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Evaluation of the effect of noanoemulsion formulation of *Myrtus communis* essential oil against date palm white scale insect *Parlatoria blanchardi*

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Abstract

The date palm white scale insect (PWS) is a serious pest that causes damage to date palm trees by feeding on the plant sap. Formulating plant-derived active ingredients into nanoemulsions can provide effective pest management options. In this study, a low-energy approach was used to formulate a water-in-oil (W/O) nanoemulsion of *Myrtus communis* L. essential oil. The primary objective was to evaluate the efficacy of a nanoemulsion pesticide against *Parlatoria blanchardi* (Targioni Tozzetti, 1892). Additionally, the study investigated the spreading capacities of the essential oil nanoformulations. The study consisted of creating three phase diagrams using methyl ester and paraffin oil as the oil phases. These oils were then combined with water and three nonionic surfactants: Tensiofix EW70, Tensiofix 96 DB10, and Termul 3540. After the phase diagram was established, three points were selected from each phase. These selected points were then mixed with 25% essential oil of *M. communis*. After conducting the stability tests, three formulations with nanoparticle sizes ranging from 28.89 to 54.65 nm exhibited excellent physical properties and demonstrated strong stability under centrifugation and storage conditions. Among these nanoformulations, FN2 (paraffin oil and DB10 surfactant) achieved the highest toxicity against *P. blanchardi*, and the largest droplet spreading area. It was followed by FN5 (methyl ester and Termul 3540 surfactant) and FN8 (methyl ester and EW70 surfactant). Based on these results, the *M. communis* essential oil nanoformulations have the potential to serve as environmentally friendly insecticides.

Keywords: Nano-pesticide, Insecticidal efficiency, Emulsion stability, Wetting performance, Eco-friendly formulation

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ارزیابی اثر فرمولاسیون نانوامولسیون اسانس گیاه *Myrtus communis* علیه سپردار سفید خرما (*Parlatoria blanchardi*)

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چکیده

سپردار سفید خرما (*Parlatoria blanchardi*)، آفت کلیدی در باغات خرما، با تغذیه از شیره گیاهی موجب خسارت قابل توجهی به درختان می‌گردد. فرمولاسیون ترکیبات فعال با منشأ گیاهی در قالب نانوامولسیون، رویکردی مؤثر در مدیریت این آفت محسوب می‌شود. در این پژوهش، با به‌کارگیری یک روش کم‌انرژی، نانوامولسیون آب در روغن (W/O) از اسانس گیاه *Myrtus communis* تهیه شد. هدف اصلی، ارزیابی کارایی این آفت‌کش نانوامولسیونی علیه *Parlatoria blanchardi* (Targioni Tozzetti, 1892) و بررسی خواص پخش‌شوندگی فرمولاسیون‌های نانوی اسانس بود. در این راستا، سه نمودار فازی با استفاده از متیل‌استر و روغن پارافین به‌عنوان فازهای روغنی تدوین گردید. این روغن‌ها به همراه آب و سه سورفکتانت غیریونی (Tensiofix EW70، Tensiofix 96 DB10 و Termul 3540) به‌کار گرفته شدند. پس از تعیین نواحی پایدار نمودار فازی، سه نقطه از هر سیستم انتخاب و با ۲۵٪ اسانس *M. communis* مخلوط گردید. آزمون‌های پایداری حاکی از آن بود که سه فرمولاسیون نهایی، با اندازه ذرات در محدوده ۲۸/۸۹ تا ٥٤/٦٥ نانومتر، دارای خواص فیزیکی مطلوب و پایداری بالا تحت شرایط سانتریفیوژ و نگهداری بودند. در میان این نانوفرمولاسیون‌ها، FN2 (حاوی روغن پارافین و سورفکتانت DB10) بیشترین سمیت را علیه *P. blanchardi* و گسترده‌ترین سطح پخش قطره را نشان داد و پس از آن، فرمولاسیون‌های FN5 (متیل‌استر و سورفکتانت Termul 3540) و FN8 (متیل‌استر و سورفکتانت EW70) قرار گرفتند. نتایج حاصل از این مطالعه نشان می‌دهد که نانوفرمولاسیون‌های اسانس *M. communis* پتانسیل بالایی برای به‌کارگیری به‌عنوان حشره‌کش‌های دوستدار محیط زیست دارا هستند.

***کلید واژه‌ها:** آفت‌کش نانو، کارایی حشره‌کشی، پایداری امولسیون، توانایی پخش‌شوندگی، فرمولاسیون سازگار با محیط زیست*

دبیر تخصصی: دکتر معصومه ضیائی

Introduction

The date palm trees are infested by many insect pest species including white scale insects *P. blanchardi*. These insects are protected by thick cuticle scales that serve as a shield. This species can attack several parts of palm trees, including racemes, wicker, and fruits (El-Shafie et al., 2018). By feeding on palm tree sap, nymphs and adult females can damage the plants, causing nutrient deficiencies and yellowing on the infested parts of the plant. These insects infest and adhere to the fruit, resulting in deformities and a reduction in quantity, quality, and marketing value in severe infestations (Babaousmail et al., 2018; Aldosary et al., 2023).

The control and management of white scale insects pose significant challenges, including the thick cuticle layer that covers their bodies and protects them from natural enemies and the penetration of pesticides (Frank, 2012; Buss & Dale, 2017). While chemical control remains the primary method for scale insect management, there has been increasing concern about the potential hazards posed by chemicals to public health and the environment. To establish sustainable pest management practices, ongoing efforts are underway to reduce reliance on harmful synthetic pesticides (Aldosary et al., 2024). Thus, there is an urgent need to explore and develop eco-friendly, efficient alternatives derived from botanical sources to control this insect pest and reduce dependence on synthetic pesticides (Rebek et al., 2012; Camacho & Chong, 2015).

Extensive research has been conducted on the insecticidal effects of the essential oils derived from various plant species against various insect pests. For example, studies have focused on the effectiveness of *M. communis* (commonly known as Myrtle) as a valuable biological resource for controlling numerous insect pests (Mossa, 2016; Hennia et al., 2019).

Despite the promising characteristics and advantages offered by plants-derived essential oils, there are several challenges that prevent them from becoming potential pesticide agents. These challenges may include limited shelf life and reduced efficacy, due to factors such as volatility, rapid degradation, high sensitivity to the light and UV radiation, susceptibility to oxidation and weak water solubility (Sendi & Ebadollahi, 2014; Peschiutta et al., 2017).

