Bulgarian Journal of Agricultural Science, 29 (No 5) 2023, 956–966

Nanoinsecticide toxicity of the essential oil of *Eucalyptus camaldulensis* toward date palm white scale insect *Parlatoria blanchardii*

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Abstract

Aldosary, N., Alnajim, I., Hasan, F. A. & Al-Darwish, M. A. (2023). Nanoinsecticide toxicity of the essential oil of *Eucalyptus camaldulensis* toward date palm white scale insect *Parlatoria blanchardii. Bulg. J. Agric. Sci., 29*(5), 956–966

The date palm white scale insect *Parlatoria blanchardii* is a serious pest of date palm trees. Traditional chemical pesticides are ineffective as these pests hide under a thick cuticle layer that protects them from insecticides and other control factors. Therefore, the study aimed to formulate nanoemulsion of *Eucalyptus camaldulensis* essential oil utilising low energy method to prepare eco-friendly insecticide. One nanoemulsion phase diagram was constructed by employing mineral oil as the oil phase, span 80 and tween 80 as the non-ionic surfactants, and water. Six points were selected from the phase diagram and mixed with 20% of *E. camadulensis* leaves and seeds essential oil, which was obtained by hydrodistillation. Two formulations exhibited high stability under centrifuge and storage conditions with good physical characterisations. These formulations were nanoemulsion formulations with particle sizes (65.44 to 54.65 nm). The toxicity of the leaves and seeds essential oil nanoemulsion formulations against *P. blanchardii* was higher than non-formulated essential oils. The toxicity increased with increasing concentration and exposure time. Depending on these findings, the nanoformulations of *E. camadulensis* essential oil can present a promising, reliable alternative to conventional pesticides to control *P. blanchardii* as an eco-friendly insecticide.

Keywords: Phoenix dactylifera; nanoemulsion; formulation; mortality; toxicity

Introduction

Date palm *Phoenix dactylifera* trees are one of the most economically significant fruit trees spread widely in semi-arid and arid regions. Like all other trees, these trees are infested by numerous pests that lead to significant production losses in quantity and quality if they are not controlled (AbdElgawad et al., 2019). The date palm white scale insect (grey-scale insect) *Parlatoria blanchardii* is considered one of the most important agricultural pests. The species spreads on fruit trees, such as date palms and ornamental palms, particularly in regions that have a tropical or semi-tropical climate (Mahmoudi et al., 2008; Salman et al., 2012). This sap-sucking insect pest is covered with a thick cuticle-like scale, and both adult females and nymphs are deleterious and damaging stages (Al-Dosary, 2009).

The damage occurs through sucking the sap from fruits and leaves using their stylets, in addition to injecting a harmful or toxic saliva substance into the host plant (Babaousmail et al., 2018). This pest infects wicker, raceme, and all fruit stages of date palms. The infestation is more severe on older and neglected date palm trees, especially in hot, dry areas (Al-Dosary, 2009; El-Shafie et al., 2017). The infestation causes serious damage to the tree in terms of growth and depleting nutrients, leading to increase transpiration, decomposition of the plant pigments, and impairing photosynthesis (Al-Dosary, 2009). Insects can infest the fruits, distorting their appearance and reducing their nutritional value. In addition, since individuals are hard to remove during washing and packaging operations in date processing factories, they result in significant quality and quantity of yield losses, as well as lower the marketing value for the fruits (Al-Dosary, 2009; Babaousmail et al., 2018).

Because of the morphological properties of the date palm white scale insects, pesticides cannot reach their bodies, making them one of the most difficult pests to control (Frank, 2012). And to eliminate the harmful effects of indiscriminately using chemical pesticides to control this pest, a botanical pesticide must be developed with a new approach that will allow the pesticide to penetrate the hard cuticle (scale) of the insect (Ebadollahi & Setzer, 2020).

Aromatic plants contain essential oils, which are among the most effective insecticides due to their insecticidal characteristics, which can serve as growth regulators, repellents, insecticides and fumigants against various insect pests (Aldosary et al., 2018; Ibrahim, 2019). Likewise, *Eucalyptus* spp. trees (Myrtaceae family) are rich in volatile oils with medicinal and commercial value, and they are considered promising biological control agents for various types of insect pests (Al-Snafi, 2017). Throughout Iraq, *E. camaldulensis* or red gum trees are spread in all regions. Their leaves and seeds are rich in volatile oils, which have diverse biological properties; therefore, they have been used in folk medicine for treating several illnesses (Kheder et al., 2020). In addition, several studies have demonstrated that volatile oils extracted from *E. camaldulensis* are effective in controlling many insect pests because they contain terpenes, such as 1,8-cineole, terpinene, α-pinene and α-terpinol, which are considered highly toxic to insects (Mubarak et al., 2015; Dogan et al., 2017; Kheder et al., 2020; Ebadollahi & Setzer, 2020).

