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Environment-Friendly Control Potential of Two Citrus Essential Oils against *Aphis punicae* and *Aphis illinoisensis* (Hemiptera: Aphididae)

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Abstract: Aphids are serious pests of a wide range of agricultural crops, including pomegranates and grapevines. In addition, due to the negative environmental impacts of chemical insecticides, these pests are developing important resistance against aphicides. Therefore, one alternative method to control aphids is the use of essential oils (EO). The present study aimed to evaluate the insecticidal activity of *Citrus aurantium* and *C. reticulata* peel EO at different concentrations and with different exposure periods to pomegranate and grapevine aphids, *Aphis punicae* and *A. illinoisensis* via the topical application method under laboratory conditions. The results reveal that *C. aurantium* L. EO had greater toxicity against pomegranate and grapevine aphids, with LC_{50} of 0.37 and 0.82 µL/mL, respectively, at 48 h after application. The highest repellence effect was estimated for *C. aurantium* EO, at 2.5 µL/cm^2, on *A. punicae*, with a value of 100% after an exposure time of 3 h, in contrast to the 88% repellence estimated for *A. illinoisensis*. The GC-MS investigation of both essential oils identified limonene, 3-carene, pinene, and p-cymene as active substances that could be attributed to the effects observed. Overall, our results offer a potential tool to control the two aphid species and could help in the development of integrated insect management in pomegranate and grapevine fields.

Keywords: Citrus aurantium; Citrus reticulata; essential oil; Aphis punicae; Aphis illinoisensis; aphicidal activity

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1. Introduction

One of the main pests in pomegranate orchards is the aphid, *Aphis punicae* Passerini (Hemiptera: Aphididae). Previously, it was considered a minor pest of pomegranate. However, in recent years, this pest has become serious and is appearing regularly all year round [1]. Nymphs and adults both feed on fruits, inflorescences, and leaves. Pomegranate aphid infestation causes pale and curled leaves, delayed growth, and dropped blossoms. In addition, the aphid spreads viral infections and secretes honey dew on which fungi grow, which affects crop quality and productivity [2]. *A. illinoisensis* Shimer, an invasive pest of grapevines, infests the undersides of young leaves, young terminal shoots [3], and fruit clusters, causing some grape berries to drop [4]. The pomegranate aphid is among the invasive pests recorded in the Mediterranean area, Asia, Africa, and the Indian subcontinent [5–9]. *A. illinoisensis* is a North American species [3]. Recently, this aphid has invaded Turkey [10], Greece [11], Iran [12], and has been discovered in Tunisia for the first

time [13]. Even in Central Europe, it has been observed more recently in Slovenia [14] and France [15]. *A. illinoisensis* appears to produce anholocycles on grape plants in the continent of Eurasia, and no sexual morphs have been detected [16]. This species has been observed in Italy, namely on the island of Sicily and in the district of Catania [17], and for the first time in mainland Italy (Rome), apterous and winged viviparous females of this pest were found in 2021 on *Vitis labrusca* L. stems [18]. In Saudi Arabia, *A. illinoisensis* is also viewed as invasive due to the potential harm it could do to the production of grapes (the second most important economic fruit), particularly Taify cultivars produced in Taif region [19,20].

Aphids can reproduce quickly, so using insecticides frequently to control them has resulted in the emergence of resistance. Effective aphicides are very expensive, very poisonous, and have adverse environmental effects [21]. Although they have more negative to moderately harmful effects than malathion and pirimicarb on predators, neonicotinoid pesticides in Egypt can be viewed as potential tools for managing the pomegranate aphid, *Aphis punicae*, as outlined by [22]. According to research on the specialized pomegranate crops in Alicante, Spain, overuse of agrochemicals might potentially result in resistant insect populations [23]. Similarly, in Iran, it appears that using chemical pesticides to suppress this pest should be avoided because they have adverse effects on the environment, in addition to being harmful to the natural enemies and other beneficial insects that cause an outbreak [24]. Therefore, scientists are looking for substitute pesticides that are stronger against the two aphid species, don't have many cytotoxic side effects on natural enemies, and are less hazardous to the environment [25].

Medicinal plants are the fundamental raw materials for various chemical drugs and food products. At present, more than one third of medical drugs are derived from botanic extracts [26]. A preliminary study on medicinal plant diversity in the flora of the Kingdom of Saudi Arabia found seven families with 254 species [27]. In particular, the Taif province of Saudi Arabia, has a wealth of such species, which are commercially and extensively cultivated in the production of essential oils (EOs). EOs from plant species are low-molecular-weight mixtures that are produced in large quantities by a variety of plant families, including the Asteraceae, Apiaceae, Lamiaceae, Rutaceae, Lauraceae, and Myrtaceae [28–30], whose monoterpenoids have received attention in recent years as potential pest control agents. These substances operate in a variety of ways, such as direct toxins, antifeedants or repellents, or by changing enzyme profiles [31]. Additionally, it was observed that a variety of parameters, including harvest year [32], cultivar [33], environmental conditions [34], and extraction system [35], had an impact on the essential oil composition of citrus fruits. The Rutaceae family includes plants of particular significance in potential applications in the medicinal [36,37], as well as the cosmetic [38,39], and industrial fields. These are the sour orange (*Citrus aurantium* L.) and mandarin orange (*C. reticulata* Blanco). It has long been speculated that the plant's production of several unique EOs and antioxidants is efficiently produced in their skin or peel [40,41]. These EOs contain several compounds, such as monoterpenes [42], flavonoids, and other phytochemicals. Limonene is known to be abundant in the peel oils of the Citrus genus [43], and GC-MS analysis results [44] have shown that limonene is the primary constituent of bergamot (46.0%), lemon (75.7%), mandarin (71.9%), and sweet orange (83.8%) oils.