To maximize the efficacy and enhance the chemical and physical properties of essential oil, the formulation process becomes crucial. This process also required reducing the costs of storage, packaging, and application processes (Chen et al., 2013; Ghosh et al., 2013; Mossa, 2016). Therefore, nanoemulsion formulation has emerged as an effective technique for the development of botanical pesticides (Bilia et al., 2014). In addition, it can enhance the physical and chemical characteristics of the essential oils, include high stability, decreased droplet size (20 –200 nm), and reduced turbidity, leading to their widespread utilization in various fields, including agriculture (Ramar et al., 2017; Azmi et al., 2019; Pavoni et al., 2020).

Previous studies indicated that a nanoemulsion formulation of plant essential oil is an effective tool for managing pests (Teles et al., 2017; Ramar et al., 2017; Pavoni et al., 2020; Aldosary et al., 2023). However, there is a research gap regarding the insecticidal effect of *M. communis* essential oil nanoemulsion against *P. blanchardi*. Therefore, the objective of the present study is to formulate a nanoemulsion of *M. communis* essential oil. In addition, to evaluate its insecticidal activity against white scale insects under laboratory conditions. The study also aimed to determine the potential of M. communis essential oil nanoemulsion as a tool for controlling *P. blanchardi*, a major pest of date palms.

Materials and Methods

Plant Material and Essential Oil Extraction

Leaves of *M. communis* were collected from Basra, Iraq in April 2024, and subsequently. After cleaning and drying in the shade at room temperature, the leaves were powdered using a commercial electric mill and stored in plastic containers. Essential oil extraction was carried out in the Medicinal Plants Unit, College of Agriculture, University of Basra. Approximately 300 g of the powdered plant sample powder was subjected to hydrodistillation using a Clevenger-type apparatus. The plant material was combined with 1.5 liters of distilled water in a rounded glass flask and boiled for 6 hours to extract essential oils. The resulting essential oil was stored at 4ºC for further use (Aldosary et al., 2023).

Chemicals

Paraffin oil and Methyl ester oil were obtained from R & M Chemicals in Malaysia and Sigma-Aldric in Germany, respectively. Three types of nonionic surfactants were used in this study. Tensiofix EW70 and Tensiofix 96 DB10 were provided by Tensiofix Agrochemical in Belgium, while Termul 3540 was supplied by KC Chemicals in Malaysia. Moreover, Triton X100 (nonionic) and paraffin wax surfactant were purchased from General Drugs House Co. Ltd., India.

Construction of Ternary Phase Diagrams

The construction of ternary phase diagrams was conducted using the titration method described by Anjali et al. (2012). Various combinations of oil and surfactant were prepared for the titration process. For each combination of oil and surfactant, water was added slowly, with the amount of water varying to cover a range from 5% to 95% of the total volume. Then, the mixtures were blended for 1 min using a vortex mixer (Bionex KML, Korea), and then centrifuged (Model: MLW T30, Germany) at 3500 rpm for 30 min at 27 ± 2°C. Visual observation was conducted to detect phase separation in each sample, considering stability, transparency, and clarity to determine whether the ingredients exhibit anisotropic or isotropic behavior. Based on these observations, the phase regions of the ingredients were defined. The ternary phase diagrams were constructed using Chemix 3.5 software, version 3.5, phase diagram plotter (UK) et al.

Selection of Formulations from Phase Diagrams

Based on specific parameters, three formulations were selected from the constructed ternary phase diagram. The parameters included achieving one-phase behavior without gel formation, optically isotropic behavior, and stability at a constant room temperature (27±2°C). These selected formulations were then mixed with 25% (w/w) M. communis essential oil and subjected to stability tests to assess their performance over time.

Stability Tests

The stability of the prepared formulations was examined using the method described by Reddy & Sowjanya (2015). In this method, 5 mL of each formulation was centrifuged at 3500 rpm for 30 min at 27 ± 2°C. Furthermore, 5 mL of each selected formulation was stored at room temperature for three months, while another set was exposed to an oven at 54 ± 2°C for two weeks to simulate a tropical climate and evaluate the stability of the formulations according to the standard protocol. The appearance formulations was visually monitored to determine any phase separation (Lee et al., 2016). The formulations (containing 25% M. communis essential oil) that passed the stability test were then submitted to additional tests, including particle size, zeta potential, and surface tension. The aim of these was to provide additional information regarding the formulation characteristics.

Characterizations of *M. communis* essential oil nanoemulsion formulation

The Ring method was used to measure surface tension of the essential oil formulations (Aldosary et al., 2023). For this purpose, the Tensiometer type KRUSS K6 (Krüss GmbH, Hamburg, Germany), equipped with a 1.9 cm De Noüy platinum ring, was used. The measurements were performed at 27 ± 2°C. Photon correlation spectroscopy (Malvern Instruments UK) with laser light scattering was used to measure the droplet size and zeta potential of the formulations. Prior to the measurements, the formulations were diluted with distilled water at a 1:20 (v/v) ratio. The particle size and zeta potential were measured at 27 ± 2°C (Choupanian et al., 2017).

Laboratory Bioassay

The spraying method described in Aldosary et al. (2023) was followed to test toxicity of *M. communis* essential oil nanoemulsion formulations against *P. blanchardi*. Distilled water was used to dilute both the non-formulated (FN0) and nanoemulsion formulation of *M. communis* essential oil at concentrations of 0, 100, 300, 500, 700, and 1000 mg/L. Additionally, to dilute the non-formulated essential oil, 0.5% Triton X-100 surfactant was added to the water. Date palm leaflets infested with the white scale insects were collected from the field and transported to the laboratory. In the laboratory, the leaflets were washed and dried before being sprayed with various concentrations using a small sprayer. After the application, the leaflets were left to dry for one hour at room temperature. Following the above steps, the lower end of each leaflet was inserted into individual test tubes containing 10 mL of water. The orifices of the tubes were sealed with cotton to secure the leaflets and prevent the evaporation of the water. The experiment was replicated four times, each replicate consisting of one leaflet. The test tubes containing the leaflets were incubated at 27 ± 2°C and 65 ± 5% RH. To determine the mortality rate, the treated leaflets were examined at 12, 24, 36, 48, and 72 h after spraying. For examination purposes, a small area measuring 2 cm^2 from each treated leaflet was randomly selected and tested under a dissecting microscope by carefully removing the insect‘s shell using a thin needle et al. Insects were confirmed dead if they appeared dry and flattened, with no signs of movement. The lethal concentration (LC_{50}), lethal time (LT50), lower and upper 95% confidence limits, as well as slope ±SE (standard error), were calculated using a probit regression analysis in SPSS. In assessing the significance of differences among LC_{50} or LT_{50} values, any overlapping in confidence limit values was considered as non-significant.