Despite the promising characteristics and advantages of essential oils, there are still several drawbacks that limit or decrease the shelf-life of these oils and prevent them from being used as pesticides. These problems include volatilisation, rapid breakdown, high sensitivity to light and UV rays, oxidation and weak water solubility (Abreu et al., 2020; Ebadollahi et al., 2020). Formulating essential oils is considered a safe, efficient, and feasible method for modulating oil release. It increases the chemical and physical stability of essential oil (Tomczykowa et al., 2018; Ibrahim, 2019). The formulation process of plant materials as nanoemulsions, with its unique properties such as high stability, reduced droplet size (20-200 nm), and reduced turbidity, makes it an effective and highly credible method for developing numerous botanical pesticides (Mustafa & Hussein, 2020). Many studies confirmed that the nanoemulsion formulation of essential oil is one of the most effective strategies for insect pest management. However, no investigations have been conducted to determine the insecticidal activity and nanoemulsion application of *E. camaldulensis* essential oil against *P. blanchardii*. Therefore, the present study aims to prepare the nanoemulsion formulation and determine the insecticidal efficiency of the essential oil extracted from seeds and leaves of *E. camaldulensis* against date palm white scale insect *P. blanchardii* under laboratory conditions.

Materials and Methods

Plant material and essential oil preparation

The leaves and seeds of *E. camaldulensis* were collected in April 2019 from Abu Ghraib, Baghdad, Iraq. For essential oil extraction, plant samples were cleaned and dried in the shade at room temperature for 7-10 days. The dried plants were ground into powder using a commercial electrical mill and stored in plastic containers. About 200 g of plant sample powder was subjected to hydrodistillation device type Clevenger with 1 L of distilled water in a rounded glass flask, which was heated to boiling for 3 h to extract essential oils. The essential oil was stored in the refrigerator at 4ºC for further use (Ebadollahi & Setzer, 2020).

Chemicals

Tween 80 (T80), Tween 20 (T20), Span 80 (S80), and Criton (Triton) X100 (X100) as the non-ionic surfactants were supplied by General Drugs House Co. Ltd. India, while the mineral oil (Mo) as an oil phase was obtained from *bioMérieux* France. The distilled water with pH neutral (7) was employed in the current study.

Screening of surfactant and oil

Phase separation was observed visually by mixing 1 g of oil, surfactant or surfactant mixture with water for one minute in a vortex mixer (Model: Bionex KML-3000v, Korea). Then, the mixtures were centrifuged at 3500 rpm for 30 min using a centrifuge device (Model: MLW T30, Germany). Combinations of oil, surfactant or surfactant mixture, and water that possessed uniformed miscibility and one phase were used to construct a ternary diagram (Thang et al., 2017).

Construction of ternary phase diagrams

The titration method was followed to construct ternary phase diagrams. The different titrations of oil and surfactant or surfactant mixtures were prepared. Slow titration of the water was performed for each mixture of oil and surfactant separately. The amount of the added water was varied to cover 5 % to 95 % of the whole volume. Then, the mixtures were blended using a vortex mixer for one min. The mixtures were centrifuged at 3500 rpm for 30 min at 27±2°C, and the phase separation of each sample was observed visually. Depending on the stability of transparency, sample clarity and phase regions of the ingredients, mixtures were defined as anisotropic or isotropic. The ternary phase diagrams were drawn and structured by Chemix software, version 3.5, phase diagram plotter (UK) (Mazonde et al., 2020).

Selection of formulations from phase diagrams

Six different formulations were selected from the phase diagram plot. The chosen formulations from the phase diagram depended on several parameters such as one-phase without gel, transparent appearance, and stability at room temperature 27±2°C.

Solubility test for essential oils of selected formulations

The solubility test was conducted to identify the appropriate amount of the essential oils to mix with the selected formulations from ternary phase diagrams to get one phase. Four ratios (1.0/9.0, 1.5/8.5, 2.0/8.0, 2.5/7.5) % (*w/w*) of leaves and seeds essential oils of *E. camaldulensis* and selected formulations that represented (10, 15, 20 and 25) % of the essential oil amount were prepared and blended using a vortex mixer for one min. Then, the mixtures were centrifuged at 3500 rpm for 30 min, and the phase separation was observed visually (Zvonar et al., 2010). The mixtures of selected formulations with essential oil that possessed good solubility, stability and one-phase transparency were chosen and submitted to characterise nanoemulsion formulation and stability tests (Ahmad et al., 2012).

Stability tests

Ten mL of each formulation with essential oil in the glass tube were centrifuged at 3500 rpm for 30 min at 27±2°C. The phase separation was observed visually (Nasr et al., 2016). In accordance with Choupanian et al. (2017) method, 10 mL of each formulation containing essential oil in a vial tube was stored at 27±2°C for three months. The combinations were then stored under oven conditions at 54±2°C for two weeks to prove the stability of formulations under tropical conditions. After that, the phase separation was observed visually. The formulations with a high ratio of *E. camaldulensis* essential oil, which passed the stability test, were submitted to the subsequent tests and analysis that included: particle size, viscosity and surface tension measurement.