Recently, laboratory research into EOs and their ingredients for use in controlling pests in fruit trees has increased. A review of the literature reveals several papers have investigated the effects of EOs on aphids and stored-product pests [45–49], but few studies have focused on the aphicidal activity of citrus EOs. Gupta et al. [50] revealed, for the first time, the insecticidal effects of lemon (*Citrus limon*) peel aqueous extract against rose aphid (*Macrosiphum roseiformis*) and its influence on nontarget predators, *Coccinella septempunctata,* and *Orius laevigatus* on rose plants. The contact and residual toxicity of eleven EOs, including *C. aurantium*, *C. sinensis,* and *C. limon*, has been tested against the woolly beech aphid, *Phyllaphis fagi* (Hemiptera: Aphididae), with *C. aurantium* recording the highest residual toxicity (40%) of the Citrus species tested against the targeted insect [51]. Oregano (*Origanum syriacum* var. bevanii L.), cumin (*Cuminum cyminum* L.), anise (*Pimpinella anisum* L.), and

eucalyptus (*Eucalyptus camaldulensis* Dehn) have all been successful as fumigants against cotton aphid (*A. gossypii* Glover) [52]. Additionally, the bioactivity of *Tagetes minuta* L. EO volatiles against the aphid species *M. persicae*, *Acyrthosiphon pisum* (Harris), and *Aulacorthum solani* (Kaltenbach) has been documented [53]. Moreover, [54] evaluated the efficacy of five plant materials on *Aphis gossypii* population in okra production and demonstrated that the aphid population was reduced more effectively by *Tagetes minuta* L. and *Carica papaya* L. Adverse effects of *Thymus*, *Veronica*, and *Agrimonia* Eos have also been demonstrated on cabbage. Although there have been numerous studies on the toxicity effects of Eos on aphids, little or no work has been carried out to find the effects of Eos on *A. punicae* and *A. illinoisensis* control. Therefore, this paper aims to investigate the biological activities and applications of *C. aurantium* and *C. reticulata* Eos as natural products on the mortality and repellency of pomegranate and grapevine aphids, *A. punicae* and *A. illinoisensis*, under laboratory conditions.

2. Materials and Methods

2.1. Plant Materials

Thirty (200 days old from blossom) sour (*Citrus aurantium* cv. Swingle) and mandarin (*C. reticulata cv.* Kinnow) full-mature orange fruits (6 fruits/plant) with orange color were picked from five plants (15 years old) each from local citrus farms (Taif, Saudi Arabia) that did not use any pesticides. The plants are grown in clay loam soil. The distance between the lines is 6 m and the distance between the trees is 5 m. The irrigation water that the plant receives (8–10 L/plant) in the drip system, and the fertilizers from soluble sources are injected with irrigation water into the drip network at the required rates in batches at a rate of once every one week. The botanical identification was conducted at the Departments of Biology and Biotechnology, Faculty of Sciences, Taif University, Saudi Arabia in January 2021.

2.2. Isolation of Essential Oils

Sour and mandarin orange fruits were completely rinsed with distilled water first, and then with absolute ethanol. Citrus fruit peels were removed. Fruit peels were allowed to air dry for two weeks at room temperature while being covered with a muslin cloth. Dried materials were pounded to powder in a mixer grinder (MRC LB20ES, Burnt Mill, Essex, CM20 2HU UK). For this experiment, 100 g of each material was added to 500 mL of distilled water and subjected to hydro-distillation for six hours using a Clevenger-type apparatus (Dolphin Labware, Mumbai, India). After distillation, 100 g of each plant's dry material produced almost 4 mL of oil. To acquire the necessary amount of oil for further uses, the distillation was repeated. The essential oil distillates were filtered, dried over anhydrous sodium sulphate, and refrigerated at −4 °C until analysis [55].

2.3. GC–MS Analysis of Eos

An Agilent-Technologies (Little Falls, CA, USA) 6890N Network gas chromatographic (GC) system, equipped with an Agilent-Technologies 5975 inert XL Mass selective detector and an Agilent-Technologies 7683B series auto injector, was used for the gas chromatography/mass spectrometry (GC-MS) analysis of the phytochemical contents of essential oils. Compounds were separated on HP-5 MS capillary columns (30 m × 0.25 mm i.d., 0.25 µm film thickness; Little Falls, CA, USA). With a split ratio of 100:1, 1.0 µL of sample was injected in the split mode. An electron ionisation system, with an ionisation energy of 70 eV, was used for GC-MS detection. The temperature program for the column oven matched the one chosen for the GC analysis. At a flow rate of 1.5 mL/min, helium acted as a carrier gas. The injector and MS transfer line temperatures were set at 220 °C and 290 °C, respectively, while the mass scanning range ranged from 50 to 550 m/z.

2.4. Compound Identification

The documentation of the oil constituents would be based on the comparison of their retention indices relative to (C9–C24) n-alkanes with authentic compounds. Compounds were identified using their MS data compared to those from the NIST mass spectra library and published mass spectra [56].

2.5. Insects

Fresh leaves and buds of pomegranate and grapevine trees infested with the aphids, *Aphis punicae* and *A. illinoisensis*, respectively (about 50 not parasitised insects, homogenised in shape, age, and size per leaf or bud) were collected in plastic bags on the same experimental day from the farms of pomegranate and grapevine trees that had not used any pesticides at Taif, Saudi Arabia.

2.6. Bioassay

2.6.1. Toxicity Test

The toxicity of *C. aurantium* and *C. reticulata* oils was evaluated on *A. punicae* and *A. illinoisensis* via a topical application method described by Eidy et al. [57] with slight modification. Quantities of 0.5, 1, 2, 4, and 6 µL for each essential oil were diluted in 1 mL acetone [48]. Then, at each concentration, five replicates (each containing 20 apterae aphid individuals of the same size of the Petri dish) were employed, totalling 100 aphid individuals. Every Petri dish (9 cm) was lined with a thick layer (4–5 mm) of moistened cotton. Four Taify pomegranate or grapevine leaf discs measuring 1.5 cm in diameter were cut out and placed on the cotton layer in each dish. On the aphid body, 1 µL of each concentration was directly poured. A delicate camel hairbrush was used to softly move the adult aphid individuals on the leaf discs in the petri dishes. In the control treatments, insects were treated with the same volume of acetone for each aphid species. The Petri dishes were then sealed with Parafilm and kept under laboratory conditions of 25 ± 1 °C, 65 ± 3% relative humidity, and a 12 L:12 D light–dark cycle. Insects were counted as dead if their appendages did not move when a fine-point brush was used to touch them after 6, 12, 24, and 48 h. According to Abbott's formula [58], mortality percentages were modified for mortality in control.

