Abdel-Aziz *et al.*
*Bulletin of the National Research Centre (2022) 46:244*
https://doi.org/10.1186/s42269-022-00932-8

Bulletin of the National
Research Centre

**RESEARCH** **Open Access**

# Characterization and insecticidal activity of two natural formulation types against the scale insect (*Parlatoria ziziphi*) and their biochemical effects on *Citrus aurantium*

Nahed Fawzy Abdel-Aziz, Hamdy Abdel-Naby Salem, Ahmed Mohamed El-Bakry* and
Elham Ahmed Sammour

## Abstract

**Background:** The scale insect, *Parlatoria ziziphi* (Lucas) (Diaspididae: Hemiptera), is one of the most serious insects in citrus orchards in Egypt. The efficiency of two different formulation types (emulsifiable concentrates (EC) and nanoemulsions) based on the essential oils *Artemisia herba-alba* (Asso.) (Asterales: Asteraceae) and *Laurus nobilis* (L.) (Laurales: Lauraceae) at two concentrations of 3 and 5%, compared with the commercial mineral oil, Active Cable, was examined against *P. ziziphi*. The green formulations were named Artemisia and Laury relative to *A. herba-alba* and *L. nobilis*, respectively. The physicochemical properties of the tested formulations have been studied.

**Results:** All the EC formulations of the essential oils (EOs) as well as the nanoemulsions with ratios of EOs to Tween 1:1.5 (Artemisia) and 1:2 (Laury) passed all the tested characteristics. The droplet sizes of the successful nanoemulsions' formulations by the ultrasonic emulsification were 153.7, 113.4 nm for Artemisia and 139.3, 89.4 nm for Laury at 3 and 5% concentrations, respectively. Laury EC caused average reductions of 92.79 and 94.94% (nymphs and females) when applied at 3 and 5%, respectively, while the same oil prepared as nanoemulsions caused average reductions of 50.02 and 55.32% at the same concentrations, compared with 91.74% reduction resulted from spraying Active Cable. Moreover, Artemisia caused reduction percentages of 74.97, 91.52 for EC and 43.7, 54.01 for nanoemulsions, sprayed at 3 and 5%, respectively. Although EC emulsions were more effective in reducing insect populations than nanoemulsion formulations, the efficiency of nanoemulsions gradually increased with time elapsed. The antioxidant activities of superoxide dismutase, catalase, and polyphenol oxidase enzymes were researched. It is recognized that insect infestations increase plant enzyme activity to defend them against insect attack. The results revealed a significant reduction of all the examined enzymes which were more obvious for EC emulsions than nanoemulsions.

**Conclusions:** The EC formulations originated from the EOs, especially Laury 3% EC and Artemisia 5% EC, could be an alternative to the traditional insecticides for controlling the scale insect, *P. ziziphi*.

**Keywords:** Insecticidal efficiency, EC emulsion, Nanoemulsion, Green formulations, *Parlatoria ziziphi*

## Background

Fruits are broadly consumed foods that are deemed nutritious and necessary for a balanced diet. Citrus is a major fruit crop in the world, with a wide abundance and

*Correspondence: ah_albakry@hotmail.com

Department of Pests and Plant Protection, Agricultural and Biological Research Institute, National Research Centre, Dokki, Cairo, Egypt

popularity that contributes to human nutrition. Citrus fruits (Family: Rutaceae) have commercial production in over 137 countries (Kahramanoğlu et al. 2020). World production is about 142.5 million tons of fruit in 2018 (Statista 2020).

The black parlatoria scale, *Parlatoria ziziphi* (Lucas) (Family: Hemiptera), is one of the most critical pests attacking citrus trees in the Mediterranean Basin and many other countries worldwide (Assouguem et al. 2021). The infestation by this insect triggers drying and weakening of the tree aerial parts, chlorosis and untimely drops of leaves, twig dieback and branching, and most prominently, a commercial loss in the fruits due to distorted fruits and fruit dropping before ripeness (Faskha 2021).

Because of the lipophilic nature of synthetic pesticides, they can gather in lipid tissues and plants, causing negative impacts on humans, non-target organisms, and the environment. Furthermore, pest resistance and tolerance have increased because of greater pesticide use (Mossa et al. 2019; Sammour et al. 2018). As a result, attention has been focused on the use of natural and biosafe pesticides for pest control (Ansari et al. 2014; Sammour et al. 2018; Sharma and Singhvi 2017).

Essential oils (EOs) are rich in biological active substances like terpenoids and phenolic acids, which possess antimicrobial, antioxidants, antiviral, and insecticidal properties (Aumeeruddy-Elalfi et al. 2015; Sammour et al. 2018). Previous studies have reported the scolicidal activity of many EOs such as almond, black seed, citrus, clove, coconut, henna, lavender, peppermint, and sesame (Al-Dosary et al. 2008; Benaissa and Belhamra 2017; Karamaouna et al. 2013). Many insect pests have been investigated by several investigators using EOs obtained from *Laurus nobilis* (L.) (Family: Lauraceae) and *Artemisia herba-alba* (Asso.) (Family: Asteraceae) for their biological activities on many insect pests (Derbalah et al. 2012; Ebrahimi et al. 2013; El-Bakry et al. 2016). On the other hand, the main disadvantages of using EOs in pest control are their unstable nature when exposed to air, light, humidity, and elevated temperatures, which can cause fast volatilization and decomposition of some active compounds (Badawy et al. 2018; Ibrahim 2019). The urgent need to bypass the sensitivity and increase the stability of biologically active ingredients encounters several potential issues correlated with the incorporation of EOs and their constituents into suitable formulations (Sugumar et al. 2013).

Previous studies referred to the usage of plant EOs as emulsions and nanoemulsions to control insects depending on their physical and chemical properties (Abdelaziz et al. 2014; Badawy et al. 2018; Sammour et al. 2018; Sundararajan et al. 2018). EC (the emulsifiable concentrates) are comprised of an oil-soluble active compound dissolved in a suitable oil-based solvent, and an emulsifier is added to manage mixing with water (Salem et al. 2016). The nanoemulsion involves an oil phase which is spread in continuous water, with each oil droplet enclosed by a thin surface molecular film, thereby helping to balance the nanomolecules' system into more steady formula (Badawy et al. 2018; Tadros et al. 2004). Surfactants can aid in the reduction of the interfacial tension between the oil phase and the aqueous phase (El-Bakry et al. 2021). As a result, surfactants play a critical role in the stabilization and enhancement of the effectiveness of EC and nanoemulsions (Sugumar et al. 2013).

To comprehend the biochemical alterations triggered by the tested insect on citrus orchards, the activity of the antioxidant enzymes should be inquired. These enzymes play a critical role in safeguarding plants from insect attack. It is clear that plants' activity generates reactive oxygen species (ROS) as signaling molecules to resist insect harm (Mittler 2002). The antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) are the main plant ROS-scavenging mechanisms (Apel and Hirt 2004). Further, the polyphenol oxidase (PPO) enzyme may have a role in the defense response encompassing plant resistance to biotic and abiotic stress (Jung 2004). Therefore, the aim of this research was to assess the efficiency of EC and nanoemulsion formulations from EOs in comparison with the commercial mineral oil, Active Cable, against the scale insect *P. ziziphi*, as well as the biochemical effects of the tested compounds on the oxidative enzymes of treated plants.

## Methods

### Plant materials

Leaves of two plant species, *A. herba-alba* and *L. nobilis*, were collected during the flowering stage from the farm of medicinal and aromatic plants at the National Research Centre, Egypt.

### Preparation of the essential oils

The collected plant materials were washed with tap water, followed by distilled water, and dried under shade for 5 days at room temperature (26 ± 1 °C). For 5 h, 1000 g of each plant's leaves were subjected to a hydro-distillation using the Clevenger apparatus. The resultant oils were dried over anhydrous sodium sulfate and kept in dark bottles at 4 °C until used for chemical analysis and the insecticides formulations.

