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ORIGINAL ARTICLE

Natural oils as botanical acaricides: linking chemical composition, enzymatic disruption, and antioxidant capacity in the control of *Tetranychus urticae*

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Abstract

The two-spotted spider mite (*Tetranychus urticae* Koch) is a major agricultural pest, with increasing resistance to synthetic pesticides thereby, driving the search for natural alternatives. This study evaluated the acaricidal and antioxidant activities of tea tree oil (TTO, *Melaleuca alternifolia* (Maiden and Betch) Cheel), pumpkin seed oil (PSO, *Cucurbita pepo* L.), and wheat germ oil (WGO, *Triticum aestivum* L.) against *T. urticae*. Laboratory trials determined LC_{50} and LC_{90} values for adults and eggs. Biochemical effects on mites surviving TTO exposure were assessed by analyzing glutathione S-transferase (GST), acetylcholinesterase (AchE), carboxylesterase (CarE), and α-esterases activities. Antioxidant activity was evaluated via DPPH and ABTS assays. Gas chromatography-mass spectrometry (GC-MS) identified 24 compounds in TTO, with 4-terpineol (36.65%) and γ-terpinene (17.66%) as major components. TTO showed the highest acaricidal activity (LC_{50} of 0.3% for adults and 1.8% for eggs), outperforming PSO and WGO. TTO exposure significantly disrupted key enzymatic activities, impairing mite survival. Among the oils, TTO exhibited the strongest antioxidant activity. The antioxidant assays revealed that while all three oils demonstrated dose-dependent antioxidant effects, TTO was markedly more effective than PSO and WGO, although less potent than vitamin C and Trolox. Additionally, TTO exposure resulted in significant reductions in detoxification enzyme activity, particularly GST and AchE, highlighting a biochemical mechanism underlying its acaricidal action. The lipophilic properties of TTO likely enhance its penetration through the mite cuticle, increasing its efficacy. These findings support the use of plant-derived oils as eco-friendly alternatives for sustainable pest management and suggest potential for further development into natural pesticide formulations.

Keywords: AchE enzymes, antioxidants activity, GC-MS, natural oils, pesticides, spider mite, *Tetranychus urticae*

Introduction

Tetranychus urticae Koch (Acari: Tetranychidae) is one of the most economically important sucking pests (Scott *et al.* 2021). It is an extremely polyphagous herbivore that feeds on more than 1,100 plant species from at least 140 families (Rakhmanov 2024). *Tetranychus urticae* has developed resistance to more than 96 different acaricidal and insecticidal active ingredients (Adesanya *et al.* 2021), including organophosphates, pyrethroids, neonicotinoids, and even newer classes like avermectins and ketoenols. This extensive resistance is largely attributed to its short life cycle, high reproductive rate, and strong detoxification enzyme systems, such as cytochrome P450 monooxygenases, glutathione-S-transferases (GSTs), and esterases. As a result, chemical control has become increasingly ineffective in many regions. Resistance cases have been reported in over 87 countries worldwide (Payumo *et al.* 2024), positioning *T. urticae* as one of the most resistant and difficult to manage agricultural pests globally. This highlights the urgent need to explore alternative, eco-friendly control strategies such as the use of plant derived essential oils. The "Green Revolution" significantly boosted crop productivity but came with high ecological costs, including environmental degradation, pesticide resistance, and threats to non-target species such as plants, animals, and humans. In response, there is growing interest in natural alternatives to synthetic agrochemicals (Mohammed *et al.* 2024). Among these, essential oils such as tea tree oil (TTO), pumpkin seed oil (PSO), and wheat germ oil (WGO) have been investigated for their potential in pest control (Siraj 2022).

Tea tree oil, derived from *Melaleuca alternifolia* (Family: Myrtaceae), has been traditionally used in Australia for its antimicrobial properties (Brun *et al.* 2019). Tea tree oil exhibits acaricidal, insecticidal, anti-inflammatory, antimicrobial, and antioxidant properties. The exact mechanism of its antiparasitic activity remains unclear, but it is known for its strong pest control capabilities. Pumpkin seed oil from *Cucurbita moschata* Duch (Family: Cucurbitaceae), is rich in bioactive metabolites such as carotenes, vitamin E, and phytosterols, all of which act as antioxidants (Šamec *et al.* 2022). Pumpkin seeds are byproducts widely available in various regions, including Mexico, South Asia, and the US. Wheat germ oil (WGO) from *Triticum aestivum* (Family: Poaceae), a byproduct of wheat milling, contains high levels of lipids and bioactive compounds, with different extraction methods such as solvent extraction or mechanical pressing. Pest resistance in *T. urticae* is largely driven by detoxification enzymes, including cytochrome P450-dependent monooxygenases and GST (Sharma *et al.* 2020). Essential oils offer alternative action mechanisms, potentially bypassing these detoxification pathways (Chaves *et al.* 2020).