2.6.2. Repellence Test

Following Isman [59], the behavioral response (food preference) of aphids was examined by offering them two options in order to test whether they had any preference for or against a specific essential oil. In brief, pomegranate leaves were cleaned, dried, and cut into equal leaf discs measuring 1.5 cm in diameter. Then, leaf discs were impregnated for 30 sec in 2 mL of each essential oil diluted in acetone at doses of 0.125, 0.25, 0.50, 0.75, and 1 µL/cm^2. The control leaf discs were treated with an equal volume of acetone. Leaf discs with and without EO treatments were air-dried for about 60 min to allow the solvent to evaporate. Two treated pomegranate leaf discs and two untreated ones were then kept in a Petri dish (9 × 1.5 cm) with moistened filter paper (Whatman No. 1, 8 cm diameter) and alternately arranged around the circumference of the Petri dish at the diagonal position. After that, 20 individuals of the *A. punicae* aphid were released at the center of the Petri dish. For *A. illinoisensis*, the previous procedures were followed. However, equal grapevine leaf discs were employed. The experiment was kept at 25 ± 1 °C and 65 ± 3% RH. At 30, 60, 90, 120, and 180 min following the release, observations on the number of aphids present on treated or untreated leaf discs were recorded. Equation used to determine repellency percentage: Repellence (%) = (Nc − Nt)/(Nc + Nt) × 100, where Nt = the number of insects on the treated discs after the exposure time And Nc = the number of insects on the untreated discs. Five replicates from each dose of the tested essential oil were taken.

2.7. Statistical Analysis

GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA) was employed for all experiments' statistical analyses. Two-way analysis of variance (ANOVA) with Duncan's test was used to compare the means among the corrected mortalities. Using SPSS Version 23 (IBM Corp., Armonk, NY, USA), a *t*-test was performed on the LC_{50} and LC_{90} values, 95% confidence intervals of the lower and upper values, slope, intercept, and chi-square for both essential oils. A *p*-value of 0.05 or less indicates statistical significance. Results are expressed as the mean ± standard error (SE).

3. Results

3.1. Toxicity Assay

The data in Figure 1 show that the oil's efficiency was directly related to concentration and exposure time, as the mortality of *A.* punicae adults ranged from 9 to 100% after topical application of *C. aurantium* oil at five concentrations for four exposure times. Median and high concentrations (2–6 µL EO/mL acetone) of the tested EO were the most efficient, causing 100% mortality after 48 h of exposure. When *A. punicae* was exposed to oil at concentrations between 2 and 6 µL/mL for a short time (8 h), the mortality percentages significantly increased, from 77 to 100%. For *A. illinoisensis*, the obtained results shown in Figure 2 illustrate that the highest significant values of mortality were 100% when a concentration of 6 µL/mL was used at 24 and 48 h exposure times followed by 98% at 6 µL/mL after 8 h exposure. On the other hand, only 2% of the mortality resulted from a 0.5 µL/mL treatment at 2 h after application.

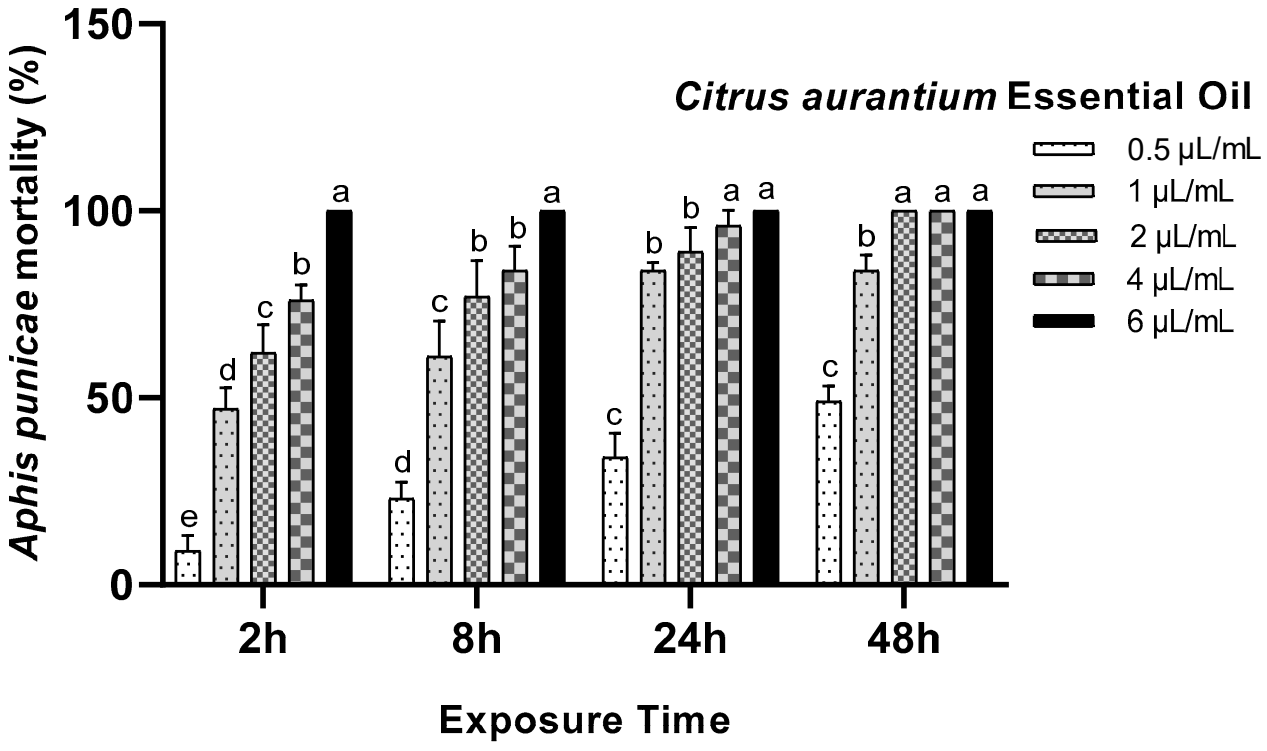

Figure 1. Mortality percentage (mean ± SE) of *Aphis punicae* exposed to different concentrations (0.5, 1, 2, 4, and 6 µL/mL) of *C. aurantium* essential oil at different exposure periods (2, 8, 24, and 48 h). The bars with the same letter annotation are not significantly different (according to the Duncan test, $p < 0.05$).