### GC–MS analysis

To identify the main constituents of the plant EOs, the extracted EOs were diluted with diethyl ether and 1 μl was injected into gas chromatography (Trace GC

ULTRA) coupled with ISQ Single Quadrupole mass spectrometry. A nonpolar 5% phenyl methylpolysiloxane capillary column (TG-5MS) (30 m × 0.25 mm ID × 0.25 um) was used. Analysis was carried out under the following conditions: oven temperature programmed from 40 °C (3 min) to 280 °C at 5 °C $min^{-1}$ and then isothermal at 280 °C for 5 min. The carrier gas was helium at a flow rate of 1 mL $min^{-1}$. Spectra were obtained in the electronic ionization mode with 70 eV ionization energy.

### Preparation of the tested formulated compounds

The compounds were naturally prepared as EC and nanoemulsion formulations. The formulations of *A. herba-alba* and *L. nobilis* were named Artemisia and Laury, respectively.

### Emulsifiable concentrate formulations (EC)

Two EOs were prepared as EC formulations by mixing each essential oil separately in appropriate amounts of an emulsifier and natural solvents (vegetable and mineral oils) (Fig. 1). Each EO was formulated at two concentrations (3 and 5%).

### Nanoemulsion formulations

For the preparation of nanoemulsions of *A. herba-alba* and *L. nobilis* oils, Tween 80 was used as an emulsifier. Two different concentrations (3 and 5%) of each EO were formulated as nanoemulsions. The nanoemulsions were prepared in two phases (Fig. 1). The organic phase (EO and surfactant) was prepared by mixing EO and Tween 80 at ratios of 1:0.5, 1:1, 1:1.5, 1:2, and 1:2.5 w/w, respectively. Then, the aqueous phase of deionized water (D.W.) was added to the organic phase and explored on a magnetic stirrer for 30 min at 4000 rpm to get an emulsion. The emulsions formed were subjected to a sonication time of 10 min using ultrasonic (Sonics & Materials, INC. 53 Church Hill Rd. Newtown, CT, USA) with a probe diameter of 13 mm at a high frequency of 20 kHz and a power output of 750 W. The energy was given through a sonicator probe, and ice was used to reduce the energy.

The commercial mineral oil compound, Active Cable (EC), was supplied by Al-Ismaelia Company for chemical products. It is a summer oil used on plants when foliage is present. The compound is a lubrication cut of petroleum oil, prepared as an emulsifiable concentrate for controlling scale insects infesting citrus crops. The compound was sprayed as a reference at the recommended rate of 1 L/100 L water.

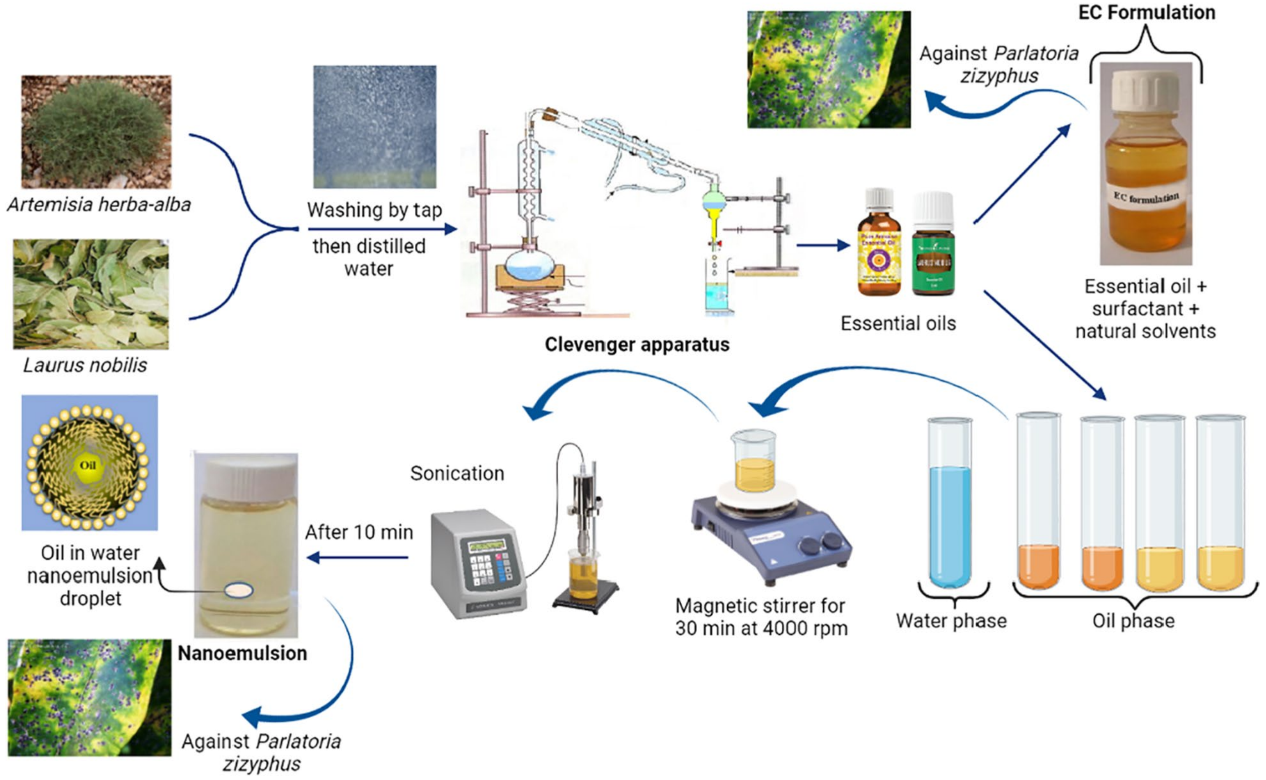

**Fig. 1** Preparation of emulsifiable concentrate and nanoemulsion formulations based on essential oils

## Characterizations of the prepared formulations

### EC emulsion stability

The test was carried out according to WHO specifications (WHO 1979). Into a 250 mL beaker, 75–80 mL of distilled water was poured. Five mL of the EC was added with a pipette while stirring with a glass rod. The beaker contents were completed to 100 mL with distilled water, while the stirring was continuous. The beaker contents were poured immediately into a graduated 100 mL cylinder. The stirring time equaled 3 min from the beginning of the addition of the EC until the emulsion was poured into the cylinder. The cylinder was kept at 30–31 °C for one hour and examined for any free oil or creaming separation.

### Foam formation

The emulsion stability test was carried out to measure the foam amounts formed on the emulsion surface in the cylinder after 5 min.

### Nanoemulsion characterization

Artemisia and Laury nanoemulsions were studied for physicochemical properties. The stability of nanoemulsions was tested with different stress factors such as centrifugation, heating–cooling, and freezing (Ghosh et al. 2013). The prepared formulations were centrifuged at 10,000 rpm for 30 min at 25 °C to observe phase separation if any, formulations that did not show any phase separation were subjected to the heating–cooling test to find out the stability of nanoemulsions at varying temperatures, i.e., at 4° C and 45° C for 48 h at each temperature. Then, the formulations that did not show any phase separation were stored under freezing conditions for 48 h. Finally, the stable nanoemulsions were stored for 4 weeks at room temperature for more observations such as separation or creaming layers. The droplet size of the final stable formulations was evaluated.

### Analysis of nanoemulsion droplet size

The size and size distribution of nanoemulsions were determined by a dynamic light scattering instrument (PSS, Santa Barbara, CA, USA) at 23 °C, using the 632 nm line of a HeNe laser as the incident light with an angle of 90°.

### Insect sampling and inspecting

Homogenated trees of the citrus orchard, *Citrus aurantium* L., infested with the black parlatoria scale, *P. ziziphi* at Barrage district, Qalyubia Governorate, were selected. Treatments of Artemisia and Laury at 3 and 5% (EC and nanoemulsions) along with the commercial compound Active Cable (EC) at 1% concentration were sprayed. The concentrations of EC and nanoemulsion formulations were established through preliminary bioassays by the preparation of a series of EO concentrations. The prepared formulations were sprayed at a rate of 1 L/200 L water. The control treatment was sprayed with water only. Samples of the infested leaves (60 leaves/treatment), i.e., 20 leaves/tree, were taken from different tree directions. Samples were transferred to the laboratory and inspected using a binocular microscope. Both the upper and lower surfaces of leaves were inspected, taking into consideration counts of live and dead individuals for both nymphs and females. Samples were taken before spraying, and after 7, 14, and 21 days from application. Percentages of reduction of nymphs and female populations were calculated according to Henderson and Tilton (1955).

$$\%\text{Reduction} = 1 - \frac{(\text{Ta} \times \text{Cb})}{(\text{Tb} \times \text{Ca})} \times 100$$

where Ta and Tb are number of insects in the treatment after and before application, respectively; Cb and Ca are number of insects in the control before and after application, respectively.