Study of the spreading area and coefficient of *M. communis* essential oil formulations

The spreading areas of both non-formulated with 0.5 % Triton X-100 (FN0) and nanoemulsion formulations of M. communis essential oil were measured using a modified paraffin wax method (Mascarin et al., 2014). To perform this, 25 mL of melted paraffin wax at 60 ± 2 °C was poured to Petri-dishes (6 ×1.5 cm), and allowed to harden. Four concentrations of each treatment (0, 200, 500, 700, and 1000 mg/L) were prepared by diluting with distilled water. Using a micropipette (Eppendorf), 10 µL of each concentration were transferred onto the surface of paraffin wax in Petri dishes. Each treatment was replicated four times. The diameters of the droplets (mm) were randomly measured at different time intervals of 0, 3, 6, 12, 18, and 24 hours after treatment, using an electronic digital caliper. The droplet spreading area was calculated using equation 1.

Equation 1. $$\pi\ (r/2)^2$$

Where r is the diameter of the droplet.

To describe the relationship between droplet spreading area, concentration, and time after treatment, the data on droplet spreading area for the M. communis essential oil nanoemulsion formulation were analyzed using a nonlinear regression model in SigmaPlot V14.0 to better describe the relationship.

Data Analysis

The experiments were conducted using a Complete Randomized Design (C.R.D). The data obtained from the experiments were analysed using a two-way analysis of variance (ANOVA). The Duncan test with a significance level of 0.05 was used to identify significant differences between the means. The analysis was performed utilizing Statistical Analysis Software version 9.3 (SAS Institute, 2011).

Results

Construction of Ternary Phase Diagrams

Figure 1 describes the ternary phase diagram of the paraffin/(DB10)/water system. The phase diagram was distinguished by the transparent, gel, and heterogeneous regions. The transparent region occupied 30% of the diagram, while the gel region covered only 4%. The remaining 66% represented a heterogeneous region. In the paraffin/ DB10/ water system, the transparent region was at the ratio 5-78.90/ 1.5-52/ 10-86.70, the gel region at the ratio 29-78/ 9-55.60/ 8-26, and the heterogeneous region at the ratio 0-90/ 0-89/ 9.90- 86.70. The phase diagram of the ternary system consisted of the methyl ester as the oil phase, Termul 3540 as the surfactant, and distilled water as the aqueous phase (Figure 2). Based on the behavior in the ternary phase diagram, the ratios 0-62/ 4-89/ 9.98-86.97 of methyl ester/ Termul 3540/ water respectively resulted in 45% isotropic region. On the other hand, the ratios of 8-61/ 23-77/ 10.7-24 and 10.7-8/ 0-18.8/ 10.6-86.6 of methyl ester/ Termul 3540/ water showed 9% gel region and 46% two-phase region, respectively. In the ternary diagram of the methyl ester/EW70/water system (Figure 3), the isotropic region occupied 43%. This isotropic region was observed in the 0-61 oil, 4-89 surfactant, and 9.8-87 water system. Additionally, the gel region in the diagram was 6% of the total area. This gel area was obtained at a ratio of 8-61% oil, 26-77% surfactant, and 12-24% water. Moreover, the two-phase region achieved 51% of the entire ternary phase diagram. This region was achieved at ratios of 10-90 methyl ester, 0-17.60 EW70, and 9.80-88 water.

Points selected from ternary phase diagrams

Three points were selected from each isotropic region of three ternary phase diagrams to formulate water-in-oil (W/O) nanoemulsions. The chosen formulations were based on three criteria: a high oil content of 35% to 45% w/w, a low water content of 25% to 35% w/w, and an equal amount of surfactant at 30% w/w. The selected formulations coded as FN1, FN2, and FN3 from the phase diagram of the paraffin/ DB10/ water system (Figure 1), FN4, FN5, and FN6 from the phase diagram of methyl ester / Termul 3540/ water system (Figure 2), and FN7, FN8, and FN9 from the phase diagram of methyl ester / EW70/ water system (Figure 3).

Stability Test of Selected Formulations with Essential Oils

The results in Table 1 present the stability test results for selected formulations containing 20% M. *communis* essential oil under different conditions. The results confirmed that the formulations FN2, FN5 and FN8 exhibited high stability during the centrifuge test, for three months at room temperature (27± 2°C) and two weeks under oven conditions (54± 2°C). These formulations did not show any signs of phase separation, gel phase, creaming, or colloidal appearance (Figure 4).

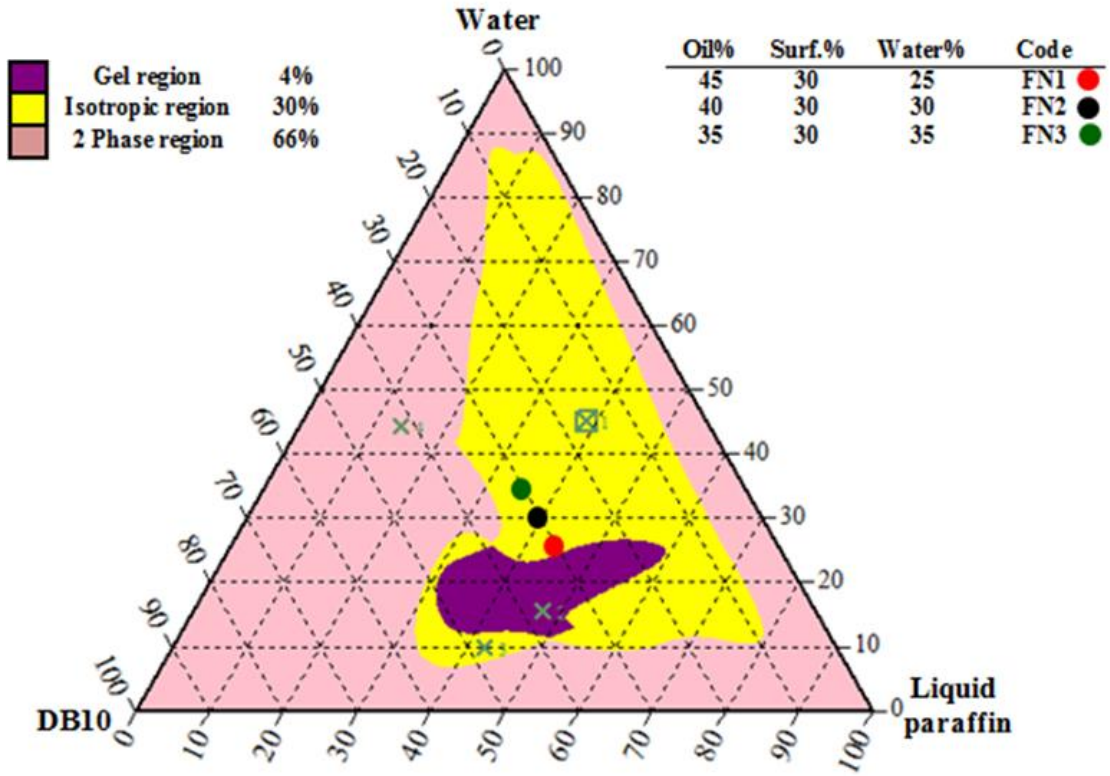


Figure 1. Phase diagram of liquid paraffin/DB10/Water system.