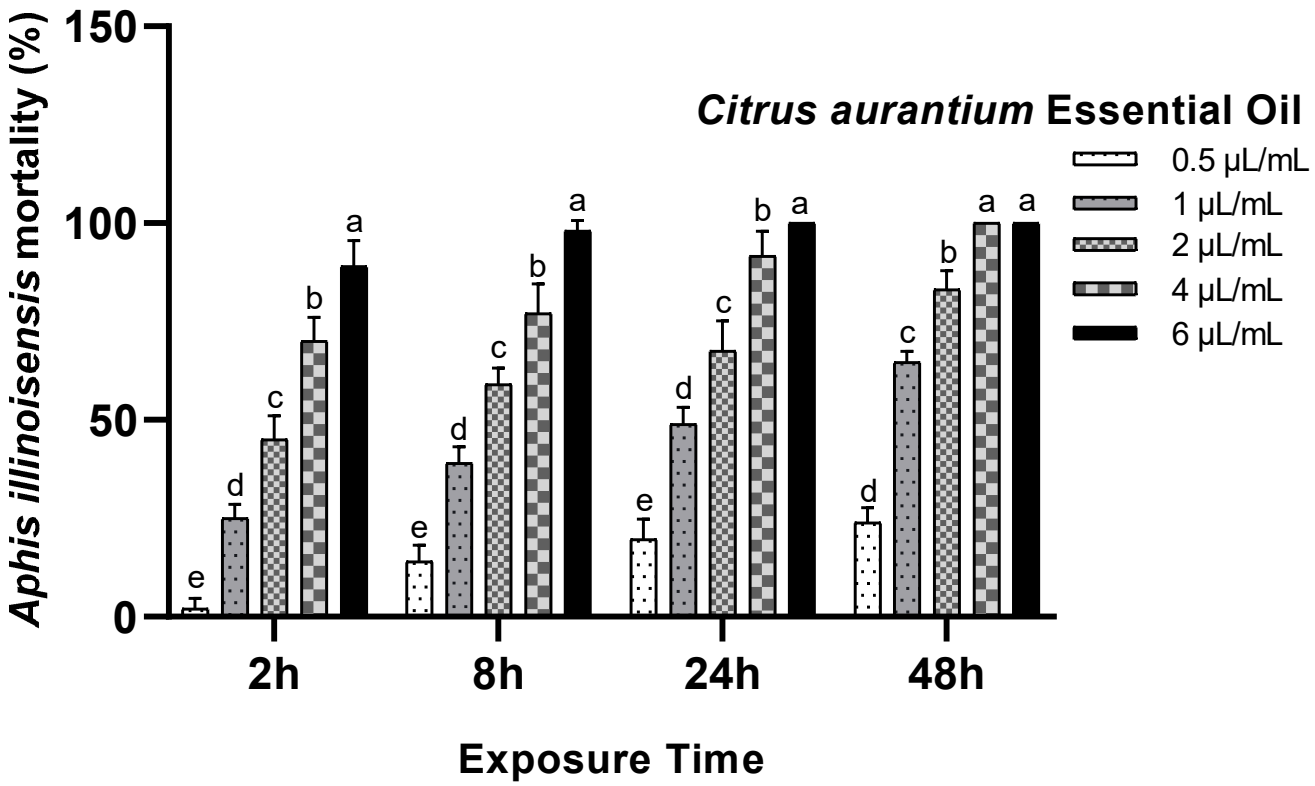

Figure 2. Mortality percentage (mean ± SE) of *A. illinoisensis* exposed to different concentrations (0.5, 1, 2, 4, and 6 µL/mL) of *C. aurantium* essential oil at different exposure times (2, 8, 24, and 48 h). Bars annotated with the same letter are not significantly different ($p < 0.05$, based on the Duncan test).

For *C. reticulata* EO, the highest significant mortality of *A. punicae* and *A. illinoisensis* adult stages were represented in the highest concentration, 6 µL/mL, at all exposure times (Figures 3 and 4). The highest percentages of *A. punicae* mortality (97% and 93%) were recorded at 6 µL/mL after 48 and 24 h (Figure 3), while those of *A. illinoisensis* mortality were 92.8% and 84.4% at 48 and 24 h, respectively (Figure 4). A statistical analysis of the *A. punicae* mortality results shows statistically significant differences ($p < 0.05$) between the mortality and the treatment of the tested EOs at all concentrations from 2 to 48 h (Table 1). The data in Table 1 show that at 48 h, *C. aurantium* EO was more effective against *A. punicae* adults than *C. reticulata*, as it recorded lower LC_{50} and LC_{90} values: 0.37 and 1.13 µL EO/mL acetone, respectively, for the former and 1.03 and 4.13 µL EO/mL acetone, respectively, for the latter. However, at 24 h, no significant difference was observed between the LC_{50} values of the two EOs. *C. aurantium* recorded 1.74 µL/mL, compared with 1.85 µL/mL for *C. reticulata*. As exposure time was extended in all treatments, the toxicity increased noticeably. As a result of the tested EOs, the individuals of *A. punicae* displayed varying degrees of homogeneity, with slope values ranging from 2.11 to 2.87 (Table 1). Similarly, Table 2 shows the toxicity of EOs, with *C. aurantium* more effective than *C. reticulata* after exposure periods of 24 h and 48 h against *A. illinoisensis*, with LC_{50} (1.09 and 0.82 µL/mL) and LC_{90} (3.47 and 2.15 µL/mL), respectively. The data in Table 2 also reveals that *C. aurantium* had the highest level of homogeneity for *A. illinoisensis*, with a slope value of 2.94.