To assess the antioxidant capacity of these oils, various spectrophotometric assays were employed, including DPPH and ABTS. Both are based on electron or hydrogen atom transfer mechanisms and quantify antioxidant activity using Trolox or vitamin C equivalents. The ABTS assay produces a blue/green ABTS$^+$ that antioxidants can reduce, while the DPPH assay measures the reduction of purple DPPH to a colorless form (Chaves *et al.* 2020). These methods allow for a comprehensive assessment of the antioxidant potential of natural oil samples. This study aimed to evaluate the acaricidal and antioxidant properties of TTO, PSO, and WGO against *T. urticae* using TSSM stock cultures, biochemical analyses, and oil extraction (Zieliński *et al.* 2025). TTO demonstrated the highest antimicrobial activity against *T. urticae*, followed by pumpkin seed oil and wheat germ oil. TTO also showed the highest antioxidant capacity, as measured by ABTS and DPPH assays, though it was lower than standard antioxidants like vitamin C and Trolox. These results underscore the potential of these essential oils as eco-friendly alternatives to synthetic pesticides and as natural sources of antioxidants (Mohammed *et al.* 2022b).

Buteler *et al.* (2021) studied tea tree oil to repel and affect the behavior of *Acromyrmex* spp. (leaf-cutting ants). Tea tree oil has been found to act as a repellent to certain insect pests. In laboratory bioassays, a repellent dosage response effect of tea tree oil was reported, where concentrations of 0.0001 to 0.1% tea tree oil did not impact ant behavior, but 1 and 10% concentrations repelled ants. Habashy *et al.* (2023) studied in the laboratory the potential acaricidal action of wheat germ oil (*Triticum vulgare* Vill.), clove oil and eucalyptus oil against *T. urticae*.

This study aimed to evaluate the acaricidal and antioxidant activities of three plant-derived oils–tea tree oil (*Melaleuca alternifolia*), pumpkin seed oil (*Cucurbita pepo*), and wheat germ oil (*Triticum aestivum*) against the two-spotted spider mite (*T. urticae*), a major agricultural pest. The objectives were: (1) to determine the lethal concentrations (LC_{50} and LC_{90}) of each oil on adult mites and eggs; (2) to assess the biochemical effects of tea tree oil on key detoxification and neurological enzymes in mites, including glutathione S-transferase (GST), acetylcholinesterase (AchE), carboxylesterase (CarE), and α-esterases (3) to investigate the antioxidant potential of the oils using DPPH and ABTS assays; (4) to identify the chemical constituents of tea tree oil through gas chromatography-mass spectrometry (GC-MS); and (5) to compare the efficacy of the oils as natural, eco-friendly alternatives to synthetic pesticides for sustainable pest management.

Materials and Methods

Tetranychus urticae stock cultures

Tetranychus urticae (Koch) was obtained from a susceptible colony that was maintained on *Acalypha marginata* (J.J.Sm.), a plant species belonging to the family Euphorbiaceae. The colony was reared in an incubator set at 25 ± 1°C and 65 ± 5% relative humidity (RH) in the Laboratory of Acarology, Plant Protection Research Institute, Giza, Egypt. Fresh *Acalypha* leaves were regularly replaced as needed to sustain the colony (Rott and Ponsonby 2000).

Plant collection and essential oils

The study investigated the acaricidal and antioxidant properties of volatile oils (VO) and fixed oils (FO) extracted from three plants species: tea tree (VO), pumpkin seed (FO) and wheat germ (FO) sourced from the National Research Center Unit, Egypt, and identified by Prof. Dr. Sabry Mahfouz (Ellaithy *et al.* 2022).

Dr. Sabry Mahfouz identified the plant species used in this study, including tea tree (VO), pumpkin seed (FO), and wheat germ (FO). The VO and FO were extracted from these plants, sourced from the National Research Center Unit, Egypt, and investigated for their acaricidal and antioxidant properties (Ellaithy *et al.* 2022).

The volatile oil was extracted from the tea tree oil (TTO). After washing, drying, and crushing, the plants underwent water distillation for 4.5 h using Clevenger instruments. The oil triple samples were dehydrated with anhydrous sodium sulfate and stored for GC/MS analysis. The mean oil triple samples content was recorded (Mohammed *et al.* 2022a).

For fixed oils extraction (PSO, and WGO) (fatty acid methyl esters – FAME), 2 g of powdered fruit were extracted with 100 ml petroleum ether for 4 h using a Soxhlet apparatus. The resultant residue was weighed, and total lipid concentration was calculated. FAMEs were produced through an alkali-catalyzed reaction between fats and methanol with 2M KOH, then injected in hexane (Olubomehin *et al.* 2020).

Toxicity tests

Toxicity of tested essential oils on *Tetranychus urticae* females ovicidal test

A Petri dish was prepared with a wet cotton bed and covered with *Acalypha marginata* leaf discs (2 cm in diameter), and placed upside down. Each disc had 60 adult *T. urticae* females. Prior to the main experiment, preliminary tests were conducted to determine the appropriate concentration ranges of the three tested materials. Bioassays were then performed by applying 2 ml of each tested material, prepared in concentrations of 0.5, 1, 2, and 3% using 0.01% Tween 80 as a surfactant, onto the leaf discs with a glass atomizer. Mortality rates were assessed at 24, 72, and 120 h post-treatment.