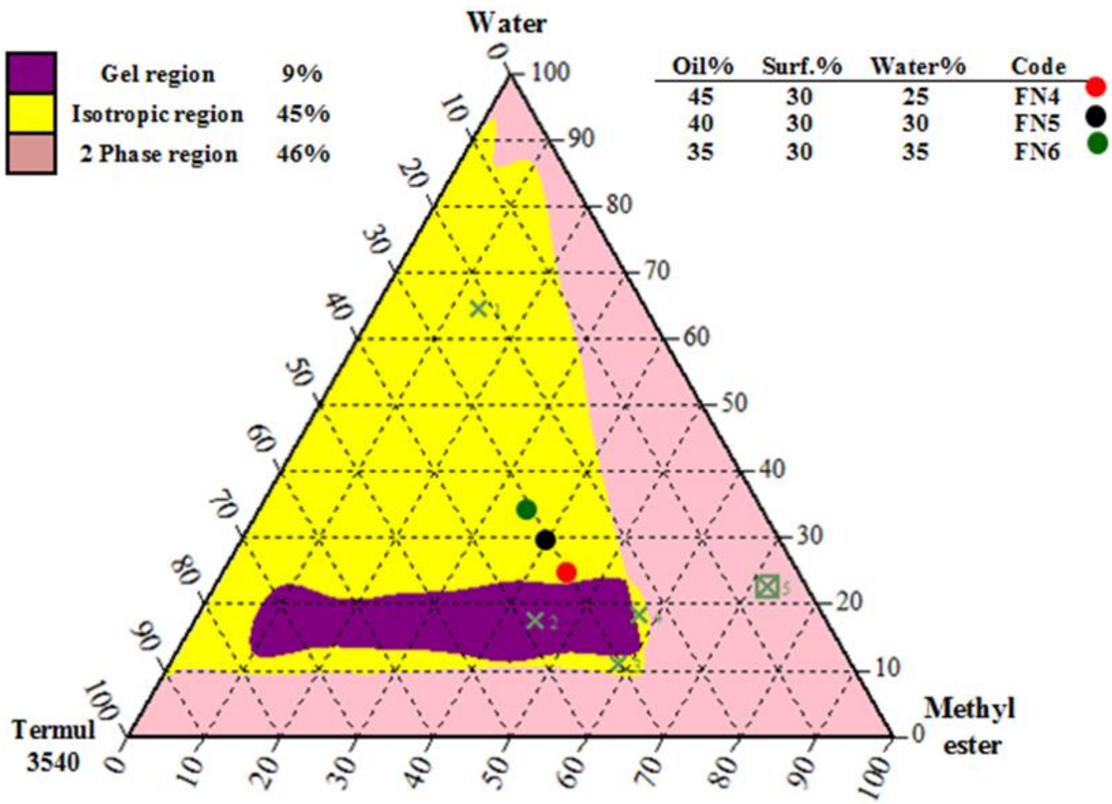


Figure 2. Phase diagram of Methyle ester/Termul 3540/Water system.

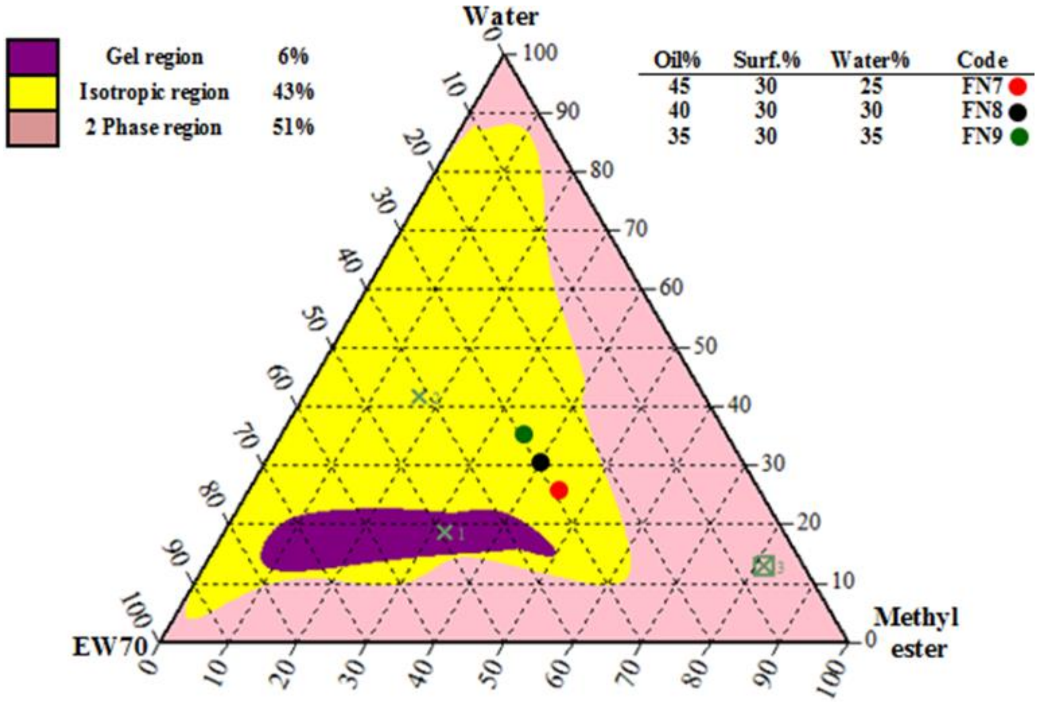


Figure 3. Phase diagram of Methyle ester/EW70/Water system.