### The side effects of the tested compounds on the oxidative enzymes of the treated plants

Samples of the tested formulations were taken before spraying, and after 7, 14, and 21 days from application. Other samples from the control were also taken after the same previously tested periods.

### Enzyme preparation

Enzyme extracts were prepared according to the method described by Chen and Wang (2006). Leaf tissues were homogenized in ice-cold phosphate buffer (50 mM, pH 7.8), followed by centrifugation at 8000 rpm and 4 °C for 15 min. The supernatant was used to determine the activities of the tested enzymes.

### Superoxide dismutase (SOD)

(SOD, EC 1.12.1.1) activity was spectrophotometrically assayed at 560 nm by the nitro-blue-tetrazolium reduction method (Chen and Wang 2006).

### Catalase (CAT)

CAT (EC 1.11.1.6) activity was determined spectrophotometrically by following the decrease in absorbance at 240 nm (Chen and Wang 2006). The enzyme activity was calculated by Kong et al. (1999).

### Polyphenol oxidase (PPO)

(PPO, EC 1.10.3.1) activity was determined using a spectrophotometric method based on an initial rate of increase in absorbance at 410 nm (Soliva et al. 2000). The absorbance at 410 nm was recorded continuously at 25 °C for 5 min.

### Statistical analysis

Data were subjected to a one-way analysis of variance followed by Duncan's multiple range test to determine significant differences among mean values at the probability level of 0.05, using SPSS 25.0 software program (SPSS 2019).

## Results

### The chemical composition of EOs

The chemical composition of the EOs isolated from two plants growing in Egypt obtained by hydro-distillation was analyzed using GC/MS (Table 1). The results of the analyses showed that the oil of *A. herba-alba* was rich in camphor (27.65%), chrysanthenone (16.48%), 1,8-cineole (8.90%), and α-thujone (7.85%), followed by camphene (4.10%) and sabinene (4.02%), while the major component of *L. nobilis* was 1,8-cineole (49.74%), followed by sabinene (12.38%) and α-terpinyl acetate (10.20%).

### Characterizations of EC formulations

Maintaining EC formulations down the environmental storage conditions is one of the most critical factors for governing their efficiency and persistence. The prepared EC formulations, Artemisia and Laury, were examined at concentrations of 3 and 5% through the emulsion stability. The cream layers of the evaluated formulations were in the range of 1.5 to 2 mL (Table 2).

Foam formation results revealed that all the prepared EC formulations passed the foam formation, where the foam layers were 0 mL for Laury and 2, 2.5 mL for Artemisia, at 3 and 5%, respectively (Table 2).

**Table 1** Main constituents (%) of the essential oils isolated from leaves of *Artemisia herba-alba* and *Laurus nobilis*

| ***A. herba-alba*** | | | ***L. nobilis*** | | |
|---|---|---|---|---|---|
| **Compound** | **Rt[a] (min)** | **Percentage** | **Compound** | **Rt (min)** | **Percentage** |
| Tricyclene | 9.64 | 0.32 | α-Pinene | 11.07 | 4.74 |
| α-Pinene | 11.09 | 0.25 | Camphene | 13.06 | 0.23 |
| Camphene | 13.09 | 4.1 | Sabinene | 13.61 | 12.38 |
| Sabinene | 13.52 | 4.02 | Myrcene | 13.9 | 0.69 |
| β-Pinene | 13.7 | 0.3 | α-Terpinene | 14.26 | 0.28 |
| β-Myrcene | 13.99 | 0.56 | p-Cymene | 14.4 | 0.95 |
| Carene | 14.15 | 0.18 | Limonene | 14.5 | 0.9 |
| α-Terpinene | 14.28 | 0.75 | Germacradienol | 14.6 | 5.23 |
| β-Phellandrene | 14.37 | 0.47 | 1,8-Cineole | 15.38 | 49.74 |
| p-Cymene | 14.51 | 1.52 | γ-Terpinene | 16.43 | 0.79 |
| 1,8-Cineole | 15.4 | 8.9 | Terpinolene | 16.91 | 0.2 |
| γ-Terpinene | 16.46 | 0.77 | Linalool | 19.1 | 0.63 |
| α-Thujone | 17.59 | 7.85 | Borneol | 19.7 | 1.13 |
| β-Thujone | 17.94 | 12.83 | α-Caryophyllene | 21.71 | 0.97 |
| Chrysanthenone | 18.13 | 16.48 | Terpinen-4-ol | 24.81 | 1.01 |
| Trans-Pinocarveol | 18.89 | 0.61 | α-Terpineol | 25.66 | 3.41 |
| Camphor | 19.13 | 27.65 | Bicyclogermacrene | 27.08 | 0.23 |
| Borneol | 19.71 | 1.72 | α-Terpinyl acetate | 28.24 | 10.2 |
| Pinocarvone | 20.01 | 0.73 | (-)-Spathulenol | 29.39 | 1.4 |
| Caryophyllene | 21.76 | 0.21 | Methyl eugenol | 31.19 | 2.96 |
| Terpinen-4-ol | 24.82 | 0.61 | | | |
| α-Terpineol | 25.66 | 0.25 | | | |
| cis-Chrysanthenyl acetate | 26.25 | 0.94 | | | |
| Germacrene D | 26.91 | 2.21 | | | |
| Trans-Nerolidol | 27.64 | 0.44 | | | |
| Davanone | 28.28 | 2.37 | | | |
| (-)-Spathulenol | 29.38 | 0.97 | | | |

[a] The retention time

**Table 2** Emulsion stability and foam formation of EC laboratory-prepared formulations

| Formulation | | Conc. of surf. % (v/v) | Conc. of solvents % (v/v) | Emulsion stability (separation mL) | Foam formation (mL) |
|---|---|---|---|---|---|
| Artemisia | 3% | 5 | 92 | 1.8 | 2 |
| | 5% | 5 | 90 | 2 | 2.5 |
| Laury | 3% | 10 | 87 | 1.5 | 0 |
| | 5% | 10 | 85 | 1.5 | 0 |

Conc. = concentration

### Characterizations of nanoemulsions

Different ratios (1:0.5 to 1:2.5 w/w) of each *A. herba-alba* and *L. nobilis* EOs and Tween 80 were mixed separately to find the best ratio of EO and the surfactant. Nanoemulsions were subjected to strict storage conditions to ensure that the prepared formulations would remain stable during storage. Among all the combinations, the nanoemulsions with ratios of EOs to Tween 1:1.5, 1:2, and 1:2.5 of Artemisia and 1:2 and 1:2.5 of Laury were found to be more stable after centrifugation, heating, cooling, and freeze cycles at 3 and 5% concentrations as there were no observations of phase separation. Therefore, the droplet sizes of the successful samples with lower surfactant concentration were measured (EOs to Tween 1:1.5 of Artemisia and 1:2 of Laury at 3 and 5% active ingredient). Results in Table 3 showed that the mean droplet size diameter was 153.7 and 113.4 nm for Artemisia (Fig. 2) and 139.3 and 89.2 nm for Laury at 3 and 5%, respectively (Fig. 3).

### Bioefficacy of EC and nanoemulsion formulations on *P. ziziphi* females' population

The insecticidal efficiency of two formulation types of EC and nanoemulsions based on the EOs, *A. herba-alba* and *L. nobilis,* against the females' population of the scale insect, *P. ziziphi*, was evaluated. Data in Table 4 cleared that EC emulsions of Laury and Artemisia at concentrations of 3 and 5% were not significantly different, as they reduced the females' population of *P. ziziphi* with high reduction of 94.15 and 97.66%, for Laury and 94.79 and 96.87% for Artemisia, respectively, compared to 95.2% for the commercial formulation, Active Cable. Meanwhile, the same oils prepared as nanoemulsions caused a lower reduction of insect females, reaching 33.61 and 38% of Laury and 35.71 and 37.39% of Artemisia at 3 and 5%, respectively.