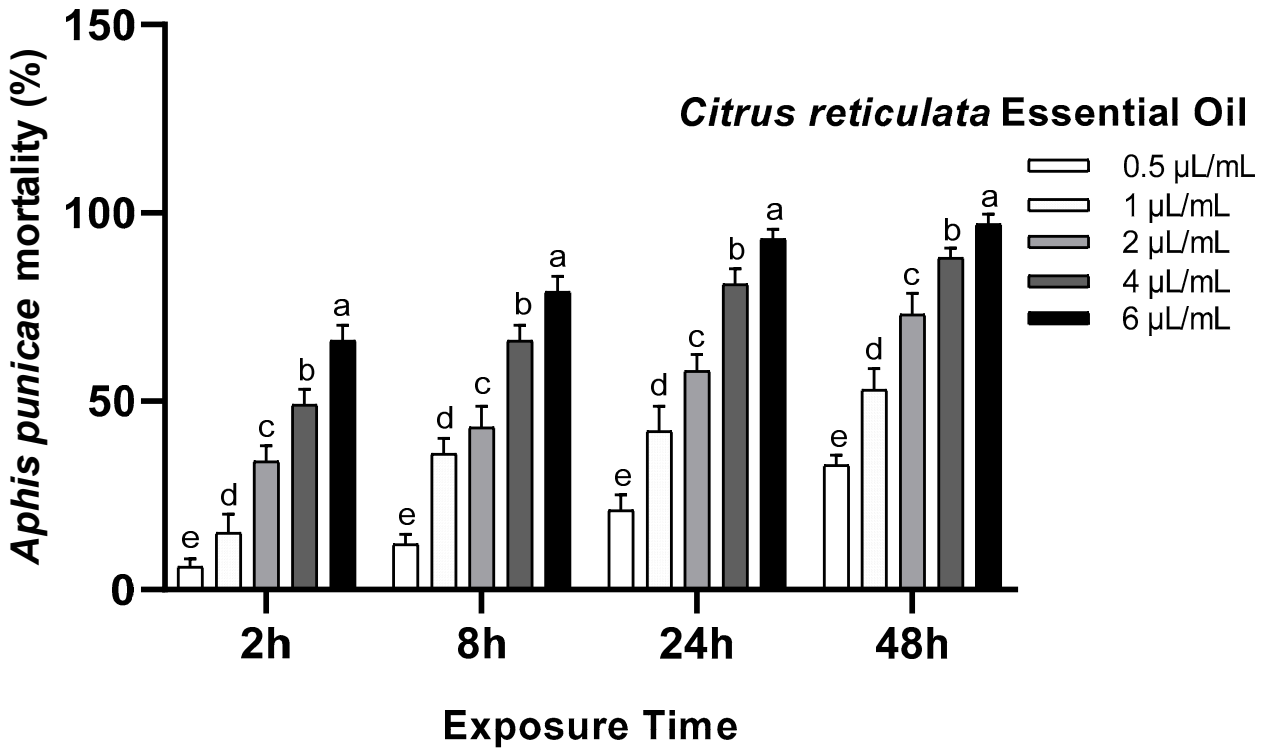

Figure 3. Mortality percentage (mean ± SE) of *Aphis punicae* exposed to different concentrations (0.5, 1, 2, 4, and 6 µL/mL) of *C. reticulata* essential oil at different exposure times (2, 8, 24, and 48 h). Bars annotated with the same letter are not significantly different ($p < 0.05$, based on the Duncan test).

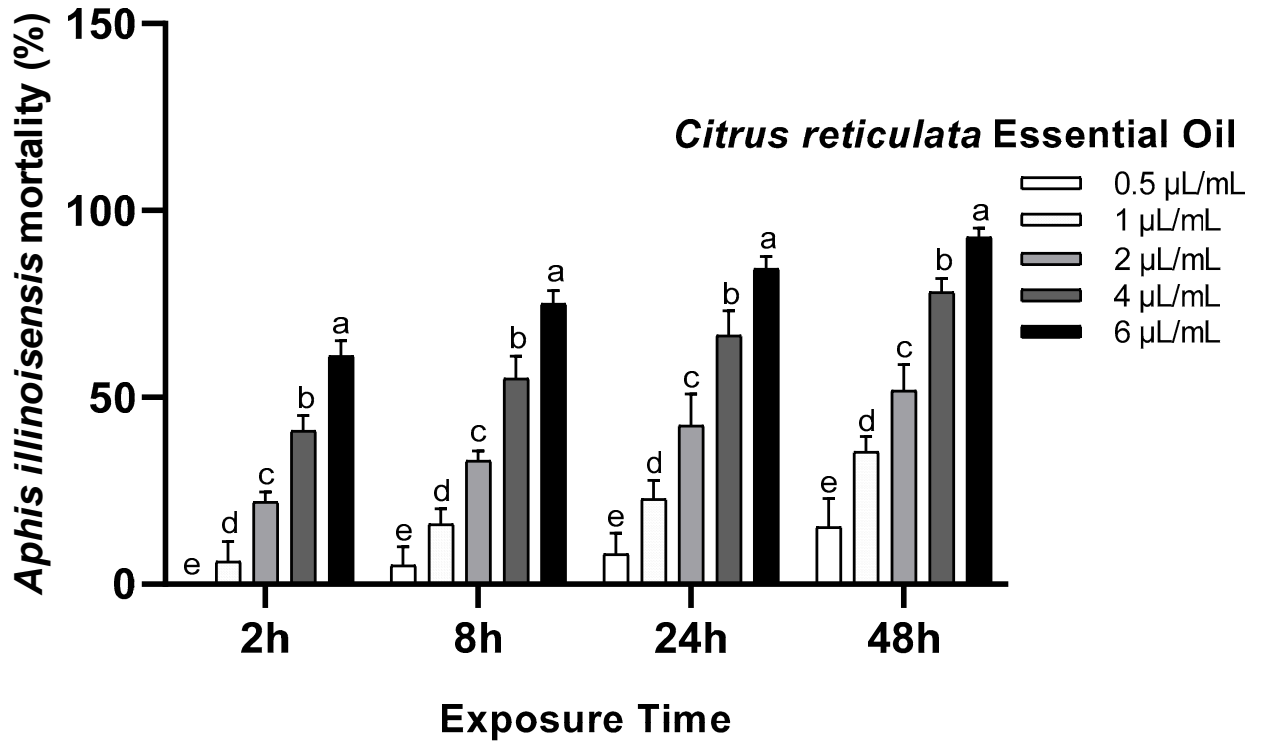

Figure 4. Mortality percentage (mean ± SE) of *A. illinoisensis* exposed to different concentrations (0.5, 1, 2, 4, and 6 µL/mL) of *C. reticulata* essential oil at different exposure times (2, 8, 24, and 48 h). Bars annotated with the same letter are not significantly different ($p < 0.05$, based on the Duncan test).

Table 1. Toxicity values of *C. aurantium* and *C. reticulata* essential oils against *Aphis punicae*.