Charactarisation of nanoformulation tests

The droplet size was determined using photon correlation spectroscopy (Model: Malvern Zetasizer Nano ZS, UK) with laser light scattering. The formulations were diluted with distilled water at a ratio (1:25) mL, (*v/v*). The particle size was measured at 27±2°C (Lobato Rodrigues et al., 2021).

Viscometer (Model: Brookfield; DV-II+Pro, UK) was utilised to evaluate the viscosity of formulations at 27±2°C. The viscosity value for each formulation was recorded after the sample equilibrium.

The ring method was followed to measure the surface tension of the essential oil formulations. The Tensiometer (Model: Krüss K6 GmbH, Germany) with a 1.9 cm platinum ring was used to estimate the surface tension of the formulations at 27±2°C (Jiang et al., 2011).

Toxicity of E. camaldulensis essential oil nanoemulsion formulations against P. blanchardii

The spraying method was followed to evaluate the toxicity of *E. camaldulensis* essential oil nanoemulsion formulations against *P. blanchardii*. Six concentrations of essential oil and essential oil nanoemulsion formulation (0, 50, 100, 200, 500, 700) mg/L were prepared separately by diluting with distilled water while 0.5 % of S80 surfactant was added to the water, which was diluted of essential oil without formulation. Besides, the same concentrations of nanoemulsion formulation without essential oil were prepared by diluting with distilled water as the second control treatment. Date palm leaflets infested with the white scale insects were collected from the field and brought to the laboratory. After washing and drying, the date palm leaflets were sprayed in different concentrations using a small sprayer and left to dry for one hour at room temperature. After that, the lower end of the leaflet was put in test tubes containing water, and the orifices of the tubes were closed with cotton to stead the leaflets and prevent evaporation. The experiment was conducted in four replicates; each replicate had one leaflet. The test tubes with leaflets were incubated at 27±2°C and 65±5 % RH. The treated leaflets were examined to calculate the mortality by cutting 2 cm^2 from treated leaflets after 12, 24, 36, 48 and 72 h of spraying using a dissecting microscope by removing the insect's shell with a very thin needle. The insects were considered dead if found dry, flat, and unmoving. The lethal concentration (LC_{50}), lethal time (LT_{50}), lower and upper confidence limits 95%, and slope ±SE, values were computed and obtained from probit regression analysis using the Polo Plus-PC program version 0.03 (LeOra Software). It was considered non-significant differences between values of LC_{50} or $LT_{50,}$ if there was any overlapping in confidence limit values.

Data Analysis

All measurements and tests of nanoemulsion formulation characterisations and the toxicity evaluation were conducted according to Complete Randomized Design (C.R.D).

All collected data were analysed using a one-way analysis of variance (ANOVA). Duncan's test at 95% probabilities separated the means with significant differences. The analysis was carried out utilising Statistical Analysis Software version 9.3 (SAS). The results were presented as mean±SE (standard error).

Results

Nanoformulation Components

Table 1 shows phase separation test results of formulation components (oil phase, aqueous phase, and surfactant or a mix of surfactants). The Mo and 40 % T80 + 60 % S80 mix of surfactants achieved one phase when mixed with water. In comparison, all remaining compositions gave two phases upon visual inspection after centrifugation.

Table 1. The components of nanoformulation

Surfactant	Oil Phase	Aqueous phase	Appearance
X100	MO	Water	2ph*
T80	MO	Water	2ph
T20	MO	Water	2ph
S80	MO	Water	2ph
Mix of surfactants % (*w/w*)			
20 % X100 + 80 % S80	MO	Water	2ph
40% X100 + 60 % S80	MO	Water	2ph
50% X100 + 50 % S80	MO	Water	2ph
60 % X100 + 40 % S80	MO	Water	2ph
20 % T80 + 80% S80	MO	Water	2ph
40 % T80 + 60 % S80	MO	Water	1ph**
50 % T80 + 50 % S80	MO	Water	2ph
60 % T80 + 40 % S80	MO	Water	2ph
20 % T20 + 80 % S80	MO	Water	2ph
40 % T20 + 60 % S80	MO	Water	2ph
50 % T20 + 50 % S80	MO	Water	2ph
60 % T20 + 40 % S80	MO	Water	2ph

* Two phases, ** one phase

Construction of ternary phase diagrams

The mixture of surfactants, including 40 % T80 + 60 % S80, Mo, and distilled water were employed as materials to construct one ternary phase diagram system to get isotropic (one phase) nanoemulsion region. In addition, these constructions of ternary phase diagrams were utilised to define the appropriate ratio range of the ingredients (aqueous phase, surfactant, and oil phase) in forming nanoemulsions. The phase diagram of the ternary system, with the distilled water as the aqueous phase, 40 % T80 + 60 % S80 as the mixture of surfactants and Mo as the oil phase, is presented in Figure 1. The behavior of the ternary phase diagram indicated that the ratios 10.21-86.96/ 2.86-89.44/ 0.00-70.51 of water/ mixture of surfactants 40 % T80 + 60 % S80, and Mo respectively gave 46 % isotropic region, in addition the ratios 10.21-72.10/ 15.21-37.56/ 11.30-70.50 of water, 40 % T80 + 60 % S80 and, Mo respectively showed 14 % transparent isotropic region. Nevertheless, 40 % two-phases region was achieved by the ratios 10.68-86.91/ 0.00-42.14/ 1.30-89.32 of water, 40 % T80 + 60 % S80 and, Mo respectively (Figure 2).