**Table 3** Ratio of the components of the successful essential oils' nanoemulsions

| Nanoemulsion | Ratio of EO and tween 80 (w/w) | Formulation (%) | | | Particle size (nm) |
|---|---|---|---|---|---|
| | | EO | Tween 80 | D.W.[a] | |
| Artemisia | 1:1.5 | 3 | 4.5 | 92.5 | 153.7 |
| | 1:1.5 | 5 | 7.5 | 87.5 | 113.4 |
| Laury | 1:2 | 3 | 6 | 91 | 139.3 |
| | 1:2 | 5 | 10 | 85 | 89.2 |

[a] Deionized water

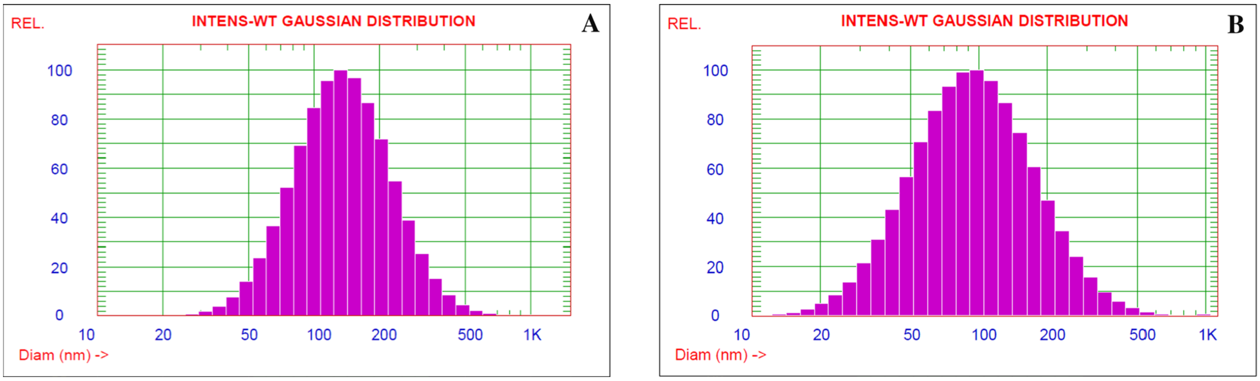

**Fig. 2** Particle size of Artemisia essential oil nanoemulsion: **A** with size 153.7 nm and **B** with size 113.4 nm at 3 and 5% concentrations, respectively

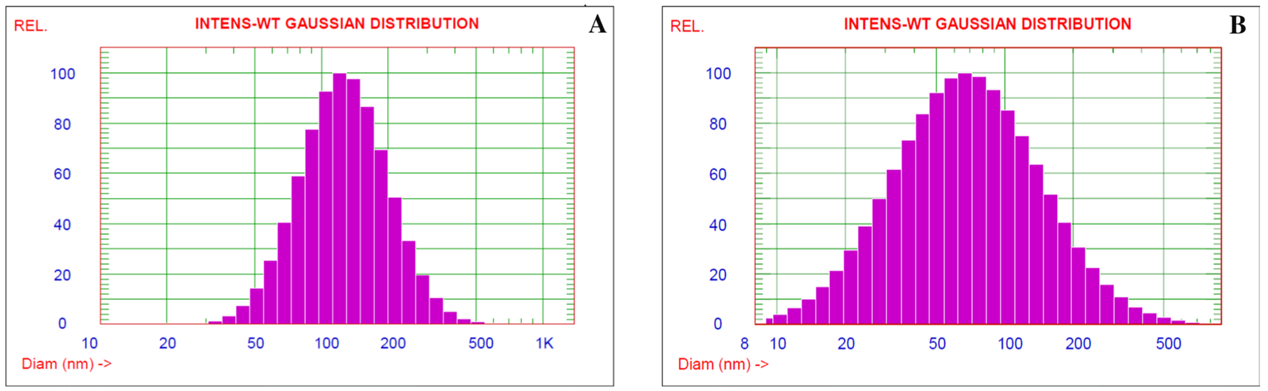

**Fig. 3** Particle size of Laury essential oil nanoemulsion: **A** with size 139.3 nm and **B** with size 89.2 nm at 3 and 5% concentrations, respectively

**Table 4** Insect females of *Parlatoria ziziphi* affected by application of two formulation types based on the plant essential oils in citrus orchards

| Treatments | Concentration | Formulation type | Live individuals | | | | Grand mean | Reduction% |
|---|---|---|---|---|---|---|---|---|
| | | | Pre spraying | After spraying (Days) | | | | |
| | | | | 7 | 14 | 21 | | |
| Laury | 3% | EC emulsions | 1040.3 | 10.93 | 22.13 | 67.2 | 62e$^{a}$ | 94.15a |
| | 5% | | 1020.93 | 12.93 | 15.74 | 44.27 | 24.31e | 97.66a |
| Artemisia | 3% | | 1017.67 | 16.73 | 59.73 | 85.67 | 54.04e | 94.79a |
| | 5% | | 1019.93 | 14.67 | 28.13 | 54.8 | 32.53e | 96.87a |
| Active Cable | 1% | | 1025 | 17.8 | 24.53 | 108.1 | 50.14e | 95.2a |
| Laury | 3% | Nanoemulsion | 1043.65 | 782.80 | 704.46 | 633.84 | 707.03b | 33.61b |
| | 5% | | 1056.71 | 739.67 | 665.29 | 598.82 | 667.93bc | 38b |
| Artemisia | 3% | | 984.78 | 985.89 | 492.39 | 458.17 | 645.48 cd | 35.71b |
| | 5% | | 944.66 | 897.42 | 472.33 | 439.18 | 602.98d | 37.39b |
| Control | | | 1026 | 1036 | 1043 | 1059 | 1046a | –– |
| $LSD_{0.05}{}^{b}$ | | | NS$^{c}$ | | | | 46.26 | 5.62 |

[a] Means coupled by the same letter in a column do not differ significantly at 5% level of probability (Duncan test)

[b] Least significant difference at 0.05 level of probability

[c] Non-significant difference at 0.05 level of probability

### The efficiency of EC and nanoemulsion formulations on *P. ziziphi* nymphs' population

Data in Table 5 cleared the insecticidal potential of two different formulation types on the nymph population of *P. ziziphi.* Laury EC emulsions of 3 and 5% concentrations were not significantly different as they gave reduction percentages of 91.44 and 92.23, respectively. On the other hand, there were significant differences between the same concentrations of Artemisia with reductions of 55.16 and 86.17%, respectively, in comparison with 88.29% of Active Cable. It is noteworthy that nymphs responded better to nanoemulsions than females, as the percent of reductions were 66.42 and 72.65 for Laury and 51.72 and 70.63 for Artemisia at the same concentrations mentioned before.