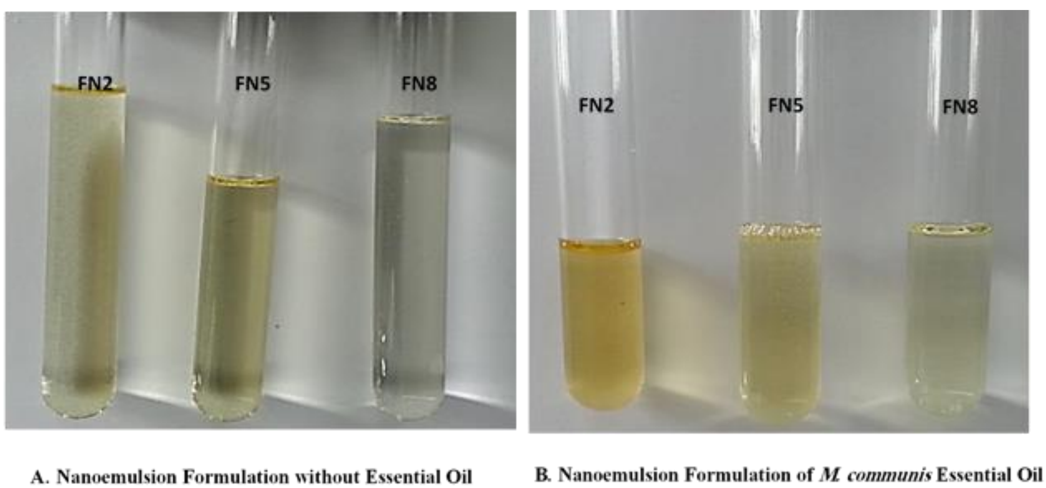


Figure 4. Nanoemulsion Formulation of *M. communis* Essential Oil.

Table 1. Stability of *M. communis* Essential Oil Formulations under Centrifuge and Storage Conditions.

Formulation Code	Centrifuge	Storage Conditions	
		Room Condition	Oven Condition
FN1	✓	✓	×
FN2	✓	✓	✓
FN3	✓	×	×
FN4	✓	×	×
FN5	✓	✓	✓
FN6	✓	✓	×
FN7	✓	✓	×
FN8	✓	✓	✓
FN9	✓	×	×

✓ = Pass, ×=Failed

Characterizations of *M. communis* Essential Oil Nanoemulsion Formulations

The results in Table 2 characterize M. communis essential oil via a nanoemulsion formulation. The results indicate significant differences (*P*<0.05) in the particle size, zeta potential, and surface tension among the different essential oil formulations. Regarding particle size, all essential oil formulations were found to be in the nanoemulsion range, with practical sizes ranging from 28.89 to 54.65 nm. In terms of zeta potential, the FN2 formulation recorded the lowest value of 9.99 mV, compared to the other essential oil formulations. Among the essential oil formulations examined, formulation FN2 achieved the lowest surface tension of 35.43 mN/m.

Evaluation of *M. communis* Essential Oil Nanoemulsion Formulation Toxicity

The LC50 and LT50 values showed that the non-formulated essential oil was less toxic than the prepared nanoemulsion, with significant differences (non-overlapping) (Tables 3 and 4). The results indicated that *M. communis* essential oil nanoemulsion formulations coded FN2, FN5, and FN8 had the highest toxicity against *P. blanchardi*. The LC_{50} for these formulations was recorded as 88.53 mg/L and 48.84 mg/L for formulation FN2 after 48 and 72 hours of treatment, and 101.24 mg/L and 60.27 mg/L for formulation FN5 after 48 and 72 hours of treatment, followed by formulation FN8, which showed 260.27 mg/L and 83.83 mg/L after 48 and 72 hours of treatment. The results confirmed that the essential oil nanoemulsion coded FN0 had low toxicity, with the highest LC50 of 5396.36 mg/L after 12 hours of treatment. Besides, the results indicated that the nanoemulsion formulation coded FN2 exhibited the shortest LT50 values at concentrations of 700 and 1000 mg/L, with values of 13.21 hours and 9.04 hours, respectively. Similarly, for the nanoemulsion formulation coded FN5, the shortest LT_{50} values were recorded at concentrations of 700 and 1000 mg/L, with values of 14.87 hours and 12.09 hours, respectively. On the other hand, the longest LT_{50} value was 86.52 hours at a concentration of 100 mg/L of the non-formulation essential oil for coded FN0.

Spreading Area Coefficient of *M. communis* Essential Oil Nanoemulsion Formulations

The results of the interaction between time and concentration revealed significant differences in droplet spreading area between the M. communis essential oil nanoemulsion formulation and the non-formulation at all tested concentrations and time points (Figure 5). The nanoemulsion formulation coded FN2 achieved the largest spreading area after 18 hours for all concentrations. At a concentration of 1000 mg/L after 18 hours of treatment, the nanoemulsion formulation code FN2 showed a spreading area of 203.50 mm^2. In contrast, no measurable spreading areas were observed for the non-formulated essential oil (FN0) after 6, 12, 18, and 24 hours of treatment and all concentrations. As a result, the droplet spreading areas of non-formulated of *M. communis* essential oil were not included in the regression equation because they did not meet the regression conditions.

Table 2. Characterisations of *M. communis* Essential Oil Formulations.

Formulation	Particle Size (nm)	Zeta Potential (mv)	Surface Tension (mN/m)
FN2	28.893±0.19a*	9.99±0.07a	35.43±0.03a
FN5	53.71±0.38ab	25.87±0.57c	36.80±0.10b
FN8	54.65±0.98b	24.83±0.26b	36.87±0.13b

* The same letters in each column are not significantly different at *P<0.05.* Duncan

Table 3. LC_{50} (mg/L) Values of *M. communis* Essential Oil Nanoemulsion formulation against *P. blanchardi*.