To evaluate ovicidal activity, 10 adult female mites were placed on 2 cm *Acalypha* leaf discs set on wet cotton wool inside Petri dishes and allowed to lay eggs for 24 h under controlled conditions (27 ± 0.2°C and 55 ± 5% RH). The females were then removed and the eggs were counted. The eggs were treated with serial concentrations (0.5, 1, 2, and 4%) of the three oils. Control discs for both adults and eggs were sprayed with 2 mL of distilled water containing 0.01% Tween 80. Each concentration was tested in four replicates. Treated Petri dishes were maintained at 27 ± 2°C, 60 ± 5% RH Kumral (2010), and a 16 : 8 h light/dark cycle. Eggs were incubated at 25 ± 0.2°C and 60 ± 5% RH for seven days, after which the number of hatched and unhatched eggs was recorded.

Biochemical analysis

Adult mites were placed on *Acalypha* leaves and sprayed with LC_{50} concentration of TTO (0.3%) after 24 h. Water was applied to the control with 0.01% Tween 80. Then living mite bodies were collected from the surviving treated and non-treated mites and frozen until biochemical analysis. Carboxylesterase activity was measured according to the method described by Simpson (1964). The activity of AchE was determined using the method reported by Simpson *et al.* (1964). GST catalyzes the conjugation of reduced glutathione (GSH) with 1-chloro 2,4-dinitrobenzene (CDNB) via glutathione's -SH group. The conjugate, S-(2,4-dinitrophenyl)-L-glutathione, may be identified using the approach described by Habig *et al.* (1974). Total proteins were determined using the Bradford technique. Van Asperen's (1962) method was used to determine α- and β-esterase levels.

Chemical composition identification

GC-MS Analysis for volatile oils

The Agilent 7890B gas chromatograph and 5977A mass spectrometer with a DB-5MS column were used for the GC/MS analysis. The system used helium as a carrier gas, and the temperature program ranged from 40 to 320°C. The mass spectra were obtained through electron ionization (EI) at 70 eV, and the results were compared with the Wiley and NIST Mass Spectral Library (MSL) data for identification (Mohammed *et al.* 2022a).

Gas chromatography for fixed oils

Gas chromatography (GC) analysis of fixed oils was conducted using an Agilent 7890B system equipped with a flame ionization detector (FID) and a Zebron ZB-FAME column. Hydrogen was used as the carrier gas, with a temperature gradient set from 100 to 240°C. The injector and detector were maintained at 250 and 285°C, respectively.

In vitro antioxidant activity of three natural oils

The antioxidant activity of three oil extracts was assessed in vitro using DPPH and ABTS assays at different concentrations (1.25, 5, 10, 15, and 25 $\mu g \cdot ml^{-1}$), with ascorbic acid and Trolox serving as positive controls. $ABTS^{+}$ radical scavenging was evaluated following the method of Dinkova-Kostova *et al.* (2007), while DPPH radical scavenging followed the protocol of Mohammed *et al.* (2024). The percentage inhibition was calculated from absorbance values (A) at 517 nm for DPPH and 734 nm for ABTS+, comparing control and test solutions side-by-side.

$$\% \text{ Inhibition} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100.$$

Statistical analysis

Mean mortality rates (± SE) of mites were calculated as a percentage of dead females. Concentration and mortality rates of adult females and eggs were calculated using Abbott's formula Abbott (1925). The LC_{50}, LC_{90}, and slope values were determined using Finney's method (Finney 1971) and analyzed with Ldp Line software as described by Bakr Dib *et al.* (2017). Biochemical study data of mite numbers was subjected to the analyses of variance test (ANOVA) with mean separation at a 5% level of significance in SAS Program version 9.1.3.

Results and Discussion

Evaluation of using essential oils and fixed oils against *Tetranychus urticae* adult females and eggs

In the current study, the acute toxicity of TTO, PSO and WGO against *T. urticae* adult females (Fig. 1) was analyzed. The four serially diluted concentrations tested were 0.5, 1, 2 and 3%, respectively. TTO had strong acaricidal activity against *T. urticae* adult females. After 24 h, TTO at 3% resulted in the greatest mean mortality rate (± SE) of 90 ± 1.92 against *T. urticae* adult females, as compared to no mortality in their corresponding control groups ($p < 0.000$). For PSO and WGO the mean mortality rate (± SE) was 86 ± 1.92 and 80 ± 2.72% at 0.3%, respectively, as compared to no mortality in their corresponding control groups ($p < 0.000$). The results showed that the LC_{50} value of TTO predicted by probit analysis for adult females at 24 h was 0.3% against *T. urticae* adult females, whereas PSO and WGO had 0.4, 0.7% LC_{50} values, respectively. After 72 h the mortality rates (± SE) were, respectively, 93.3 ± 4.71, 88.3 ± 3.19 and 86.6 ± 2.7 with TTO, PSO and WGO. After 72 h, LC_{50} values were recorded as 0.28, 0.4 and 0.5% respectively.