Concerning the average percentage of reduction of the insect population (females and nymphs) of *P. ziziphi*, data cleared that Laury 3 and 5% of EC emulsions reduced the insect population by 92.79 and 94.94, respectively, while the corresponding values of their nanoemulsions were 50.02 and 55.32% (Table 6). As for Artemisia EC treatments, data revealed that the concentration of 5% increased the efficiency by 91.52%, compared with 74.97% for the concentration of 3%. In the same trend, the percentage of reduction

**Table 5** Insect nymphs of *Parlatoria ziziphi* affected by application of two formulation types based on the plant essential oils in citrus orchards

| Treatments | Concentration | Formulation type | Live individuals | | | | Grand mean | Reduction% |
|---|---|---|---|---|---|---|---|---|
| | | | Pre spraying | After spraying (Days) | | | | |
| | | | | 7 | 14 | 21 | | |
| Laury | 3% | EC emulsions | 616.7a$^{a}$ | 16.73 | 13.53 | 90.67 | 40.31f | 91.44a |
| | 5% | | 595.73a | 12.07 | 17.93 | 76.07 | 35.36f | 92.23a |
| Artemisia | 3% | | 618.27a | 9.73 | 39.53 | 585.7 | 211.65b | 55.16c |
| | 5% | | 617a | 28.6 | 47.4 | 119.4 | 65.13e | 86.17a |
| Active Cable | 1% | | 615.87a | 136.27 | 25.73 | 3.31 | 55.04ef | 88.29a |
| Laury | 3% | Nanoemulsion | 621.66a | 188.99 | 160.64 | 128.46 | 159.36c | 66.42b |
| | 5% | | 608.39a | 138.71 | 124.80 | 117.67 | 127.06d | 72.65b |
| Artemisia | 3% | | 521.98b | 357.03 | 119 | 101.18 | 192.40b | 51.72c |
| | 5% | | 502.83b | 267.51 | 38.21 | 32.57 | 112.76d | 70.63b |
| Control | | | 595a | 218.07 | 597.07 | 547.67 | 454.27a | – |
| $\text{LSD}_{0.05}{}^{b}$ | | | 63.19 | | | | 20.64 | 8.05 |

[a] Means coupled by the same letter in a column do not differ significantly at 5% level of probability (Duncan test)

[b] Least significant difference at 0.05 level of probability

**Table 6** Average percentages of reduction in insect population of *Parlatoria ziziphi* as a result of spraying certain essential oils as emulsions and nanoemulsions

| Tested formulations | Insect stage | Concentration | EC emulsions | | Nanoemulsions | |
|---|---|---|---|---|---|---|
| | | | Reduction% | Average of reduction | Reduction% | Average of reduction |
| Laury | Females | 3% | 94.15 | 92.79a$^{a}$ | 33.61 | 50.02c |
| | Nymphs | | 91.44 | | 66.42 | |
| | Females | 5% | 97.66 | 94.94a | 38 | 55. 32c |
| | Nymphs | | 92.23 | | 72.65 | |
| Artemisia | Females | 3% | 94.79 | 74.97b | 35.71 | 43.71d |
| | Nymphs | | 55.16 | | 51.72 | |
| | Females | 5% | 96.87 | 91.52a | 37.39 | 54.01c |
| | Nymphs | | 86.17 | | 70.63 | |
| Active Cable | Females | 1% | 95.20 | 91.74a | 95.20 | 91.74a |
| | Nymphs | | 88.29 | | 88.29 | |
| $\text{LSD}_{0.05}{}^{b}$ | | | | 5.66 | | 5.66 |

[a] Means coupled by the same letter in rows or columns do not differ significantly at 5% level of probability (Duncan test)

[b] Least significant difference at 0.05 level of probability

for Artemisia nanoemulsions was 43.71 and 54.01 at 3 and 5%, respectively, compared with 91.74% for Active Cable.

## The biochemical impacts of the examined compounds on the citrus orchards' enzymatic antioxidants

### *Superoxide dismutase enzyme (SOD)*

The effect of Laury and Artemisia formulations (EC and nanoemulsions) compared with Active Cable oil on SOD enzyme activity at different intervals (7, 14, and 21 days) is summarized in Table 7. The treated plants significantly differed in their activity during all testing intervals. Before spraying, the activity of SOD ranged between 794 and 827 U/g fresh weight/hour. Laury 5% (EC) exhibited the lowest significant decrease in enzyme activity with 225.33 U/g f.w/h, while the control had the highest activity (828) at 7 days after treatment (DAT). Laury 3% and Artemisia 5% EC emulsions were

**Table 7** Effect of the treatment with tested compounds on superoxide dismutase (SOD) enzyme of citrus orchard

| Enzyme activity (U/g fresh w./h) | Treatment | Days after treatment | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Before spray | 7 | | 14 | | 21 | |
| | | | EC | Nano | EC | Nano | EC | Nano |
| SOD | Control | 800 ± 8 | 828 ± 16a[a] | | 812 ± 52a | | 826 ± 20a | |
| | Active Cable | 827 ± 29 | 445.33 ± 132c | | 465 ± 41ef | | 527 ± 17d | |
| | Laury 3% | 811 ± 23 | 397.33 ± 9.9 cd | 638 ± 15.1b | 495 ± 3de | 628 ± 14c | 593 ± 45 cd | 602 ± 56c |
| | Laury 5% | 813 ± 7 | 225.33 ± 7.1e | 628 ± 10.6b | 426 ± 10f | 616 ± 10c | 587 ± 41c | 592 ± 52 cd |
| | Artemisia 3% | 794 ± 26 | 432 ± 79.7c | 659.33 ± 20.8b | 596 ± 16c | 686 ± 2b | 690 ± 10b | 650 ± 30bc |
| | Artemisia 5% | 823 ± 63 | 322 ± 64.1d | 633.33 ± 23.2b | 510 ± 20d | 620 ± 2c | 583 ± 53 cd | 612 ± 42c |
| | $\text{LSD}_{0.05}$[b] | NS[c] | 92.69 | | 66.62 | | 110.99 | |

Each value represents the mean of three replicates ± standard deviation

[a] Means with different letters within each interval were significantly different at the 0.05 level (Duncan's multiple range test)

[b] Least significant difference at 0.05 level of probability

[c] Non-significant difference at 0.05 level

the following compounds in reducing the enzyme activity with no significant differences (397.33 and 322 U/g. f.w./h, respectively). The activity of EC emulsions was increased, while it was the opposite of nanoemulsions after 14 and 21 DAT. Our findings revealed that there were no significant differences between the nanoemulsions of Laury 3 and 5% at all the tested intervals. In addition, the activity of Laury 3 and 5% and Artemisia 5% nanoemulsions decreased with time elapsed. Furthermore, a variation in the SOD activity resulted from the Active Cable application was observed; the paramount decrease in activity was obtained after 7 DAT (445.33 U/g f.w./h).

### *CAT enzyme*

Regarding the effect of the examined formulations on CAT, the same tendency of activity was observed as SOD (Table 8). All the treated plants were significantly different compared with the control at all measured intervals. The enzyme activity of EC formulations increased with time elapsed, and it was less than nanoemulsions, while the activity of nanoemulsions decreased gradually over time. For example, the activity of Laury 5% nanoemulsion was decreased from 19.84 U/g f.w./h at 7 DAT to 16.95 at 21 DAT. As for Laury 5% EC emulsion, which was the most influential compound in reducing the enzyme activity, a significant decrease in CAT activity was obtained at

**Table 8** Effect of the treatment with tested compounds on catalase (CAT) enzyme of citrus orchard

| Enzyme activity (U/g fresh w./h) | Treatment | Days after treatment | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Before spray | 7 | | 14 | | 21 | |
| | | | EC | Nano | EC | Nano | EC | Nano |
| Catalase | Control | 36.13 ± 0.15b[a] | 39.04 ± 0.53a* | | 38.34 ± 0.14a | | 38.23 ± 0.03a | |
| | Active Cable | 37.65 ± 0.43a | 13.78 ± 8.20e | | 16.39 ± 3.70d | | 16.50 ± 2.01e | |
| | Laury 3% | 35.91 ± 0.31b | 12.78 ± 4.57e | 22.69 ± 1.55bc | 20.42 ± 1.33 cd | 22.98 ± 6.28bc | 21.64 ± 6.38bcd | 21.92 ± 1.15bcde |
| | Laury 5% | 36.03 ± 0.45b | 5.26 ± 1.09f | 19.84 ± 1.03 cd | 16.09 ± 2.44d | 18.97 ± 0.13 cd | 19.39 ± 5.28cde | 16.95 ± 1.10de |
| | Artemisia 3% | 37.72 ± 0.83a | 15.55 ± 0.28de | 27.59 ± 2.10b | 19.86 ± 0.07 cd | 26.72 ± 0.55b | 24.01 ± 3.14bc | 26.54 ± 0.20b |
| | Artemisia 5% | 37.65 ± 0.01a | 10.99 ± 1.18e | 25.35 ± 0.85b | 17.94 ± 7.69 cd | 23.33 ± 0.11bc | 22.86 ± 6.65bcde | 22.64 ± 0.02bcd |
| | $\text{LSD}_{0.05}$[b] | 0.79 | 5.37 | | 5.91 | | 6.12 | |