Formulations	Time (hours)	LC_{50} (95% C.L.)*	Slope ±S.E.	X^2
non-formulation code FN0	12	5396.36 (1897.00 to 30242.13)	0.63±0.19	2.2
	24	1963.12 (1040.07 to 11197.28)	0.72±0.18	2.84
	36	734.19 (522.89 to 1288.30)	0.90±0.18	1.76
	48	323.16 (221.93-872.119)	1.00±0.11	2.34
	72	148.18 (153.38-520.49)	1.02±0.20	2.07
Nanoemulsion code FN2	12	488.21 (344.71 to 767.40)	0.88±0.17	5.41
	24	193.45 (117.56 to 266.21)	0.97±0.18	2.47
	36	107.77 (58.810 to 154.11)	1.15±0.19	8.32
	48	88.53 (61.04 to 113.87)	2.02±0.26	12.69
	72	48.84 (23.20 to 73.73)	1.76±0.29	10.39
Nanoemulsion code FN5	12	946.78 (675.70 to 1686.72)	1.03±0.18	5.95
	24	448.64 (342.77 to 609.19)	1.09±0.18	7.98
	36	173.24 (119.46 to 224.71)	1.28±0.18	8.32
	48	101.24 (68.45 to 132.00)	1.72±0.22	16.95
	72	60.27 (31.93 to 88.45)	1.59±0.77	13.87
Nanoemulsion code FN8	12	3632.54 (1214.80-138356.80)	1.61±0.15	2.01
	24	1024.61 (583.50-7098.72)	1.67±0.22	2.11
	36	730.22 (396.78-7210.43)	1.34±0.17	2.93
	48	260.27 (207.25 to 315.85)	1.58±0.18	6.27
	72	83.83 (47.30 to 118.78)	1.40±0.20	16.66

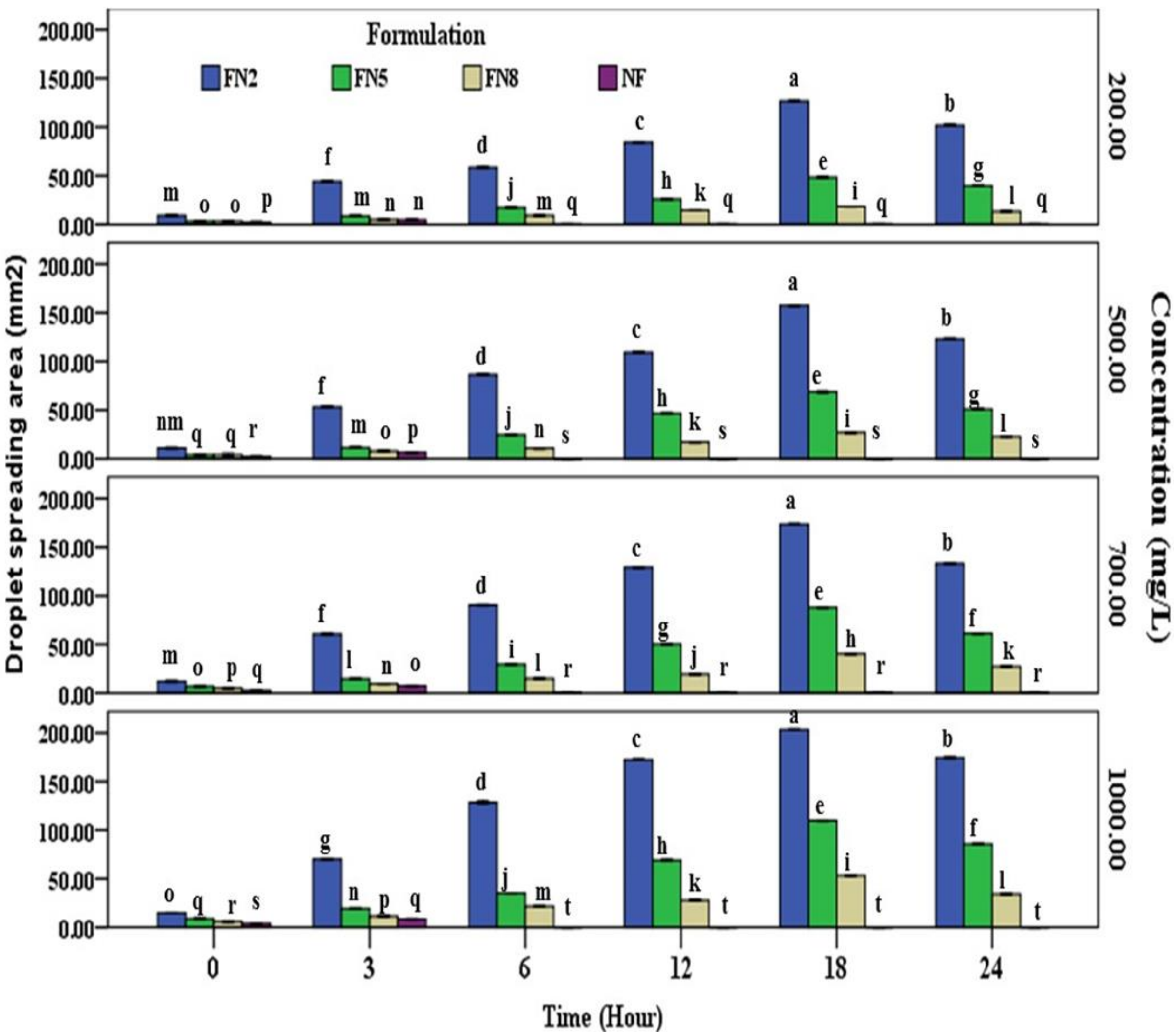
*C.L. (confidence limit) which has been calculated with 95% confidence.

Table 4. LT_{50} (hour) Values of *M. communis* Essential Oil Nanoemulsion formulation against PWS *P. blanchardi*.

Formulations	Concentration mg/L	LT50 (95% C.L.)*	Slope ±S.E.	X2
Non-formulation code FN0	100	86.52 (53.87-448.61)	1.15±0.38	1.23
	300	59.36 (42.96-122.93)	1.98±0.83	2.31
	500	45.67 (31.61-97.88)	1.29±0.37	2.41
	700	30.75 (19.44-46.68)	1.21±0.37	0.94
	1000	22.37 (18.39 to 26.15)	2.75±0.29	27.69
Nanoemulsion code FN2	100	32.54 (26.10 to 40.52)	1.41±0.25	3.56
	300	20.26 (15.56 to 24.43)	1.71±0.26	5.67
	500	15.76 (11.91 to 19.08)	2.00±0.27	5.66
	700	13.21 (10.60 to 15.47)	2.93±0.34	16.71
	1000	9.04 (6.01 to 11.56)	2.51±0.37	9.89
Nanoemulsion code FN5	100	44.40 (37.32 to 55.48)	1.72±0.26	3.56
	300	32.15 (27.82 to 37.06)	2.16±0.26	5.35
	500	24.02 (20.31 to 27.62)	2.22±0.26	7.95
	700	14.87 (11.89 to 17.48)	2.55±0.30	13.99
	1000	12.09 (9.41 to 14.37)	2.87±0.35	13.98
Nanoemulsion code FN8	100	78.15 (60.69 to 121.30)	1.650±0.28	12.43
	300	53.89 (44.49 to 71.36)	1.69±0.26	10.40
	500	37.65 (32.23 to 44.76)	1.91±0.26	6.92
	700	26.46 (22.80 to 30.17)	2.32±0.27	16.45
	1000	21.15 (8.66-30.87)	1.01±0.37	1.21