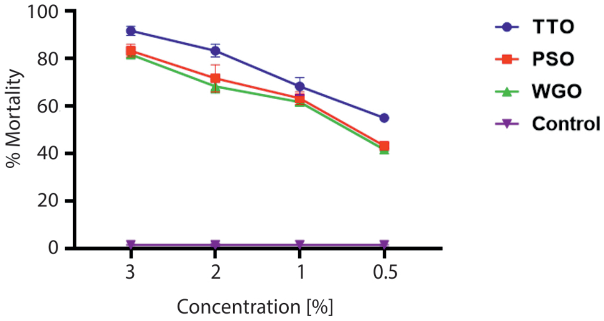

Fig. 1. Mean mortality rate (±SE) of *Tetranychus urticae* adult females treated with tea tree oil (TTO), pumpkin seed oil (PSO) and wheat germ oil (WGO) 24 h after treatment

After 24 h the three oils recorded LC_{90} values of 4.2, 6.9 and 8.6%, respectively. Increased times of treatment increased the mortality rates. Many natural plant oils and phytochemicals have been proven to possess acaricidal action – in part due to their lipophilic nature and high vapor pressure and have no or few negative effects on non-target organisms or the environment (Anholeto *et al.* 2024). The present study revealed that all tested natural oils had acaricidal effects on *T. urticae*. The activity of the essential oils may be attributable to recognized main components. This theory is in accordance with Hussein *et al.* (2013) who reported that the most efficient natural oil was *Triticum aestivum* oil (LC_{50} = 0.995% and LC_{90} = 3.085%).

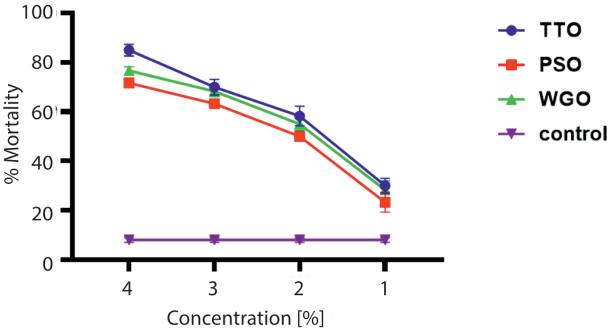

Fig. 2. Mean mortality rate (±SE) of *Tetranychus urticae* eggs treated with tea tree oil (TTO), pumpkin seed oil (PSO) and wheat germ oil (WGO)

The result in Figure 2 showed that TTO was more efficient against *T. urticae* eggs at 4% with the greatest mean mortality rate (± SE) of 85 ± 1.67 followed by PSO and WGO 75 ± 4.19 and 70 ± 3.3, respectively. The LC_{50} was 1.8, 2.2 and 2.5% in TTO, PSO and WGO, respectively. The LC_{90} recorded were 5.2, 7.7 and 7.4% for TTO, PSO and WGO, respectively. Afify *et al.* (2012) reported that the LC_{50} values after 24 h for eggs were 1.17, 6.26 and 7.33% after seven days for chamomile essential oil extract, marjoram, and *Eucalyptus*, respectively. The most potent acaricidal activities were seen with chamomile essential oil extract. The results are consistent with those of Topuz *et al.* (2018) who found that the oils of the aromatic plants tested in the present reseach significantly decreased the number of laid eggs.

Effect of tea tree oil on enzyme analysis in *Tetranychus urticae* adults

Changes in particular enzymes were examined in surviving mites after exposing adult females to the LC_{50} of TTO (LC_{50}, 0.3% concentration). Data in Table 1 show that a substantial reduction was found in treated mites and non-treated mites. The GST had a high significant reduction in comparison to the control that increased from 9.97 to 32.53 mmol sub.conjugated/min/mg protein. The AchE decreased from 29.50 to 21.30 µg AchBr/min/mg protein. The CarE increased from 3.95 to 8.71 µg Meb/min/mg protein. α-esterases increased from 271 to 299.33 µg α-naphthol/ min/mg protein while there was no significant reduction in β-esterases between treated and control mites. Adly and Bakr (2016) found that no remarkable changes were observed in total proteins, alkaline phosphatase, and acid phosphatase between treated and control mites. However, treated mites that survived showed a considerable reduction in α-esterases, β-esterases and G. S-transferase on *T. urticae* with LC_{50} of menthol.