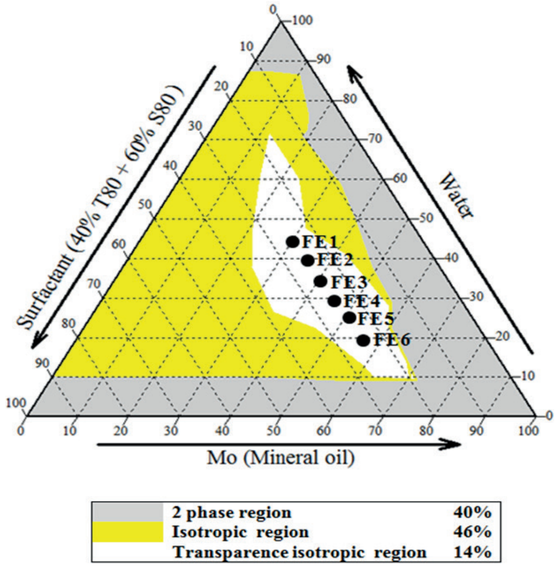


Fig. 1. Phase diagram and the selected points of water/ 40% T80 + 60% S80 / Mo (Mineral oil)

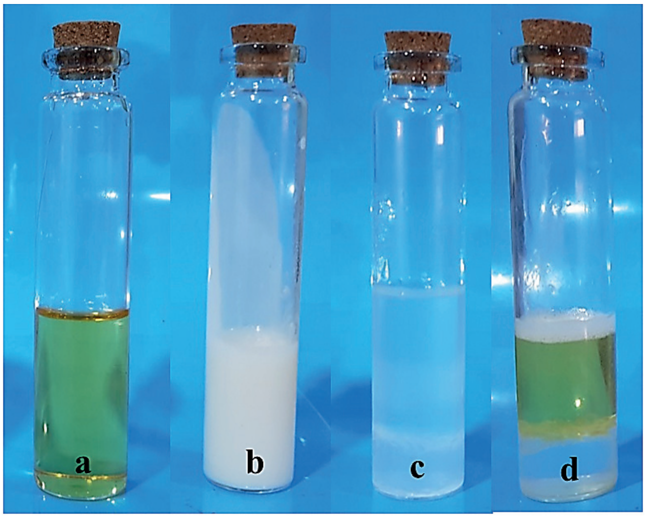


Fig. 2. Appearances of formulations based on phase diagrams:
a. 1 phase transparent, b. 1 phase cloudy, c. 2 phases cloudy, d. 2 Phases transparent

Selection of formulations from phase diagrams

Six different formulation points were selected from water/ 40 % T80 + 60 % S80 / Mo phase diagram plot. These formulation points were chosen depending on three criteria, including the high amount of oil (30-55) % *w/w*, the high amount of water (20-45) % *w/w*, and the low amount of surfactant 25 % *w/w*, to formulate nanoemulsion. The selected formulations were coded as FE1, FE2, FE3, FE4, FE5, and FE6 (Table 2).

Table 2. Ingredients and ratios of selected formulation points

Formulation code	Ingredients ratios % *(w/w)*		
	Water	The mixture of surfactants	Oil
FE1	45	25	30
FE2	40	25	35
FE3	35	25	40
FE4	30	25	45
FE5	25	25	50
FE6	20	25	55

Solubility test of essential oil in selected formulations

Table 3 exhibits the solubility test results of essential oils extracted from the leaves and seeds of *E. camaldulensis* with selected formulations. The formulation codes FE3 and FE4 were more suitable with leaves and seeds essential oils of *E. camaldulensis* plant in different amounts. In contrast, the gel phase or/and two-phases appeared when mixing all amounts of FE1, FE2, FE3, FE5, and FE6 formulation with various percentages of essential oils.

Stability test of selected formulations with* E. camaldulensis *essential oils

Table 4 shows the stability test results under centrifugation and storage conditions of selected formulations with different amounts of *E. camaldulensis* leaves and seeds essential oils. The stability test results confirmed that the leaves and seeds essential oils of *E. camaldulensis* at ratios of 10/90 and 15/85 % *w/w* with formulation codes FE3 and FE4 possessed high stability. In comparison, the ratio of 20/80 % w/w of seeds essential oil with formulation code FE3 and the ratio of 20/80 % w/w of leaves essential oil with formulation code FE4 presented high stability under centrifugation, storing three months at 27± 2°C, and two weeks under oven condition (54 ± 2°C), without phase separation or/and gel phase or/and creaming or/and colloid appearances (Figure 3). Although the stability test of essential oils extracted from leaves and seeds of *E. camaldulensis* at a ratio of 25/75 % *w/w* with formulation codes FE3 and FE4 had high stability under centrifugation, the same compositions were unstable under storage conditions. These compositions suffered from phase separation or/and gel phase or/and creaming or/and colloid appearances during stability tests of storage conditions.