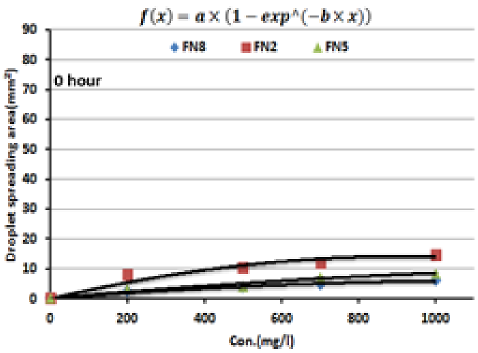
*C.L. (confidence limit) which has been calculated with 95% confidence.

The results of the non-linear regression model demonstrated a strong positive relationship between the concentration of nanoformulation type of *M. communis* essential oil and droplet spreading area on date palm leaflets. The regression analysis produced high r2 values ranging from 0.95 to 0.99 and r values ranging from 0.87 to 0.99 (Fig. 6). The droplet spreading area increased significantly as concentration and time period increased after treatment for all nanoemulsion formulations. The relationships could be expressed using an exponential function $(\boldsymbol{x})=\boldsymbol{a}\times(\mathbf{1}-\boldsymbol{exp}^{(-\boldsymbol{b}\times\boldsymbol{x})})$ (McPherson, 2024), where: $\boldsymbol{f}(\boldsymbol{x})$ is the spreading area mm^2, $\boldsymbol{x}$ is concentration (mg/L) of treatment, $\boldsymbol{a}$, $\boldsymbol{b}$, and $\boldsymbol{y0}$ are the regression coefficients. The coefficient $(\boldsymbol{a})$ represents the highest spreading capacity of essential oil formulations and is proportional to the spreading area of all essential oils nanoemulsion formulations. Furthermore, the current findings indicate significant differences (*P < 0.05*) in droplet spread area among the treatments, including formulation type, concentration, and time after treatment.

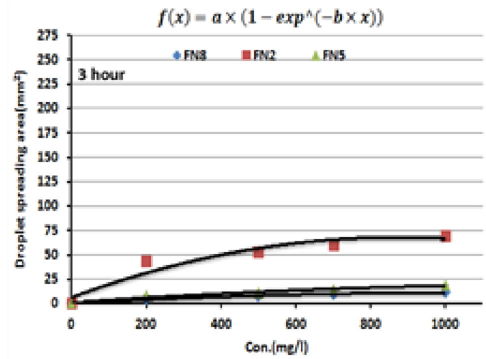


* The same letters in each column are not significantly different at *P<0.05.* Duncan

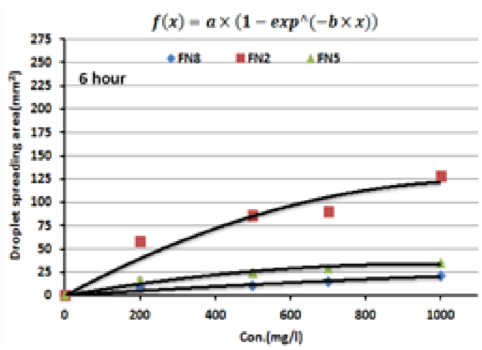
Figure 5. The droplet spreading area at different concentrations of formulation types of *m. communis* after various treatment periods.



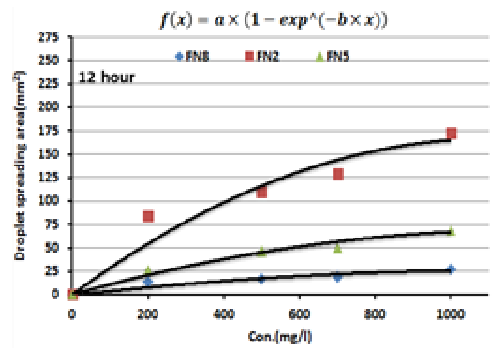
For. Code	Parameter estimates (± SE)		R	R^2
	a	b		
F5	15.14±0.86	0.003±0.0004	0.98	0.96
F8	20.27±12.71	0.0006±0.0004	0.95	0.90
F2	8.07±1.03	0.001±0.0003	0.98	0.96



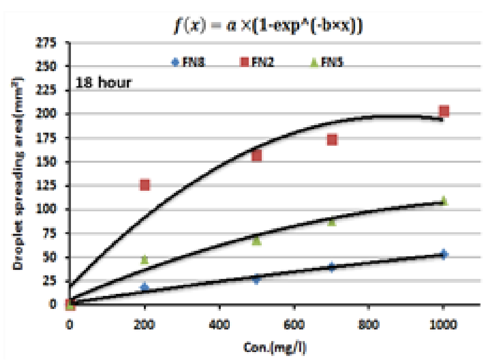
For. Code	Parameter estimates (± SE)		R	R^2
	a	b		
F5	67.86±2.40	0.004±0.0004	0.99	0.87
F8	24.85±4.17	0.001±0.0004	0.97	0.94
F2	14.42±1.14	0.002±0.0002	0.99	0.98



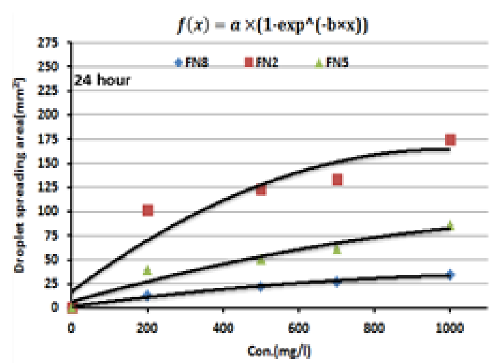
For. Code	Parameter estimates (± SE)		R	R2
	a	b		
F5	142.52±15.33	0.002±0.0004	0.98	0.94
F8	38.59±2.48	0.002±0.0003	0.98	0.97
F2	48.13±24.31	0.0006±0.0004	0.97	0.93



For. Code	Parameter estimates (± SE)		R	R2
	a	b		
F5	192.45±16.53	0.002±0.0003	0.98	0.96
F8	89.78±10.43	0.001±0.0003	0.99	0.97
F2	29.46±3.44	0.002±0.0005	0.96	0.92



For. Code	Parameter estimates (± SE)		R	R2
	a	b		
F5	188.89±5.42	0.005±0.0006	0.98	0.97
F8	125.33±9.30	0.002±0.0003	0.99	0.97
F2	108.08±32.86	0.0007±0.0003	0.98	0.96



For. Code	Parameter estimates (± SE)		R	R2
	a	b		
F5	157.08±7.32	0.004±0.0007	0.97	0.94
F8	96.36±11.73	0.002±0.0004	0.97	0.93
F2	42.25±4.40	0.002±0.0003	0.98	0.96

Figure 6. The non-linear regression of droplet spreading area at different concentrations of nanoemulsion formulations of *M. communis* after various treatment periods.