A total of 24 compounds were identified in the volatile oil of tea tree (*Melaleuca alternifolia*), representing 100% of its composition (Table 1). The main constituents were terpinen-4-ol (36.65%) and γ-terpinene (17.66%), with retention times of 8.441 and 6.461 min, respectively (Fig. 1). These results are consistent with previous international studies (Homer *et al.* 2000). Six chemotypes of *M. alternifolia* were described, each with a distinct chemical profile, including terpinen-4-ol, terpinolene, and four types rich in 1,8-cineole. The terpinen-4-ol chemotype, which contains 30–40% terpinen-4-ol, is the most frequently used in commercial tea tree oil production (Fig. 3).

A total of 11 metabolites were identified in the fixed oils extracted from WGO and PSO, collectively constituting 100% of their total composition (Table 2). Linoleic acid was the predominant constituent, representing 56.65 and 63.89% of the total oil content, with a retention time of 35.126 min (Fig. 2). These results agree with previously reported data (Shelenga *et al.* 2020) (Figure 4).

Cucurbita pepo, a member of the Cucurbitaceae family, is recognized for its nutrient-rich seeds, which are processed into PSO. Pumpkin soil oil is characterized by high levels of bioactive constituents, including essential fatty acids namely palmitic (C16:0), stearic (C18:0), oleic (C18:1), and linoleic acid (C18:2) as well as significant amounts of vitamin E and tocopherols (Younis *et al.* 2000). Additionally, it contains diverse phytochemicals such as tyrosol, vanillic acid, vanillin, ferulic acid, luteolin, amino acids, phytosterols,

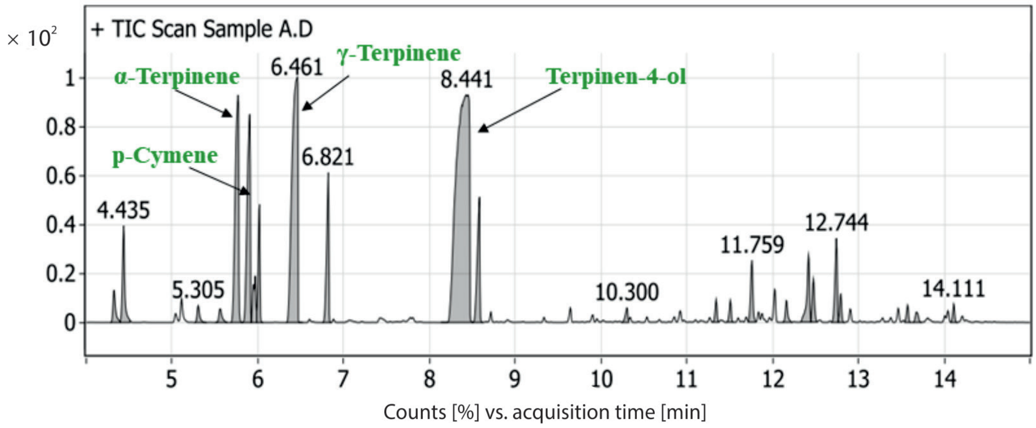

Fig. 3. TIC chromatogram of the volatile oil of *Melaleuca alternifolia*

Table 1. Glutathione S-transferase (GST), acetylcholinesterase (AchE), carboxylesterase (CarE), α-esterases, and β-esterases enzymes in the mites which survived from LC_{50} of tea tree oil (TTO) and control mites

	GST [mmol] sub. conjugated · min^{-1} · mg^{-1} protein	AchE [ug] AchBr · min^{-1} · mg^{-1} protein	CarE [ug] Meb. min^{-1} · mg^{-1} protein	α-esterases [ug] α-naphthol · min^{-1} · mg^{-1} protein	β-esterases [ug] β- naphthol · min^{-1} · mg^{-1} protein
Treated	32.53 ± 2.55 a	21.30 ± 1.61 b	8.71 ± 0.44 a	299.33 ± 7.37 a	72.43 ± 3.23 a
Control	9.97 ± 1.15 b	29.50 ± 2.0 a	3.95 ± 0.23 b	271.00 ± 8.54 b	67.53 ± 2.37 a
p-value	0.0002	0.0052	0.0001	0.0122	0.1018

Means±SD there is no noticeable change when the same letter appears in the same column are not significant difference at level 0.05

Table 2. The primary components of the volatile oil from *Melaleuca alternifolia*