Table 3. Solubility test of *E. camaldulensis* essential oils with selected formulations

Formulations		Essential oils of E. *camaldulensis*		
Code	Amount % (*w/w*)	Amount % (*w/w*)	Leaves	Seeds
FE1	90	10	×	×
	85	15	×	×
	80	20	×	×
	75	25	×	×
FE2	90	10	×	×
	85	15	×	×
	80	20	×	×
	75	25	×	×
FE3	90	10	✓	✓
	85	15	✓	✓
	80	20	✓	✓
	75	25	✓	✓
FE4	90	10	✓	✓
	85	15	✓	✓
	80	20	✓	✓
	75	25	✓	✓
FE5	90	10	×	×
	85	15	×	×
	80	20	×	×
	75	25	×	×
FE6	90	10	×	×
	85	15	×	×
	80	20	×	×
	75	25	×	×

× =Failed, ✓ = Pass

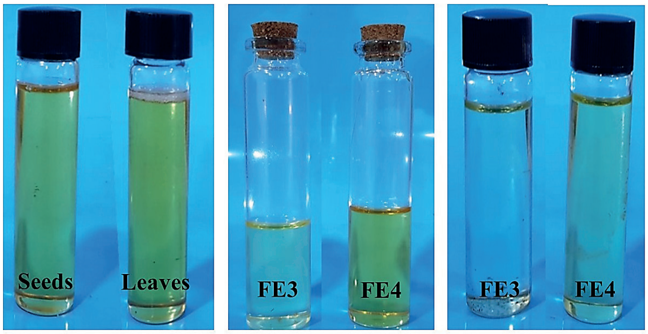
Characterisations of E. camaldulensis essential oil nanoemulsion formulations

The results of the characterisation of *E. camaldulensis* essential oil via nanoemulsion formulation are presented in Table 5. The results underscored significant differences ($p < 0.05$). They were observed on the particle size, surface tension, and viscosity. The particle size of the essential oil formulations revealed that essential oil formulations from both seeds and leaves were nanoemulsions in the range of (65.44 to 54.65 nm). In addition, the formulation FE4 with leaves essential oil achieved the lowest surface tension of 36.97 mN/m, followed by the formulation FE3 with seeds

Table 4. Stability tests of *E. camaldulensis* leaves and seeds essential oil formulations under centrifugation and storage conditions

Formulation		Essential oil of leaves % (*w/w*)	Centrifugation	Storage conditions	
Code	% (*w/w*)			Room condition	Oven condition
FE3	90	10	✓	✓	✓
	85	15	✓	✓	✓
	80	20	✓	×	×
	75	25	✓	×	×
FE4	90	10	✓	✓	✓
	85	15	✓	✓	✓
	80	20	✓	✓	✓
	75	25	✓	×	×
Formulation		Essential oil of seeds % (*w/w*)	Centrifugation	Storage conditions	
Code	% (*w/w*)			Room condition	Oven condition
FE3	90	10	✓	✓	✓
	85	15	✓	✓	✓
	80	20	✓	✓	✓
	75	25	✓	×	×
FE4	90	10	✓	✓	✓
	85	15	✓	✓	✓
	80	20	✓	×	×
	75	25	✓	×	×

× =Failed, ✓ = Pass



a b c

Fig. 3. The essential oil of *E. camaldulensis* before and after nanoemulsion formulation:
a. The essential oil without formulation, b. Nanoformulatiom without essential oil, c. The essential oil after nanoemulsion formulation

essential oil, which was 37.10 mN/m. As for the viscosity, the FE4 formulation with the leaves essential oil composition recorded the lowest viscosity of 22.77 mPa s. The FE3 formulation with seeds essential oil composition recorded a higher viscosity of 31.20 mPa s at room temperature.

Toxicity evaluation of E. camaldulensis essential oil nanoemulsion formulation

The results presented in Tables 6 to 9 show the toxicity of *E. camaldulensis* essential oil without and with nanoemulsion formulation against date palm white scale insects *P. blanchardii*. Depending on log-probit analysis results, the lethal concentration (LC_{50}) values gradually decreased with the time after treatment. The lethal time (LT_{50}) decreased with increasing the concentration at 95 % confidence level of all treatments. In addition, the toxicity results showed an increase in the toxicity of the essential oils after the nanoformulation compared to the non-formulated oils, with significant differences. In addition, the results indicated that the nanoformulations (codes FE3 and FE4) without *E. camaldulensis* essential oil showed the non-toxic activity of all concentrations and times after treatment, as these formulations did not cause any mortality against *P. blanchardii*