Discussion

Based on our knowledge, there is no existing literature that mentions the insecticidal activity of M. communis essential oil nanoemulsion formulations against the date palm white scale insect, P.

blanchardi. The ternary phase diagrams were employed in this study to understand the phase behavior as the ratio of emulsion ingredients was gradually varied. The phase behavior provided a significant indication of the proportions of the formulation components, which is a crucial factor in achieving a nanoemulsion formulation with enhanced thermodynamic stability (Qadir et al., 2016; Pavoni et al., 2020). Methyl esters and paraffin oils used in this study typically have low solubility in water. However, the addition of nonionic surfactants such as EW70, Termul 3540, and DB10 significantly improved the solubility and distribution of the oils. This is because the primary function of surfactant is to reduce the interracial energy and create a mechanical barrier between formulation particles. As a result, surfactant agents minimized cloudiness and droplet size in the emulsion system, which agrees with the findings of Anjali et al. (2012). These findings are consistent with previous studies that have demonstrated the possibility of preparing and formulating a nanoemulsion of essential oil using paraffin and methyl ester as the oil phase, along with non-ionic surfactants, through a low-energy method (Ramar et al., 2017; Moss et al., 2017; Pavoni et al., 2020).

Numerous studies have revealed that non-ionic surfactants can generate nanoemulsion formulations with small droplet sizes and high stability using a low-energy emulsification process. These surfactants can form films around the particles of the formation, effectively preventing and/or protecting them from aggregation, thereby creating a separate layer (Kale & Deore, 2017; et al. Prajapati et al., 2017). The addition of a surfactant is crucial in achieving small-sized droplets as it reduces the interfacial tension between oil and aqueous phases of the emulsion. Hence, they contribute to the formulation of nanoemulsions with prolonged stability (Che Marzuki et al., 2019). Nevertheless, it is important to note that surfactants may lose effectiveness under high temperatures and rapid movement. Increased particle motion can lead to bias, collisions, and particle convergence, ultimately destabilizing the emulsion system (Pavoni et al., 2020; Aldosary et al., 2023).

It was observed that particle size has a crucial impact on the stability and turbidity of the emulsion formulation, consistent with other studies (Gupta et al., 2016; Kale & Deore, 2017). In this study, the formulations had particle sizes considered to be within the nanoformulation range of the emulsion, with a range of 26.94 to 108.00 nm (Pereira et al., 2016; Pavoni et al., 2020). To achieve a suitable drop size that enhances the stability of a nanoemulsion formulation, several factors were considered. These factors included the amounts of the ingredients, the concentration of the surfactant, and the preparation method, which are considered crucial in nanoemulsion formulation (Zuccari & Alfei, 2023).

Another important factor to increase the stability of particles in a system, by causing particles dispersal or aggregation, is the zeta potential. Generally, particles with zeta potential values ranging from -30 to +30 mV exhibit aggregation potential. It means that particles with zeta potential values higher than +30 mV and lower than -30 are stable. Studies indicated that zeta potential is influenced by several factors, including particle type, background electrolyte, particle concentration, water pH, and the type and concentration of additives (surfactants, oils, etc.) (Feng et al., 2020).

The results indicate that non-ionic surfactants also play an important role in reducing surface tension. During emulsification, surface tension decreases due to an increase in interfacial area (Posocco et al., 2016; Haghighi et al., 2020).

Consistent with studies that reported an enhancement in the activity of essential oils against various pests (Moss et al., 2017; Ibrahim et al., 2022), the present study demonstrated that the nanoemulsion formulations of *M. communis* essential oil enhanced the toxicity of the essential oil against *P. blanchardi*. The toxicity increased with higher nanoemulsion concentrations and longer post-treatment exposure times. It is believed that the nanoformulation system used in this study increased the effectiveness of the essential oil, resulting in higher mortality through several mechanisms, as reported in previous studies (Bilia et al., 2014; Ribeiro et al., 2014). It might be that nanoemulsion improved the essential oil's effect by several mechanisms, including increasing its controlled release, increasing its solubility, and reducing its volatility. These factors allowed for longer-lasting effects of the essential oil, better distribution on the treated surface, and prevented rapid evaporation. As a result, the nanoemulsion extended the exposure time and increased the oil's penetration into the target insects, leading to an increase in mortality. In the present study, higher mortality was observed with the smallest particle size compared to the other formulations with larger particle sizes. This is because with smaller particle sizes, better penetration and distribution through and on the treated surface can be achieved (Thakur et al., 2013).

The toxicity of *M. communis* essential oil against white scale insects can be attributed to its composition of compounds known for their toxic effects on insects (Hennia et al., 2019; Laghzaoui et al., 2022; Gad et al., 2022). These compounds, including monoterpenes and monoterpenoids, are mostly volatile and lipophilic, can penetrate insect bodies, and negatively affect their physiological functions (Aldosary et al., 2018; Chaudhari et al., 2021).

The differences in the spreading areas can be attributed to the physical properties of nanoemulsion formulation droplets, such as surface tension and particle size (Aksoy et al., 2020; Argentou et al., 2022). As a result, the nanoemulsion formulation with lower surface tension and smaller particle sizes in this study achieved larger spreading areas. The increase in nanoemulsion concentration also increases droplet spreading areas (Marhamati et al., 2021; Argentou et al., 2022).

Conclusion

Using the low-energy method, the essential oil of *M. communis* was successfully synthesized as a nanoemulsion insecticide. In vitro, the nanoformulation of *M. communis* essential oil showed strong stability under both room and oven conditions. It also showed high insecticidal properties against the date palm white scale insect. Overall, this study demonstrated that *M. communis* essential oil nanoemulsion formulations improved the essential oil's stability and efficacy as a pesticide. As such, they can be considered reliable and environmentally friendly alternatives to chemical pesticides for the control of date palm white scale insect.

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