Each value represents the mean of three replicates ± standard deviation

[a] Means with different letters within each interval were significantly different at the 0.05 level (Duncan's multiple range test)

[b] Least significant difference at 0.05 level of probability

**Table 9** Effect of the treatment with the two different tested formulations on polyphenol oxidase (PPO) enzyme of citrus orchard

| Enzyme activity (U/g fresh w./h) | Treatment | Days after treatment | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Before spray | 7 | | 14 | | 21 | |
| | | | EC | Nano | EC | Nano | EC | Nano |
| PPO | Control | 10.98 ± 0.62 | 11.49 ± 1.22a[a] | | 11.89 ± 1.3a | | 11.44 ± 0.2a | |
| | Active Cable | 10.37 ± 0.76 | 5.73 ± 0.12c | | 5.89 ± 0.4c | | 6.06 ± 0.6f | |
| | Laury 3% | 11.00 ± 0.84 | 5.28 ± 0.87c | 8.32 ± 0.24b | 5.38 ± 0.8 cd | 8.21 ± 0.2b | 6.34 ± 0.3ef | 8.05 ± 0.6bc |
| | Laury 5% | 10.02 ± 1.76 | 3.73 ± 0.28d | 7.89 ± 0.83b | 4.64 ± 0.6d | 7.73 ± 0.8b | 5.98 ± 0.6f | 6.69 ± 0.3def |
| | Artemisia 3% | 10.34 ± 1.40 | 5.84 ± 0.65c | 8.26 ± 0.83b | 5.81 ± 0.7 cd | 8.05 ± 0.7b | 7.22 ± 0.2 cd | 8.56 ± 0.8b |
| | Artemisia 5% | 10.13 ± 0.92 | 4.74 ± 0.65 cd | 7.97 ± 1.03b | 5.2 ± 0.7 cd | 7.86 ± 0.6b | 6.24 ± 0.2f | 7.17 ± 0.7de |
| | $LSD_{0.05}$[b] | NS[c] | 1.29 | | 1.24 | | 0.86 | |

Each value represents the mean of three replicates ± standard deviation

[a] Means with different letters within each interval were significantly different at the 0.05 level (Duncan's multiple range test)

[b] Least significant difference at 0.05 level of probability

[c] Non-significant difference at 0.05 level

7 DAT with 5.26 U/g f.w./h compared with the control (39.04 U/g f.w./h).

#### *Polyphenol oxidase (PPO)*

Data in Table 9 elucidated that the variations in PPO activity depend on the effectiveness of the tested compounds. Generally, the results of PPO enzyme activity before spraying cleared that the insect infestation induced a significant increase in PPO activity which ranged between 10.02 and 11.00 U/g f.w./h. EC emulsions were the most influential in reducing PPO activity, where the compounds of Laury and Artemisia at 5% concentration caused the lowest enzyme activity (3.73 and 4.74 U/g f.w./h, respectively, at 7 DAT). Also, Laury and Artemisia at a concentration of 3% and Active Cable oil caused a significant decrease in the enzyme activity (5.28, 5.84, and 5.73 U/g f.w./h, respectively, after 7 DAT) compared with the control (11.49 U/g f.w./h).

## Discussion

The major constituents of the isolated EOs from *A. herba-alba* and *L. nobilis* were significantly and/or slightly different from the previous reports (Dahmani-Hamzaoui and Baaliouamer 2015; Mathlouthi et al. 2021; Mohsen and Ali 2009; Salido et al. 2001). The variations of the EO concentrations and components might be attributed to many factors, including the geographic site, season, the condition of the environment, the plant nutrition level, and might be some additional factors as well (Badawy et al. 2018; El-Bakry et al. 2016, 2019). EC formulations of *A. herba-alba* and *L. nobilis* EO were prepared in the current research utilizing suitable emulsifiers and natural solvents (vegetable and mineral oils). Previous investigators prepared successful EC formulations from different EOs by using appropriate amounts of some emulsifiers and natural solvents (mineral and vegetable oils) (Abdel-Aziz et al. 2015; 2018; Salem et al. 2016; Sammour et al. 2018). The physical and chemical properties of the studied EC formulations showed that all the formulations passed the emulsion stability and the foam formation, where the maximum cream layer should not exceed 2 mL (WHO 1979) and the foam layer, if any, should not override 5 mL (WHO 1979). This suggests that the evaluated EC formulations could be employed without separation and foaming troubles (Abdel-Aziz et al. 2018; Sammour et al. 2018). At a sonication time of 10 min, nanoemulsions of the aforementioned EO were prepared by the combination of the EO and Tween 80 with different ratios. Earlier investigations reported that the optimal sonication process time is 10 min, where the additional sonication does not supply further reduction in the droplet size of nanoemulsion (Adak et al. 2020; Pratap-Singh et al. 2021). The results of the mean droplet diameter demonstrated that all the nanoemulsion prepared formulations were in the nanosize range. Many researchers have generated nanoemulsions from some EOs like rosemary, eucalyptus, and cinnamon (Adak et al. 2020; Kaur et al. 2021; Mossa et al. 2019) and evaluated their biological activities against insect pests. In non-balanced systems, emulsions tend to lower their interfacial area and free energy throughout some breakdown techniques, like creaming, sedimentation, flocculation, and coalescence (Badawy et al. 2018). The present study revealed that the prepared nanoemulsions with the highest surfactant concentration exhibited the smallest particle size, which was in correspondence with Arancibia et al. (2017) and Kaur et al. (2021). High surfactant concentration aids in reducing the interfacial tension between the oil and aqueous phases, resulting in smaller particle sizes (Yildirim et al. 2017). The current research elucidated that treatments with EC emulsions were more efficient than nanoemulsions for increasing the reduction percent of infestations. All the tested stages, except crawlers, were fixed, settled on the plant surface, and their bodies were covered with scale. The higher insect responsibility to EC emulsions than to nanoemulsions may be due to the fact that EC emulsions kill scale insects via contact by making a thin film from oil solution around the insect wax cover. This film prevents insects from breathing oxygen (Mead et al. 2016; Salem et al. 2016). They also kill scale insects by disrupting their feeding on the oil-covered surfaces; therefore, their toxic action on scale insects is more physical than chemical. Consequently, EC formulations which have a droplet size diameter greater than nanoemulsions are more efficient than nanoemulsions. Campolo et al. (2017) revealed that higher mortality on eggs (as a settled stage) of *Tuta absoluta* (Meyrick) (Gelechiidae: Lepidoptera) by citrus peel essential oil emulsions was done through contact toxicity, but on larvae (as a mobile stage) higher mortality by EOs emulsions was done through contact and ingestion toxicity, respectively. The effect of nanoemulsions depends on the penetration of the insect's waxy cuticle and the plant tissue, which leads to the movement through the plant sap and sucking by scale insects, causing kill (Mustafa and Hussein 2020). The obtained results showed that the efficiency of EC compounds decreased after 7 d, which was the opposite of nanoemulsions. These results indicated that nanoemulsions had persistent properties compared to EC formulations. Sabbour and Abd El-Aziz (2019) reported that nanoemulsion formulations avoid the rapid degradation and evaporation and improve insecticidal persistence. Further, Yang et al. (2009) found that the efficacy of garlic EO nanoparticles on *Tribolium castaneum* was lower relative to its free essential oil in the first month, but this became gradually stronger in the following months compared to the free essential oil. Data also cleared that EC formulations of Laury and Artemisia 5% along with the commercial mineral oil, Active Cable, were the most potent as they were not significantly different. Previous research elucidated the role of essential oil formulations in reducing the scale insects' infestations. Abdelaziz et al. (2014) mentioned that a laboratory-prepared formulation based on rosemary EO was effective in reducing the population of *Aulacaspis tubercularis* (Newstead) (Hemiptera: Diaspididae). Moreover, Salem et al. (2016) revealed that the laboratory EO formulations of *Cymbopogon citratus* (Stapf.) (Poales: Poaceae), *C. nardus* (L.) (Poales: Poaceae), and *Origanum minutiflorum* (Lamiales: Lamiaceae) were more potent on *A. tubercularis* than the commercial neem oil, Trilogy.