The toxicity results of *E. camaldulensis* leaves essential oil (Table 6) indicated that the essential oil with nanoformulation code FE4 was the most toxic with the lowest LC_{50} values which were 74.45, 207.80 mg/L after 72 and 48 h of treatment, respectively. On the other hand, the leaves essential oil without formulation recorded the lowest toxicity against date palm white scale insects with the highest LC_{50} values, which were 6442.85, 4159.73 mg/L after 12 and 24 h of treatment, respectively. The results also indicated that the lowest LT_{50} values (highest toxicity) were obtained from the leaves essential oil with nanoformulation were 17.87, 23.01 h at 700 and 500 mg/L concentrations, whilst the most extended LT_{50} values was 161.07 h at 50 mg/L concentration of leaf essential oil without formulation (Table 7).

The LC_{50} and LT_{50} values showed significant differences (non-overlapping of confidence intervals) of *E. camaldulensis* seeds essential oil without and with nanoemulsion formulation toxicity results on date palm white scale insect *P. blanchardii* (Tables 8 and 9). The results indicated that *E. camaldulensis* seeds essential oil nanoemulsion formulation

Table 5. Characterisations of *E. camaldulensis* essential oil nanoformulations

Essential oil source	Formulation code	Particle Size (nm)	Surface Tension (mN/m)	Viscosity (mPa s)
		Mean±SE.	Mean± SE.	Mean± SE.
Leaves	FE4	54.65 ±1.00a	36.97±0.28a	22.77±0.30a
Seeds	FE3	65.44±0.82b	37.10±0.32a	31.20±2.64b

* = similar letters in each column are not significant differences at $P<0.05$. Duncan

Table 6. LC_{50} (mg/L) values of *E. camaldulensis* leaves essential oil without and with the nano emulsion formulation against date palm white scale insects *P. blanchardii*

Treatments	Time (hours)	LC_{50}	Confidence limit*		Slope ±SE
			Lower	Upper	
Essential oil without formulation	12	6442.85	2209.67	135102.50	0.94±0.24
	24	4159.73	1684.38	40084.64	0.89±0.20
	36	3479.59	1246.73	83469.74	0.62±0.17
	48	1469.92	714.84	9300.04	0.65±0.16
	72	556.19	367.54	1149.20	0.81±0.16
Essential oil with nanoemulsion formulation	12	3190.82	1420.28	22034.90	0.90±0.20
	24	2786.39	1171.90	26750.94	0.73±0.17
	36	1212.95	642.035	5350.205	0.70±0.16
	48	207.80	144.70	299.58	0.85±0.15
	72	74.45	46.28	102.370	1.10±0.16

*Confidence limit has been calculated with 95% confidence

Table 7. LT_{50} (hour) values of *E. camaldulensis* leaves essential oil without and with the nano emulsion formulation against date palm with scale insects, *P. blanchardii*

Treatments	Concentration mg/L	LT_{50}	Confidence limit*		Slope ±SE
			Lower	Upper	
Essential oil without formulation	50	161.07	89.18	682.30	1.40±0.31
	100	138.60	75.76	617.77	1.19±0.27
	200	143.23	76.37	703.46	1.03±0.24
	500	88.17	54.80	253.00	1.05±0.22
	700	36.02	27.82	53.27	1.22±0.21
Essential oil with nanoemulsion formulation	50	70.58	2.29	119.25	1.68±0.27
	100	45.55	36.75	62.47	1.74±0.24
	200	35.47	30.65	53.42	1.47±0.22
	500	23.01	19.27	27.85	1.70±0.21
	700	17.87	14.84	21.27	1.73±0.21

*Confidence limit has been calculated with 95% confidence

Table 8. LC_{50} (mg/L) values of *E. camaldulensis* seeds essential oil without and with the nano emulsion formulation against date palm white scale insects, *P. blanchardii*

Treatments	Time (hours)	LC_{50}	Confidence limit *		Slope ±SE
			Lower	Upper	
Essential oil without formulation	12	11871.67	2979.72	1566534.82	0.88±0.25
	24	8616.58	2475.37	444733.91	0.81±0.22
	36	3950.39	1519. 50	50477.23	0.76±0.18
	48	1645.45	867.27	6586.54	0.83±0.17
	72	695.97	473.96	1323.82	0.98±0.16
Essential oil with nanoemulsion formulation	12	6260.47	2134.10	133978.63	0.89±0.23
	24	5112.49	1722.67	125014.15	0.71±0.10
	36	2631.06	977.45	70462.40	0.56±0.16
	48	387.35	274.16	642.35	0.87±0.16
	72	172.30	131.62	221.31	0.19±0.16

*Confidence limit has been calculated with 95% confidence

was the highest toxic to white scale insect *P. blanchardii*. Their LC_{50} recorded 172.30, 387.35 mg/L after 72 and 48 h of treatment, respectively. However, the results confirmed that the lowest toxicity of non formulated *E. camaldulensis* seeds essential oil recorded the highest LC_{50} value of 11871.67 mg/L after 12 h of treatment. Besides, the results mentioned the shortest LT_{50} values, which were 23.19, 29.80 h, at 700 and 500 mg/L of *E. camaldulensis* seed's essential oil nanoemulsion formulation respectively. In contrast, the longest LT_{50} value of 412.42 h was recorded with 50 mg/L concentration of the seeds essential oil without nanoemulsion formulation (Table 9).

