لیتر نشان داد که از ویتامین C (۱/۶۶۴ میکروگرم بر میلی‌لیتر) و ترولوکس (۲/۰۷۳ میکروگرم بر میلی‌لیتر) نیز بیشتر بود. در آزمون ABTS، فرمولاسیون مخلوط روغن‌ها با نسبت (۱:۱:۱) بیشترین ظرفیت مهار رادیکال را نشان داد ($IC_{50} = 1.337$ میکروگرم بر میلی‌لیتر) که بیانگر اثر هم‌افزایی احتمالی میان سه روغن است. این نتایج نشان‌دهنده توانایی این روغن‌ها در خنثی‌سازی رادیکال‌های آزاد بوده و در صورت استفاده در راهبردهای حفاظت از محصولات، می‌تواند مزایای اضافی از نظر سلامت و افزایش ماندگاری نیز داشته باشد. آزمون‌های زیست‌سنجی کنه‌کشی در شرایط آزمایشگاهی نشان داد که با افزایش غلظت روغن‌های اسانسی، میزان مرگ‌ومیر کنه‌ها به طور معنی‌داری افزایش یافت و روغن *Annona* بیشترین کارایی را نشان داد ($LC_{50} = 0.17\%$). آزمایش‌های مزرعه‌ای انجام‌شده بر روی بادمجان و کدو در استان‌های قلیوبیه و فیوم، کارایی مخلوط روغن‌های اسانسی را تأیید کرد؛ به طوری که این مخلوط بیشترین و پایدارترین کاهش جمعیت *T. urticae* را تا ۷۶/۳۷ ٪ در کدو و ۷۰/۴۷ ٪ در بادمجان نشان داد. این یافته‌ها نشان می‌دهد که فرمولاسیون‌های مبتنی بر روغن‌های اسانسی، به‌ویژه در حالت ترکیبی، می‌توانند به عنوان جایگزین‌های سازگار با محیط زیست برای کنه‌کش‌های سنتزی مورد استفاده قرار گیرند. ویژگی‌های آنتی‌اکسیدانی این روغن‌ها نیز ارزش آن‌ها را در مدیریت پایدار آفات و سامانه‌های حفاظت از محصولات کشاورزی افزایش می‌دهد.

واژگان کلیدی: فعالیت کنه‌کشی، فعالیت آنتی‌اکسیدانی، جایگزین‌های سازگار با محیط زیست، شناسایی GC–MS، روغن‌های طبیعی، کنهٔ تارتن دولکه‌ای

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