Essential Oils	Exposure Time (h)	LC_{50} µL/mL (95% LCL–UCL)	LC_{90} µL/mL (95% LCL–UCL)	Slope ± SE	Intercept	X^2	*p*-Value
Citrus aurantium	2	3.95 (2.32–5.98)	4.8 (2.98–6.69)	2.42 ± 0.46	5.05	6.06	0.0131
	8	2.31 (1.04–4.69)	3.87 (1.6–5.1)	2.87 ± 0.57	4.59	7.01	0.0154
	24	1.74 (1.11–3.4)	2.89 (1.36–4.47)	2.26 ± 0.36	5.55	7.86	0.0082
	48	0.37 (0.047–4.04)	1.13 (0.89–2.33)	2.25 ± 0.56	5.97	0.02	0.0276
Citrus reticulata	2	4.28 (3.37–5.79)	8.47 (6.63–12.97)	2.11 ± 0.24	1.11	1.99	0.0030
	8	2.92 (2.01–3.97)	7.14 (5.58–10.91)	2.20 ± 0.38	3.50	2.42	0.0110
	24	1.85 (0.99–2.56)	5.1 (4.07–7.28)	2.44 ± 0.42	5.21	1.37	0.0100
	48	1.03 (−0.09–1.73)	4.13 (3.21–6.21)	2.12 ± 0.49	8.03	1.28	0.0220

Lethal concentrations (LC_{50}, L_{C90}) are those that kill 50% of insects and 90% of insects, respectively. LCL is for lower confidence limit, UCL for higher confidence limit, X^2 stands for chi-square value, SE for standard error, and *p*-value for probability.

Table 2. Toxicity values of *C. aurantium* and *C. reticulata* essential oils against *A. illinoisensis*.

Essential Oils	Exposure Time (h)	LC_{50} µL/mL (95% LCL–UCL)	LC_{90} µL/mL (95% LCL–UCL)	Slope ± SE	Intercept	X^2	*p*-Value
Citrus aurantium	2	2.29 (1.91–3.69)	5.71 (4.74–7.51)	2.8 ± 0.28	4.0	4.03	0.0022
	8	1.41 (1.23–3.92)	4.69 (3.8–6.44)	2.57 ± 0.42	4.62	2.74	0.0088
	24	1.09 (1.88–3.68)	3.47 (2.77–5.0)	2.78 ± 0.28	4.9	1.83	0.0022
	48	0.82 (2.0–3.87)	2.15 (1.69–3.44)	2.94 ± 0.29	5.25	2.12	0.0021
Citrus reticulata	2	4.89 (4.06–6.31)	8.33 (6.77–11.81)	2.2 ± 0.12	−0.75	2.55	0.0050
	8	3.82 (3.06–4.89)	7.35 (5.96–10.29)	2.48 ± 0.19	0.67	1.62	0.0010
	24	3.05 (2.34–3.89)	6.31 (5.16–8.32)	2.68 ± 0.30	1.72	1.82	0.0030
	48	2.21 (1.49–2.91)	5.24 (4.25–7.22)	2.67 ± 0.38	3.73	1.32	0.0060

Lethal concentrations (LC_{50}, L_{C90}) are those that kill 50% of insects and 90% of insects, respectively. LCL is for lower confidence limit, UCL for higher confidence limit, X^2 stands for chi-square value, SE for standard error, and *p*-value for probability.

3.2. Repellent Activity

The repellent percentage in aphid adults was increased by increasing both the doses of the two tested EOs and exposure times. Regarding the *A. punicae* results shown in Table 3, the highest significant repellency of the individuals was recorded as 100% and 98% for 2.5 µL *C. aurantium* oil/cm^2, at 2 h and 3 h, respectively, followed by 92% for the same dose at 1.5 h, while 8% was the least significant repellency rating at 0.156 µL *C. aurantium* oil/cm^2 after 30 min. For *C. reticulata* EO, the data presented in the same table indicate that the highest significant repellency of *A. punicae* adults after 3 h was 70% and 58% at 2.5 and 1.25 µL oil/cm^2, respectively. For *A. illinoisensis*, the results presented in Table 4 show that the highest repellency percentage of adults increased when the dose of EOs was increased at all exposure times. *C. aurantium* showed its greatest substantial repellent activity at 2.5 µL oil/cm^2 after 3 h, with a value of 88%, versus a value of 62% for *C. reticulata* after the same exposure time. However, the lowest significant repellency value was 2%, at 0.156 µL of *C. aurantium* oil/cm^2. Furthermore, it was clear that *C. reticulata* EO at low doses does not perform well as an aphicide, since no repellency of *A. illinoisensis* was recorded at 0.156 and 0.312 µL/cm^2 after a half-hour exposure.

Table 3. Repellent activity of five doses of *C. aurantium* and *C. reticulata* essential oils against *Aphis punicae*.

Essential Oils	Dose (μL/cm^2)	[a] Repellence %				
		30 min	60 min	90 min	120 min	180 min
Citrus aurantium	0.156	[b] 8 ± 3.7	20 ± 3.2	30 ± 3.2	36 ± 2.4	40 ± 0
	0.312	12 ± 3.7	26 ± 2.4	40 ± 3.2	50 ± 3.2	52 ± 3.7
	0.625	28 ± 2	44 ± 2.4	56 ± 2.4	60 ± 0	64 ± 2.4
	1.25	50 ± 3.2	66 ± 2.4	72 ± 3.7	76 ± 2.4	86 ± 2.4
	2.5	70 ± 3.2	82 ± 2	92 ± 3.7	98 ± 2	100 ± 0
Citrus reticulata	0.156	0 ± 0	4 ± 2.4	8 ± 2	22 ± 4.9	36 ± 2.4
	0.312	2 ± 2	8 ± 2	16 ± 2.4	34 ± 2.4	46 ± 2.4
	0.625	6 ± 2.4	24 ± 2.4	38 ± 2	46 ± 2.4	50 ± 0
	1.25	16 ± 2.4	38 ± 2	46 ± 2.4	52 ± 2	58 ± 3.7
	2.5	22 ± 2	48 ± 2	52 ± 2	56 ± 2.4	70 ± 3.2

[a] In this experiment, each treatment was represented by five replicates, each containing twenty adult insects. [b] Each column's numbers showed the repellence ± standard error.

Table 4. Repellent activity of five doses of *C. aurantium* and *C. reticulata* essential oils against *A. illinoisensis*.