Table 9. LT_{50} (h) values of *E. camaldulensis* seeds essential oil without and with the nanoemulsion formulation against date palm white scale insects, *P. blanchardii*

Treatments	Concentration mg/L	LT_{50}	Confidence limit*		Slope ±SE.
			Lower	Upper	
Essential oil without formulation	50	412.42	142.81	23216.37	1.15±0.35
	100	230.13	106.17	2097.28	1.18±0.30
	200	199.90	95.65	1492.64	1.07±0.26
	500	125.98	71.02	495.344	1.08±0.24
	700	41.53	32.21	61.52	1.34±0.22
Essential oil with nanoemulsion formulation	50	93.42	63.98	195.81	1.63±0.30
	100	85.77	58.00	184.56	1.38±0.26
	200	74.56	50.80	156.82	1.24±0.23
	500	29.80	24.90	37.20	1.71±0.22
	700	23.19	19.61	27.75	1.80±0.22

*Confidence limit has been calculated with 95% confidence

Discussion

There is no investigation on the insecticidal activity of *E. camaldulensis* essential oil nanoemulsion formulations against the date palm scale insects. Hence, the present study is a preliminary investigation on applying nanoemulsion formulation of *E. camaldulensis* essential oil as a botanical insecticide to control date palm scale insects. The significance of the study is derived from the importance of the formulation of essential oils as a practical method improve and physical characteristics of the essential oil (Libs & Salim, 2017; Khoshraftar et al., 2019; Mustafa & Hussein, 2020). Thus, the formulation process of essential oils as a botanical insecticide is the pivotal aspect of our study.

The selection of appropriate ingredients for nanoformulation affects the formulation preparation, characterisations and stability (Mahdi et al., 2011). Therefore, materials used to prepare and formulate nanoemulsion of essential oils were

Mo as the oil phase, and T80, S80 as the surfactant. These were chosen based on their solubility with water and essential oil to obtain one stable phase that was obtained in our study. This because the solubility of formulation ingredients in water is one of the most influential factors in the preparation and properties of a nanoemulsion formulation (Thang et al., 2017). The ingredient solubility differences in the current study may be attributed to the variation in the physical and chemical properties of oils and other components (Pavoni et al., 2020; Shao et al., 2021).

In our study, the low energy method was applied to synthesis the nanoemulsion formulation of essential oil. In this regards, several studies have confirmed the use of different oils in combination with non-ionic surfactants in preparing the nanoemulsion of essential oils using the low energy method (titration method) (Mazonde et al., 2020; Lobato Rodrigues et al., 2021; Hien et al., 2022). As a result, the ternary phase diagrams in Figure 1 provided an insight into the phase behavior as the ratio of emulsion ingredients started changing gradually. This allows us to choose the combination that includes water as the aqueous phase, 40 % T80 + 60 % S80 as the mixture of surfactants and mineral oil (Mo) as the oil phase. This is because formulation elements should be proportioned based on the phase behavior, which is a key factor in preparing nanoemulsion formulations with higher thermodynamic stability (Lobato Rodrigues et al., 2021; Hien et al., 2022).

In fact, essential oil and mineral (paraffin) oil possess low solubility when mixed with water. However, the addition of the non-ionic surfactants (Tween 80 and Span80) can influence the solubility and distribution of oils immensely. Besides, the primary function of surfactants is to reduce the free energy and produce a mechanical barrier among formulation particles. Thus, surfactant agents minimise cloudiness and droplet size in the emulsion system (Vilasau et al., 2011; Moradi & Barati, 2019). This characteristic reduces the surface tension of the two immiscible liquid molecules, which leads to the dispersion of the molecules of the two liquids and the formation of a film between them that prevents them from gathering again (Kale & Deore, 2017; Barradas et al., 2020). Therefore, the addition of these surfactants is critical in the production of nanoemulsion with high stability and long-term through making films around the particles of the formation. These films prevent and/or protect the particles from gathering to create a separate layer (Pavoni et al., 2020; Nirmala et al., 2020). Investigations revealed that the non-ionic surfactants possess the ability to produce nanoemulsion formulations with tiny droplet size and high stability by a low-energy emulsification process (Moradi & Barati, 2019; Lobato Rodrigues et al., 2021; Hien et al., 2022). The particle size of the emulsion has a significant impact on the stability and turbidity of the emulsion formulation (Kale & Deore, 2017; Nirmala et al., 2020; Pavoni et al., 2020). In this study, the particle size of all essential oil formulations manifested in the range of 65.44 to 54.65 nm, which are considered to characterise nanoformulation of emulsion (Mustafa & Hussein, 2020; Lobato RodriguesI et al., 2021). Many factors affect droplet sizes of an emulsion, such as; chemical and physical properties of ingredients, ingredients amount, surfactant concentration, and preparation method (Kotta et al., 2015; Maphosa & Jideani, 2018; Pavoni et al., 2020).