Peak	Retention time	Name	Formula	Classification	Area sum [%]
1	4.326	α-Thujene	$C_{10}H_{16}$	monoterpenes	1.04
2	4.435	α-Pinene	$C_{10}H_{16}$	monoterpenes	2.75
3	5.305	β-Myrcene	$C_{10}H_{16}$	monoterpenes	0.53
4	5.562	α-Phellandrene	$C_{10}H_{16}$	monoterpenes	0.52
5	5.768	α-Terpinene	$C_{10}H_{16}$	monoterpenes	9.63
6	5.906	p-Cymene	$C_{10}H_{14}$	monoterpenes	7.87
7	5.969	ψ-Limonene	$C_{10}H_{16}$	monoterpenes	1.65
8	6.02	Eucalyptol	$C_{10}H_{18}O$	monoterpenes	2.52
9	6.461	γ-Terpinene	$C_{10}H_{16}$	monoterpenes	17.66
10	6.821	Terpinolene	$C_{10}H_{16}$	monoterpenes	3.89
11	8.441	4-Terpineol	$C_{10}H_{18}O$	monoterpenoid alcohol	36.65
12	8.584	L-α-Terpineol	$C_{10}H_{18}O$	monoterpenoid alcohol	3.85
13	10.3	1-acetyl-1,2-epoxy-2-methylcyclohexane	$C_9H_{14}O_2$	monoterpenes	0.43
14	11.342	1H-Cyclopropa[a]naphthalene	$C_{15}H_{24}$	sesquiterpenes	0.52
15	11.508	Caryophyllene	$C_{15}H_{24}$	sesquiterpenes	0.52
16	11.759	Aromandendrene	$C_{15}H_{24}$	sesquiterpenes	1.76
17	12.16	Isoledene	$C_{15}H_{24}$	sesquiterpenes	0.61
18	12.417	(+)-Ledene	$C_{15}H_{24}$	sesquiterpenes	2.25
19	12.475	β-Cyclogermacrane	$C_{15}H_{24}$	sesquiterpenes	1.17
20	12.744	δ-Cadinene	$C_{15}H_{24}$	sesquiterpenes	2.27
21	12.795	cis-Calamenene	$C_{15}H_{22}$	sesquiterpenes	0.69
22	13.573	(-)-Globulol	$C_{15}H_{26}O$	sesquiterpenes	0.43
23	13.676	Viridiflorol	$C_{15}H_{26}O$	sesquiterpenes	0.38
24	14.111	(-)-Spathulenol	$C_{15}H_{24}O$	sesquiterpenes	0.41
Total identification					100
Total monoterpenes					88.99
Total sesquiterpenes					11.01

β-carotenes, and selenium (Neamah *et al.* 2023; Singh and Kumar 2024). Pumpkin seed oil (PSO) was analyzed using GC-MS in full scan mode (m/z 60–600), and compounds were identified based on retention times and mass spectra compared to the NIST library (Mwangi *et al.* 2024) (Figure 4).

The present findings are consistent with those of Wang and Johnson (2001). US-based analyses of WGO revealed a similar fatty acid profile: linoleic acid (58.0%), oleic acid (16.4%), palmitic acid (16.4%), linolenic acid (6.4%), stearic acid (0.7%), arachidonic acid (0.2%), and eicosenoic acid (1.4%), which closely match those found in Turkish WGO (Dunford and Zhang 2003; Kan 2012). Furthermore, Zargar *et al.* (2023) identified 12 major constituents in WGO using GC-MS, accounting for 99% of its total composition. Notable components included trans-13-octadecenoic acid (99%), squalene (93%), and octadecane (94%), along with various fatty acids and terpenoids such as elaidic acid and linoleic acid, all exhibiting narrow peak widths (< 0.463), indicative of high analytical resolution. Kan (2012) summarized the fatty acid composition of solvent-extracted WGO, reporting a yield of 10.97%. The oil was comprised of seven main fatty acids, 80.1% of which were unsaturated. Linoleic acid (56.1%), palmitic acid (17.4%), and oleic acid (17.4%) were predominant, with minor fractions of linolenic acid (6.4%), eicosenoic acid (1.4%), stearic acid (0.9%), and arachidonic acid (0.2%) (Figure 4).

In this study, the antioxidant activity of three plant samples was evaluated using the DPPH assay, which showed that these plants exhibited relatively high antioxidant activity compared to other samples, though still lower than that of vitamin C and Trolox at the tested concentrations (see Fig. 1). The $ABTS^{\cdot+}$ assay measured antioxidant activity by assessing the reduction of the radical cation, represented as the percentage inhibition of absorbance at 734 nm. Figure 2 illustrates