Essential Oils	Dose (μL/cm^2)	[a] Repellence %				
		30 min	60 min	90 min	120 min	180 min
Citrus aurantium	0.156	[b] 2 ± 2	4 ± 2.4	14 ± 4	26 ± 2.4	32 ± 2
	0.312	6 ± 2.4	10 ± 3.2	18 ± 2	30 ± 3.2	40 ± 0
	0.625	8 ± 2	18 ± 2	28 ± 2	36 ± 2.4	46 ± 2.4
	1.25	18 ± 2	28 ± 2	44 ± 2.4	44 ± 2.4	56 ± 4
	2.5	32 ± 2	42 ± 2	50 ± 3.2	68 ± 3.7	88 ± 3.7
Citrus reticulata	0.156	0 ± 0	0 ± 0	2 ± 2	6 ± 2.4	14 ± 2.4
	0.312	0 ± 0	2 ± 2	6 ± 2.4	6 ± 2.4	18 ± 2
	0.625	4 ± 2.4	8 ± 3.7	12 ± 3.7	18 ± 2	32 ± 2
	1.25	8 ± 2	14 ± 2.4	14 ± 2	24 ± 2.4	42 ± 2
	2.5	22 ± 2	26 ± 2.4	34 ± 2.4	44 ± 2.4	62 ± 3.7

[a] Each treatment in this experiment was represented by five replicates, each containing twenty adult insects. [b] The numbers in each column are indicated to repellence ± standard error.

3.3. Chemical Compositions of Essential Oils

The screened molecules found in the peels of both sour orange and mandarin samples containing EO compounds are limonene, 3-carene, α-pinene, and p-cymene (Table 5). Among all the identified compounds, limonene was the most prevalent, with a 96.98% area coverage and retention time of 4.738 and 91.86% and 4.768 for *C. aurantium* and *C. reticulata* oils, respectively. Some compounds were identified in only one plant, such as beta-guaiene and 2,7-bis(spirocyclopropane)bicyclo [2.2.1]heptan-5-one in *C. aurantium*, and carveol, cis-p-mentha-2,8-dien-1-ol, doconexent, methyl 6,8-octadeca diynoate, methyl 8,10-octadecadiynoate, (-) carvone, 3-cyclohexen-1-ol, 5-methylene-6-(1-methylethenyl)-, acetate, oxacyclotetradeca-4,11-diyne, and 5,8-dimethylene bicyclo [2.2.2]oct-2-ene in *C. reticulata*.

Table 5. The identified chemical compounds contained in the peel of *C. aurantium* and *C. reticulata*.

No.	Compounds	*C. aurantium*			*C. reticulata*		
		Detect	R.T (Min.)	Area %	Detect	R.T (Min.)	Area %
1	Limonene	+	4.738	96.98	+	4.768	91.86
2	α-Pinene	+	3.020 3.930	0.42 0.88	+	3.026 3.946	0.33 0.40
3	3-Carene	+	3.629 4.285 6.371	0.31 0.27 0.63	+	3.640 13.846	0.04 0.17
4	p-Cymene	+	4.605	0.06	+	4.620	0.04
5	beta-Guaiene	+	16.570	0.23	-	-	-
6	2,7-Bis (spirocyclopropane)bicyclo [2.2.1]heptan-5-one	+	8.785	0.22	-	-	-
7	cis-p-Mentha-2,8-dien-1-ol	-	-	-	+	7.150	1.08
8	Carveol	-	-	-	+	7.264 9.454 9.808	0.94 0.86 0.41
9	Doconexent	-	-	-	+	8.801 8.914 13.294 13.732	0.19 0.34 0.53 0.72
10	Methyl 6,8-octadeca diynoate and Methyl 8,10-octadecadiynoate	-	-	-	+	6.887 11.851 12.193	0.26 0.56 0.28
11	(-) Carvone	-	-	-	+	10.089	0.29
12	3-Cyclohexen-1-ol, 5-methylene-6 (1methylethenyl)-, acetate	-	-	-	+	12.466	0.19
13	Oxacyclotetradeca-4,11-diyne	-	-	-	+	13.080	0.13
14	5,8-Dimethylene bicyclo [2.2.2]oct-2-ene	-	-	-	+	8.359	0.10

4. Discussion

The results of the present laboratory investigation shed light on the promising use of citrus Eos to suppress aphid insects attacking pomegranate and grapevine plants. Our results showed that both of the tested EOs at all concentrations and exposure times have been recorded as the toxicants against either *A. punicae* or *A. illinoisensis*. *C. aurantium* EO was more effective than *C. reticulata*. The results obtained were in accordance with [60,61], who evaluated the aphicidal effects of peel extracts of orange, *C. sinensis*, and *C. sinensis*, and *C. paradise* against wheat and cowpea aphids, respectively. They recorded that orange peel extract treatment consistently caused the highest level of wheat aphid mortality (65.69%) and the lowest LC_{50} value (62.3 µL/mL) for cowpea aphid. Furthermore, in the present study, *A. punicae* was more sensitive to both EOs than *A. illinoisensis*, which may be due to different insect morphology, behaviors, and inhibitory activity changes in biochemical biomarkers in *A. punicae* than in *A. illinoisensis*, as previously mentioned by [46,62]. In *A. craccivora*, it was found that the detoxification enzymes, Glutathione S-transferase and acetylcholinesterase, had significantly lower activities in insects treated with EOs than control.

The present investigation revealed for the first time that EOs of selected two citrus species possessed repellency towards *A. punicae* and *A. illinoisensis*. There were noticeable differences in the repellent actions of *C. aurantium* and *C. reticulata* essential oils, and all treatment doses efficiently repelled both aphids upon their instant application. *C. aurantium*

EO also resulted in higher repellency than *C. reticulata*. In the same context, Fiaz et al. [63] found that lemon oil (at 5% concentration) had repellent and phago-deterrent effects against jassids and thrips. Numerous complex chemicals, including terpenoids and phenols, are responsible for the repellent properties of EOs. These compounds either prevent or disrupt insect feeding by rendering treated leaf surfaces unpleasant or unattractive [64]. As an alternative, these EOs might change the insects' diet or interfere with their hormone balance, rendering their diet inedible [65].