The antioxidant enzymes SOD, CAT, and PPO play an important role in protecting plants from various stresses (including herbivorous insects) as they induce in plants in response to herbivory attack (He et al. 2011). Also, they are the main enzymes in the ROS cleaning system; they can efficiently restrict ROS from damaging the body of living organisms and play an important role in the induced protective response (War et al. 2013). The activity of SOD, CAT, and PPO in the current research was increased before spraying, which indicated that the plants were indeed under insect infestation stress (biotic stress), while the application of the tested formulations declined their activities. Further, EC emulsions inhibited these three enzymes more than nanoemulsions. The lower-enzyme activity may be attributed to the lower survival of insects on the treated plants as mentioned by Adamczyk et al. (2003). Chaman et al. (2001) stated that insect infestations induce changes in the levels of plants' oxidative enzymes. This infestation triggers biotic stresses which stimulate ROS creation, causing an increase in the intrinsic antioxidant enzymes activity such as SOD, catalase, and polyphenol oxidase compared to uninfected plants (Afzal et al. 2014; Kaur et al. 2017).

## Conclusions

At 3 and 5% concentrations, EC and nanoemulsion formulations of two plant EOs (*A. herba-alba* and *L. nobilis*) were prepared. The droplet size of nanoemulsion formulations ranged from 89.4 to 153.7 nm by ultrasonic emulsification. EC formulations, especially Laury 3% and Artemisia 5%, which originated from the EOs *L. nobilis* and *A. herba-alba* along with the commercial formulation Active Cable were the most potent against the scale insect, *P. ziziphi*, with 92.79, 91.52, and 91.74% reduction of infestation, respectively. All the tested formulations reduced the activity of plant enzymes as an indication of reducing insect infestation. The effect of EC formulations was more obvious than nanoemulsions.

### Abbreviations

D.W.: Deionized water; DAT: Days after treatment; EC: Emulsifiable concentrates; EOs: Essential oils; ROS: Reactive oxygen species; SOD: Superoxide dismutase; CAT: Catalase; PPO: Polyphenol oxidase.

### Author contributions

NFA, HAS, AME, and EAS designed experiments, carried out the experiments, analyzed the data, and wrote the article. All authors have read and approved the manuscript.

### Funding

his research was financed by the National Research Centre, Egypt, Project No. 12050114

### Availability of data and materials

All the data generated or analyzed during this study were included within the article.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that there is not any conflict of interests.

Received: 23 September 2021 Accepted: 22 August 2022

## References

Abdel-Aziz NF, Abdou WL, Abdel-Hakim EA, El-Hawarya FM, El-Bakry AM, Sammour EA (2015) The effect of some green insecticides from essential oils on *Aphis craccivora*, and their side effects. J Entomol Res 39(4):275–286. https://doi.org/10.5958/0974-4576.2015.00034.1

Abdel-Aziz NF, El-Bakry AM, Metwally NS, Sammour EA, Farrag ARH (2018) Insecticidal efficiency of some green-based formulations on *Spodoptera littoralis* and their side effects on *Albino rats*. Asian J Crop Sci 10(4):198–206. https://doi.org/10.3923/ajcs.2018.198.206

Abdelaziz NF, Salem H, Sammour E (2014) Insecticidal effect of certain ecofriendly compounds on some scale insects and mealybugs and their side effects on antioxidant enzymes of mango nurslings. Arch Phytopathol Plant Protect 47(1):1–14

Adak T, Barik N, Patil NB, Gadratagi BG, Annamalai M, Mukherjee AK et al (2020) Nanoemulsion of eucalyptus oil: An alternative to synthetic pesticides against two major storage insects (*Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst)) of rice. Ind Crops Prod 143:111849. https://doi.org/10.1016/j.indcrop.2019.111849

Adamczyk J, Williams M, Reed J, Hubbard D, Hardee D (2003) Spatial and temporal occurrence of beet armyworm (Lepidoptera: Noctuidae) moths in Mississippi. Fla Entomol 86(3):229–232

Afzal F, Khurshid R, Ashraf M, Kazi AG (2014) Reactive oxygen species and antioxidants in response to pathogens and wounding. In: Ahmad P (ed) Oxidative damage to plants: antioxidant networks and signaling. Academic Press, an imprint of Elsevier, United States of America

Al-Dosary NH, Al-Najim IA, Al-Mansory NA, Ali HM (2008) Evaluate sufficiency of some plants oils to control on white scale insect *Parlatoria blanchardi* (Coccocide: Homoptera) on date palm (*Phoenix dactylifera* L.). Basrah J Date Palm Res 7(1):48–61

Ansari MS, Moraiet MA, Ahmad S (2014) Insecticides: impact on the environment and human health. Environmental deterioration and human health. Springer, pp 99–123

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:373–399. https://doi.org/10.1146/annurev.arplant.55.031903.141701

Arancibia C, Riquelme N, Zúñiga R, Matiacevich S (2017) Comparing the effectiveness of natural and synthetic emulsifiers on oxidative and physical stability of avocado oil-based nanoemulsions. Innov Food Sci Emerg Technol 44:159–166. https://doi.org/10.1016/j.ifset.2017.06.009

Assouguem A, Kara M, Mechchate H, AlZain MN, Noman OM, Imtara H et al (2021) Evaluation of the effect of different concentrations of spirotetramat on the diaspine scale *Parlatoria ziziphi* in citrus orchards. Agronomy 11(9):1840. https://doi.org/10.3390/agronomy11091840

Aumeeruddy-Elalfi Z, Gurib-Fakim A, Mahomoodally F (2015) Antimicrobial, antibiotic potentiating activity and phytochemical profile of essential oils from exotic and endemic medicinal plants of Mauritius. Ind Crops Prod 71:197–204

Badawy MEI, Abdelgaleil SAM, Mahmoud NF, Marei AE-SM (2018) Preparation and characterizations of essential oil and monoterpene nanoemulsions and acaricidal activity against two-spotted spider mite (*Tetranychus urticae* Koch). Int J Acarol 44(7):330–340. https://doi.org/10.1080/01647954.2018.1523225

Benaissa K, Belhamra M (2017) Contribution to a study of the effect of the essential oil of henna (*Lawsonia inermis* L), on the biological aspect of white scale (*Parlatoria blanchardi* targ) of date palm. Indian J Pharm Educ 51(3):S309–S312

Campolo O, Cherif A, Ricupero M, Siscaro G, Grissa-Lebdi K, Russo A et al (2017) Citrus peel essential oil nanoformulations to control the tomato borer, *Tuta absoluta*: chemical properties and biological activity. Sci Rep 7(1):1–10. https://doi.org/10.1038/s41598-017-13413-0

Chaman ME, Corcuera LJ, Zuniga GE, Cardemil L, Argandoña VH (2001) Induction of soluble and cell wall peroxidases by aphid infestation in barley. J Agric Food Chem 49(5):2249–2253. https://doi.org/10.1021/jf0011440

Chen J, Wang X (2006) Plant physiology experimental guide. Higher Educ Press, Beijing 24(25):55–56

Dahmani-Hamzaoui N, Baaliouamer A (2015) Volatile constituents of Algerian *Artemisia herba-alba* essential oils. J Essent Oil Res 27(5):437–446

Derbalah A, Morsey S, El-Samahy M (2012) Some recent approaches to control *Tuta absoluta* in tomato under greenhouse conditions. Afr Entomol 20(1):27–34

Ebrahimi M, Safaralizade MH, Valizadegan O, Amin BHH (2013) Efficacy of three plant essential oils, *Azadirachta indica* (Adr. Juss.), *Eucalyptus camaldulensis* (Dehn.) and *Laurus nobilis* (L.) on mortality cotton aphids, *Aphis gossypii* Glover (Hem: Aphididae). Arch Phytopathol Plant Protect 46(9):1093–1101

El-Bakry AM, Abdel-Aziz NF, Sammour EA, Abdelgaleil SAM (2016) Insecticidal activity of natural plant essential oils against some stored product insects and their side effects on wheat seed germination. Egypt J Biol Pest Control 26(1):83–88