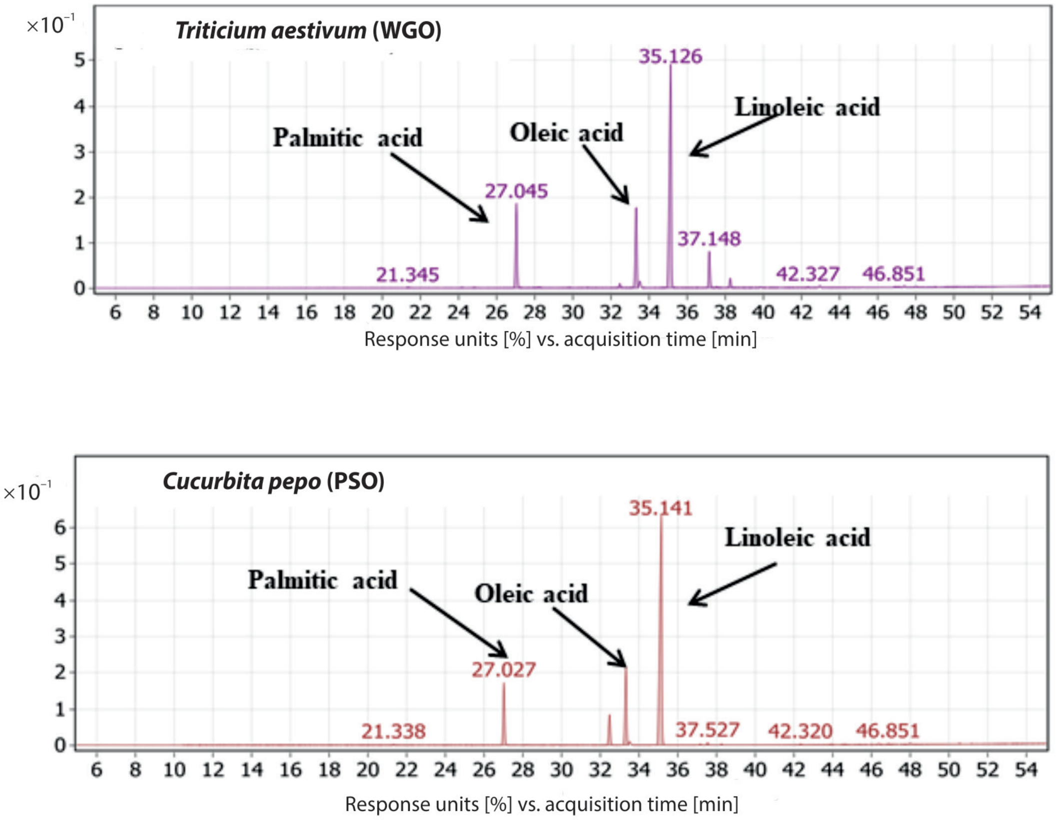

Fig. 4. Primary components of the fixed oil are *Triticum aestivum* and *Cucurbita pepo*. WGO – wheat germ oil, PSO – pumpkin seed oil

the effect of the interaction duration of specific antioxidants, such as Trolox and ascorbic acid (reference standards), on the suppression of the ABTS$^{\cdot+}$ radical cation at 734 nm. The results of the two standards were compared with those of the three plant samples (Figure 5).

The antioxidant activity was found to be concentration-dependent, with activity increasing as concentration rose. This was true for both reference standards and all three plant samples. The DPPH antioxidant activity (IC$_{50}$ values, from highest to lowest) was as follows: vitamin C (6.231), Trolox (8.478), *M. alternifolia* (17.70), *C. pepo* (178.4), and *T. aestivum* extract (178.4). Similarly, the ABTS antioxidant activity IC50 values followed the same pattern: Trolox (7.450), vitamin C (8.847), *M. alternifolia* (9.251), *C. pepo* (57.50), and *T. aestivum* extract (121.8) (Figure 4).

The two methods used in this study to measure antioxidant activity largely confirmed each other when considering all fractions. This suggests that the extracts examined may contain similar chemical groups, and their effects can be attributed to these groups (Mohammed *et al.* 2024). In this respect, determination and characterization of such volatile oil and fatty acid profiles are useful in developing natural antioxidant substances from *M. alternifolia, C. pepo* and *T. aestivum*.

The study focused on the extraction and chemical composition analysis of volatile and fixed oils from *M. alternifolia***,** *T. aestivum*, and *C. pepo*. Volatile oils were extracted through water distillation and analyzed using GC-MS, while fixed oils were analyzed with gas chromatography. Antioxidant activities of the oils were evaluated using DPPH and ABTS assays. Results indicated that *M. alternifolia* had the highest antioxidant activity among the three oils, although lower than standard antioxidants like vitamin C and Trolox.

In Table 3, the enzymatic responses of surviving mites exposed to the LC$_{50}$ concentration (0.3%) of tea tree oil were analyzed. The results indicated a substantial reduction in the activity of GST, AchE, CarE, and α-esterases, suggesting that these enzymes play a role in detoxification and metabolism in response to the oil's active constituents. The activity of GST decreased from 9.97 to 32.53 mmol substrate conjugated/min/mg protein, while AchE activity dropped from 29.50 to 21.30 μg acetylcholine bromide/min/mg protein. The noted reductions in α-esterases and CarE reinforce the impact of tea tree oil on biochemical pathways essential for mite survival, although no significant change was observed in β-esterase activity.