Fourteen compounds were recorded in the screened molecules detected in the EOs of both sour orange and mandarin peels, of which limonene was the most prevalent in both *C. aurantium* and *C. reticulata* oils, with a higher percentage in *C. aurantium* than in *C. reticulata*. On the other hand, p-cymene appeared the least in the two tested oils. In the present study, results confirmed that the oil from *C. aurantium* and *C. reticulata* comprises mostly limonene, which is probably the reason for the insecticidal activities. Similarly, Sreepian et al. [66] identified 12 and 25 compounds in the EO of *C. reticulata* and *C. aurantium* fruit peels, respectively, revealing the potential of these citrus EOs as natural antibacterial agents, with the most prevalent component being limonene (62.9–72.5%). In China, monoterpene hydrocarbons were the main constituents of the EOs extracted from the fruit peels of physiologically dropped navel oranges (*Citrus sinensis* Osbeck cv. Newhall), Yangshuo kumquats (*Citrus japonica* Thunb), Nanfeng mandarins (*Citrus reticulata* Blanco cv. Kinokuni), Xunwu mandarins (*Citrus reticulata*), and limonene was the predominate compound for all citrus EOs [67]. Boughendjioua et al. [68] recorded the presence of 28 compounds in Algerian mandarin (*Citrus reticulata*) essential oil, constituting mainly D-limonene (85.10%), sabinene (2.49%), linalyl acetate (2.00%), copaene (1.80%), and α-pinene (1.75%). In Tunisia, [69] identified 37 compounds from *C. aurantium* EO, of which limonene was the most abundant (62.2%) and recorded the antimicrobial activity against a panel of pathogenic bacteria. However, analyzing the crude extract of peels of *C. aurantium* in Thailand by GC-MS revealed the presence of limonene as the major compound, accounting for 93.7% of the total and demonstrating the antiviral activity of L-limonene for the first time [70]. Furthermore, Sevindik et al. [71] in Turkey recorded (72.51%) limonene in the essential oil obtained from the *C. aurantium* central population, (77.27%) in samples originated from Germencik region, (79.77%) in samples from the Koçarlı population, and (95.70%) in samples from the Nazilli population.

Both limonene and linalool are poisonous for insects; they amplify the sensory nerve, causing considerable over-stimulation of the motor nerves, which may lead to convulsion and paralysis [50]. These findings were in conformity with the results of [72,73], who mentioned that the complexity of the chemical composition of the majority of unstable oils gives them low specificity because biological activity is not assigned to a single action mechanism since the wide variety of chemical groups allows for multiple targets in the cell. As recorded by [74] in Argentina, a mixture of ethanol extract of *C. aurantium* albedo and peel essential oil deterred *Spodoptera frugiperda* feeding by 46% and had the highest larval mortality (100%), attributing this action to the presence of D-limonene, which may be responsible for the feeding behaviour and toxic effects. *C. aurantium* has a higher potential for the production of terpenes than other plants in the citrus family, such as the sweet orange (*C. sinensis*) [75]. Many of these monoterpenes have been shown to have potential in medicinal applications to reduce inflammation [76] and as natural antioxidants [77] with numerous antimicrobial and antifungal properties [78–85]. Our GC-MS analysis also detected carene in the EO of both tested citrus species, whereas carvone was found in *C. reticulata* only. Carene and carvone are cyclic monoterpenes with an immense range of naturally occurring variations produced by many plants in the Rutaceae family as secondary metabolites [86]. They are used in the plant's defence system and, as such, increase under oxidative stress. Many studies have also shown them to be experimentally inducible [87,88]. Methyl 8,10-octadecadienoate, oxacyclotetradeca-4,11-diyne, methyl 4,6-tetradecadiynoate, and methyl-4,7,10,13,16,19-docosahexaenoate are esters produced by the sour orange plant that exhibit antimicrobial/antifungal and biodiesel applications,

while doconexent are omega-3 fatty acids prized for their medicinal properties and use as flavorants in several food products, such as cereals [89].

Our results were also in line with those recorded by [47], who mentioned that the EOs, especially those from *Salvia* sp., have been shown to be promising natural aphicides, repellents, and deterrents against *A. punicae*, and they are safe for important insect predators. In another study by [45], results indicated that arugula oil was the best treatment against two aphid species, *Macrosiphum rosae* and *A. fabae*. Abdelaal et al. [46] revealed that EO and nanoemulsion of *Basilicum ocimum* exhibited potential toxic activities against both laboratory and field strains of cowpea aphid, *A. craccivora*. Our techniques were used by Ezeonu et al. [90], Mansour et al. [91], and Akram et al. [92] to corroborate the mosquito-repelling and toxic effects of peel extracts of sweet orange (*C. sinensis*), lime (*C. aurantifolia*), rough lemon (*C. jambhiri*), and lemon (*C. limon*). As in this investigation, others have shown that EOs can be efficient insecticides for pest control in stored grains. Abad and Besheli [93] recorded the remarkable fumigant toxicity, repellent activity, and persistency effect of EO from the leaves of *C. aurantium* on three coleopteran stored-product pests. With topical treatment on the maize weevil, *S. zeamais*, Estrela et al. [94] evaluated the EOs of both *Piper hispidinervum* and *P. aduncum* and revealed 90–100% mortality. Restello et al. [95] evaluated the insecticidal influences of the EO from *Tagetes patula* on maize weevils and observed its effective influence at concentrations of 10 µL. Moreover, Changbunjong et al. [96] indicated that *C. aurantium* EO exhibits insecticidal activity against the stable fly *Stomoxys calcitrans* based on contact and fumigant toxicity, which can be achieved as an alternative to synthetic insecticides for controlling this pest, as well as chemical analysis of the essential oil showed the dominance of limonene (93.79%). The present results indicate the high toxicity of *C. aurantium* and *C. reticulata* towards aphids. Moreover, the presence of various monoterpenes (limonene, pinene, carene, p-cymene, and carvone), proven with mass spectrometry data are responsible for the possible insecticidal property.

5. Conclusions

In the current study, the two EOs extracted from peel displayed strong insecticidal activity against pomegranate and grapevine aphids at low concentrations and with shorter exposure times, showing higher toxicity of *C. aurantium* over *C. reticulata* and less sensitivity of *A. punicae* to both EOs than *A. illinoisensis*. Therefore, our results offer a reliable basis for promising and safe methods to develop insecticides based on these two EOs used in managing piercing–sucking insects, particularly pomegranate and grapevine aphids. More investigations should be completed on these two EOs or other plant products to evaluate their efficacy against other pests in the greenhouse and field.

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