El-Bakry AM, Abdou GY, Abdelaziz NF, Shalaby SM (2021) Improvement of expired insecticides and their effectiveness against cotton leafworm, *Spodoptera littoralis* (Boisd.). J King Saud Univ Sci 33:101649. https://doi.org/10.1016/j.jksus.2021.101649

El-Bakry AM, Youssef HF, Abdel-Aziz NF, Sammour EA (2019) Insecticidal potential of Ag-loaded 4A-zeolite and its formulations with *Rosmarinus officinalis* essential oil against rice weevil (*Sitophilus oryzae*) and lesser grain borer (*Rhyzopertha dominica*). J Plant Prot Res 59(3):324–333

Faskha SM (2021) Natural enemies associated with black parlatoria scale, *Parlatoria ziziphi* (Lucas) (Homoptera: Diaspididae), in citrus orchards at El-Qualubia governorate, Egypt. Entomologia Hellenica 30(2):33–42. https://doi.org/10.12681/eh.26102

Ghosh V, Saranya S, Mukherjee A, Chandrasekaran N (2013) Cinnamon oil nanoemulsion formulation by ultrasonic emulsification: investigation of its bactericidal activity. J Nanosci Nanotechnol 13(1):114–122

He J, Chen F, Chen S, Lv G, Deng Y, Fang W et al (2011) Chrysanthemum leaf epidermal surface morphology and antioxidant and defense enzyme activity in response to aphid infestation. J Plant Physiol 168(7):687–693

Henderson CF, Tilton EW (1955) Tests with acaricides against the brown wheat mite. J Econ Entomol 48(2):157–161

Ibrahim S (2019) Essential oil nanoformulations as a novel method for insect pest control in horticulture horticulture. IntechOpen

Jung S (2004) Variation in antioxidant metabolism of young and mature leaves of *Arabidopsis thaliana* subjected to drought. Plant Sci 166(2):459–466

Kahramanoğlu İ, Nisar MF, Chen C, Usanmaz S, Chen J, Wan C (2020) Light: an alternative method for physical control of postharvest rotting caused by fungi of citrus fruit. J Food Qual 2020:ID 8821346. https://doi.org/10.1155/2020/8821346

Karamaouna F, Kimbaris A, Michaelakis A, Papachristos D, Polissiou M, Papatsakona P et al (2013) Insecticidal activity of plant essential oils against the vine mealybug *Planococcus ficus*. J Insect Sci 13(1):142

Kaur G, Singh P, Sharma S (2021) Physical, morphological, and storage studies of cinnamon based nanoemulsions developed with Tween 80 and soy lecithin: a comparative study. J Food Meas Charact 15:1–13. https://doi.org/10.1007/s11694-021-00817-w

Kaur H, Salh P, Singh B (2017) Role of defense enzymes and phenolics in resistance of wheat crop (*Triticum aestivum* L.) towards aphid complex. J Plant Interact 12(1):304–311. https://doi.org/10.1080/17429145.2017.1353653

Kong F, Hu W, Chao W, Sang W, Wang L (1999) Physiological responses of Mexicana to oxidative stress of $SO_2$. Environ Exp Bot 42:201–209

Mathlouthi A, Saadaoui N, Ben-Attia M (2021) Essential oils from *Artemisia* species inhibit biofilm formation and the virulence of *Escherichia coli* EPEC 2348/69. Biofouling 37(2):174–183. https://doi.org/10.1080/08927014.2021.1886278

Mead HM, El-Shafiey SN, Sabry HM (2016) Chemical constituents and ovicidal effects of mahlab, *Prunus mahaleb* L. kernels oil on cotton leafworm, *Spodoptera littoralis* (Boisd.) eggs. J Plant Prot Res 56(3):279–290. https://doi.org/10.1515/jppr-2016-0044

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7(9):405–410

Mohsen H, Ali F (2009) Essential oil composition of *Artemisia herba-alba* from southern Tunisia. Molecules 14(4):1585–1594

Mossa A-TH, Afia SI, Mohafrash SM, Abou-Awad BA (2019) Rosemary essential oil nanoemulsion, formulation, characterization and acaricidal activity against the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). J Plant Prot Res 59(1):102–112

Mustafa IF, Hussein MZ (2020) Synthesis and technology of nanoemulsion-based pesticide formulation. Nanomaterials 10(8):1608. https://doi.org/10.3390/nano10081608

Pratap-Singh A, Guo Y, Ochoa SL, Fathordoobady F, Singh A (2021) Optimal ultrasonication process time remains constant for a specific nanoemulsion size reduction system. Sci Rep 11(1):9241. https://doi.org/10.1038/s41598-021-87642-9

Sabbour MM, Abd El-Aziz SE-S (2019) Impact of certain nano oils against *Ephestia kuehniella* and *Ephestia cutella* (Lepidoptera-Pyralidae) under laboratory and store conditions. Bull Natl Res Centre 43(1):1–7. https://doi.org/10.1186/s42269-019-0129-3

Salem HA, Abdel-Aziz NF, Sammour EA, El-Bakry AM (2016) Semi-field evaluation of some natural clean insecticides from essential oils on armored and soft scale insects (Homoptera: Diaspididae and Coccidae) infesting mango plants. Int J ChemTech Res 9(8):87–97

Salido S, Altarejos J, Nogueras M, Sánchez A (2001) Chemical composition of the essential oil of *Artemisia herba-alba* Asso ssp. *valentina* (Lam) Marcl. J Essent Oil Res 13(4):221–224

Sammour EA, Kandil MAH, Abdel-Aziz NF, Agamy EAM, El-Bakry AM, Abdel-maksoud NM (2018) Field evaluation of new formulation types of essential oils against *Tuta absoluta* and their side effects on tomato plants. Acta Sci Agric 2(6):15–22

Sharma N, Singhvi R (2017) Effects of chemical fertilizers and pesticides on human health and environment: a review. Int J Environ Agric Biotech 10(6):675–680

Soliva RC, Elez P, Sebastián M, Martı́n O, (2000) Evaluation of browning effect on avocado purée preserved by combined methods. Innov Food Sci Emerg Technol 1(4):261–268

SPSS (2019) Statistical package for the social sciences. SPSS. 26 edn. Chicago, IL, USA

Statista (2020) World production of citrus fruits in 2018, by region. In. https://www.statista.com/statistics/264002/production-of-citrus-fruits-worldwide-by-region/#statisticContainer

Sugumar S, Nirmala J, Ghosh V, Anjali H, Mukherjee A, Chandrasekaran N (2013) Bio-based nanoemulsion formulation, characterization and antibacterial activity against food-borne pathogens. J Basic Microbiol 53(8):677–685. https://doi.org/10.1002/jobm.201200060

Sundararajan B, Moola AK, Vivek K, Kumari BR (2018) Formulation of nanoemulsion from leaves essential oil of *Ocimum basilicum* L. and its antibacterial, antioxidant and larvicidal activities (Culex *quinquefasciatus*). Microb Pathog 125:475–485. https://doi.org/10.1016/j.micpath.2018.10.017

Tadros T, Izquierdo P, Esquena J, Solans C (2004) Formation and stability of nano-emulsions. Adv Colloid Interface Sci 108:303–318

War AR, Paulraj MG, Ignacimuthu S, Sharma HC (2013) Defensive responses in groundnut against chewing and sap-sucking insects. J Plant Growth Regul 32(2):259–272

WHO (1979) World Health Organization. Specifications for pesticides used in public health: insecticides, molluscicides, repellents, methods, 1st edn

Yang F-L, Li X-G, Zhu F, Lei C-L (2009) Structural characterization of nanoparticles loaded with garlic essential oil and their insecticidal activity against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). J Agric Food Chem 57(21):10156–10162

Yildirim ST, Oztop MH, Soyer Y (2017) Cinnamon oil nanoemulsions by spontaneous emulsification: Formulation, characterization and antimicrobial activity. LWT-Food Sci Technol 84:122–128. https://doi.org/10.1016/j.lwt.2017.05.041

## Publisher's Note