The acaricidal activity of essential oils can be attributed to their lipophilic properties and high vapor

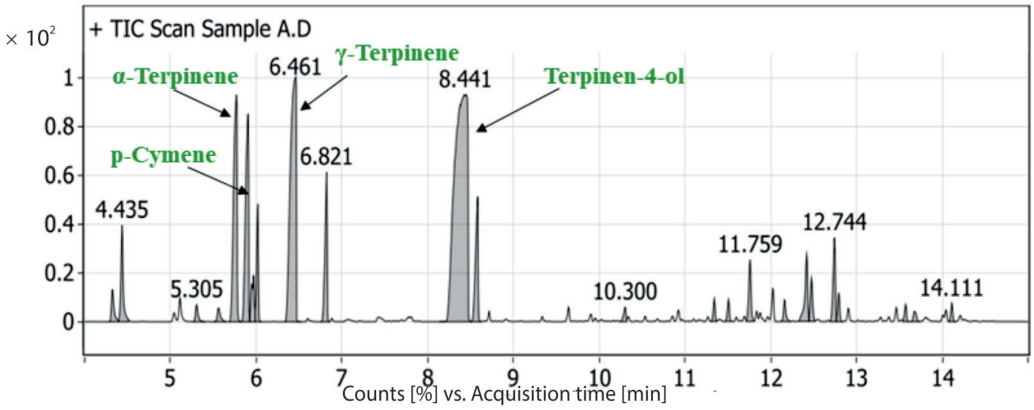

Fig. 5. *In vitro* $\mathrm{DPPH}^{\cdot}$ and $\mathrm{ABTS}^{\cdot+}$ antioxidant activity of standards with three natural oil plants

pressure, which enable efficient penetration through the cuticle of arthropods, resulting in toxicity. Previous studies have highlighted the low toxicity of these compounds to non-target organisms, making them favorable for pest management. The presented findings corroborate those of Abdelgaleil *et al.* (2019), who also noted significant acaricidal effects associated with essential oils.

Regarding the efficacy against eggs of *T. urticae*, Table 3 and Figure 2 reveal that tea tree oil shows an LC_{50} value of 1.8%, outperforming pumpkin seed oil and wheat germ oil, which had LC_{50} values of 2.2 and 2.5%, respectively. The LC_{90} values corroborate this trend, with tea tree oil achieving 5.2%, compared to 7.7 and 7.4% for wheat germ and pumpkin seed oils. This is consistent with findings by Afify *et al.* (2012), who reported significant acaricidal activity in essential oils from other plants.

Tea tree oil (*M. alternifolia*) exhibited the highest acaricidal efficacy against *T. urticae*, with an LC_{50} of 0.3% after 24 h, outperforming pumpkin seed oil and wheat germ oil (0.4 and 0.7%, respectively). After three days, the LC_{50} values for all oils were 0.28, 0.4, and 0.5%, respectively. For eggs, tea tree oil had an LC_{50} of 1.8%, again surpassing the others. Enzymatic analysis revealed significant reductions in GST, AchE, and CarE activities in treated mites, indicating a disruption in detoxification processes. The primary components of tea tree oil were 4-terpineol (36.65%) and γ-terpinene (17.66%). These results suggest that essential oils can serve as effective, eco-friendly pest management alternatives. Further research is needed to optimize their application in agriculture.

TTO exerts its acaricidal effects on *T. urticae* by inhibiting key detoxification enzymes, including GST, AchE, CarE, and α-esterases, leading to metabolic

Table 3. The main constituents of the fixed oil of *Tricium aestivum* and *Cucurbita pepo*

Peak	Retention time	Name	Area sum [%]	
			Tricium aestivum	*Cucurbita pepo*
1	21.345	myristic acid	0.08	0.12
2	27.045	palmitic acid	16.9	12.51
3	28.206	palmitoleic acid	0.16	0.07
4	32.459	stearic acid	0.8	6.64
5	33.319	oleic acid	16.39	16.02
6	35.126	linoleic acid	56.65	63.89
7	37.148	linolenic acid	6.91	0.12
8	37.537	arachidic acid	0.19	0.34
9	38.244	*cis*-11-Eicosenoic acid	1.71	0.12
10	42.327	behenic acid	0.11	0.07
11	46.851	lignoceric acid	0.1	0.1

disruption (Abdelgaleil *et al.* 2019). The inhibition of AchE interferes with neurotransmission, causing paralysis and death. TTO's lipophilic nature enables efficient penetration through the mite cuticle, enhancing toxicity. The primary bioactive compounds, terpinen-4-ol and γ-terpinene, contribute to oxidative stress and membrane disruption. These mechanisms collectively impair mite survival and reproduction, supporting the use of TTO as a natural pesticide.

Conclusions

This study highlighted the potent acaricidal effect of tea tree oil (*M. alternifolia*) against *T. urticae*, with the lowest LC_{50} values among the tested oils. The observed enzymatic inhibition suggests disruption of mite detoxification pathways, likely due to key compounds such as terpinen-4-ol. These results support the use of natural oils as eco-friendly pest control agents, warranting further research to refine their agricultural application.

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A list of abbreviations

Abbreviation	Meaning
GSTs	glutathione-S-transferases
TTO	tea tree oil
PSO	pumpkin seed oil
WGO	wheat germ oil
AchE	acetylcholinesterase
CarE	carboxylesterase
DPPH	2,2-diphenyl-1-picrylhydrazyl
ABTS	2,2′-azino-bis(3-ethylbenzothiazoline -6-sulfonic acid)
GC-MS	Gas Chromatography-Mass Spectrometry

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