The physical chractaristics of essential of nanoemulsion formulation including viscosity and surface tension were singnificantly different. Nanoemulsions viscosity depends on materials and their ratios in the formulation. The low viscosity of essential oil nanoformulation may be due to the employment of non-ionic surfactants which increase the hydrated surfactant's hydrophilic tail and consequently reduce the viscosity (Bhosale et al., 2014; Pavoni et al., 2020). The low viscosity of the nanoemulsion provides high stability and greater dispersal of nanoparticles on treated surfaces (Shaker et al., 2019; Nirmala et al., 2020). The non-ionic surfactants also play a vital role in reducing surface tension which is consistent with our results. During the emulsification process, the interfacial area increases and leads to a decrease in surface tension. The reduced size of the particles, decreased viscosity and surface tension values provide stability to the final nanoemulsion (Aziz et al., 2019; Abreu et al., 2020; Pavoni et al., 2020).

The study findings confirmed that the materials (non-ionic surfactants and Mo) utilised to prepare the nanoemulsion were non-toxic against *P. blanchardii*. These results are in agreement with other previous studies that indicated to the reduced or non-toxic effect of paraffin oil (European Food Safety Authority, 2008; Dokukins & Muter, 2016). The low toxicity of paraffin (long to medium chain oil C20-C60) on insects may be interpreted to the fact that they are the medium-chain oils that make them less toxic compared to the long chain oils (Zhang et al., 2011) . Also, the well-known non-ionic surfactants have lower toxicity to the organisms (Setya et al., 2014). Furthermore, Volpato *et al.* (2016) mentioned that the treatments with nanoemulsions (without active ingredients) did not make toxic effects on targeted insects because the nanoformulation process allows for decreased toxicity, increased bioavailability and activity, and in some cases targets the release of compounds incorporated with nanoparticles.

The present study found that the nanoemulsion formulations enhanced the toxicity of *E. camaldulensis* essential oil compared with non formulated essential oil against the white scale insect *P. blanchardii*. The nanoformulation enhances the activity of essential oils by improving the controlled release, increasing solubility, decreasing volatility of essential oil (Sugumar et al., 2014; Shaker et al., 2019; Lobato Rodrigues et al., 2021). Furthermore, the nanoemulsion system improves the penetration and spreading of the essential oils particles into insect cuticle. The higher mortality after essential oil formulations might be due to the decrease in particle size. Choupanian et al. (2017) and Lobato Rodrigues et al. (2021) found that increasing insect mortality can be achieved by decreasing the particle size of the formulations. Thus, the nanoemulsion of essential oils in the present study has achieved higher mortality and toxicity than non formulated essential oil. Furthermore, the toxicity results revealed that non-formulated essential oils had a reduced insecticidal effectiveness as compared to nanoemulsions of essential oils. The rapid breakdown of non-formulated essential oils during application due to oxidation, photolysis, and other factors such as weak water solubility and penetration may lead to a decrease in their insecticidal effectiveness (Abreu et al., 2020; Ebadollahi et al., 2020). The results also showed an increase in the effectiveness of the essential oils nanoemulsion by increasing their concentration. As demonstrated in many studies, the formulation's efficacy improves with increased concentrations of the active substance, surfactant, and oils (Khoshraftar et al., 2019; Mossa et al., 2019; Lobato Rodrigues et al., 2021). The essential oil of *E. camaldulensis* is toxic to the white scale insect *P. blanchardii* because it contains monoterpenes and monoterpenoids compounds which are lethal to insects (Dogan et al., 2017; Kheder et al., 2020; Ebadollahi & Setzer, 2020). These substances are volatile and lipophilic and can quickly penetrate the insect cuticle and intervene with their physiological functions (Aldosary et al., 2018; Gaire et al., 2019).

Conclusion

In the current study, we successfully formulated *E. camaldulensis* seeds and leaves essential oil as a nanoemulsion insecticide utilising low energy method. In laboratory tests, the nanoformulation of *E. camaldulensis* essential oil exhibits high stability under room and oven conditions. It also showed insecticidal effects against the date palm white scale insect *P. blanchardii*. Generally, the present study confirmed that the nanoemulsion formulation has contributed to increasing the stability and toxicity of the essential oil of *E. camaldulensis* as a pesticide. Hence, it can be a reliable, eco-friendly alternative to chemical pesticides for date palm white scale insects.

Declaration of competing interests

The authors declare that there is no conflict of interest.

Funding

This research recieved no funding.

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Received: **November, 30, 2022**; *Approved*: **December, 12, 2022;** *Published:* **October, 2023**