Arab Universities Journal of Agricultural Sciences
(2024) 32 (2) 283-296
Website: http://ajs.journals.ekb.eg

Jojoba Oil-based Nano-emulsion as Promise Bio-Pesticide Against *Myzus persicae* and *Tetranychus urticae*, and Its Biosafety on *Coccinella undecimpunctata* and Aphid Mummies

Doaa F El Sherif*, Sherin HM Safar
Plant Protection Dept, Fac of Agric, Fayoum Univ, Fayoum 63514, Egypt

*Corresponding author: dfs00@fayoum.edu.eg

https://doi.org/10.21608/AJS.2024.291637.1569

Received 21 May 2024; Accepted 7 August 2024

Keywords:

Acaricidal activity,
Aphicidal activity,
Jojoba oil,
Nano-emulsion,
Natural enemies

Abstract: Nano-emulsion-based essential oils are considered the most effective applications for controlling pests. Jojoba oil-based nano-emulsion and bulk emulsion formulations were examined against *Tetranychus urticae* and *Myzus persicae* and their natural enemies. The prepared jojoba oil-based nano-emulsion showed superior stability in centrifuging and freezing tests and had non-foaming properties. The droplet size of the Jojoba oil-based nano-emulsion was as small as 45±5 nm with a zeta potential of 4.79 mV. Results confirmed that jojoba oil-based nano-emulsion possesses better acaricidal and algicidal activity than bulk emulsion. The LC_{50} of nano-emulsion was 0.103 and 0.06%, while in bulk emulsion, it recorded 4.06 and 4.76% against *T. urticae* after 24 h of spraying at temperatures of 20 and 30ºC, respectively. Under the same conditions, nano-emulsion had an LC_{50} value of 0.23 and 0.35% while bulk emulsion had 5.14 and 3.61% against *M. persicae*. Furthermore, the use of jojoba oil-based emulsion had no significant negative impact on aphid mummies or *Coccinella undicempunctata*. These encouraging findings confirmed that jojoba-based nano-emulsions possess potential eco-friendly and effective bio-pesticides against mites and aphids as well as being safe as biological control agents. Additionally, its physical properties are suitable for commercial use.

1 Introduction

The green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), and the red spider mite, *Tetranychus urticae* (Koch) (Acari, Tetranychidae), are the most important pests worldwide that attack many economically important vegetable and field crops. Furthermore, the major damage caused by green peach aphids is through the transmission of plant viruses where more than 100 viruses are transferred by this species. Additionally, quarantine issues may arise due to the contamination of vegetables by aphid colonies (Aboalfayah and Samara 2022).

The control of *M. persicae* and *T. urticae* is based on pesticide application (Seki 2016, Çağatay et al 2018, Assouguem et al 2022). Pesticides are well known to reduce yield loss and ensure food safety (Cooper and Dobson 2007). Conventional agrochemicals remain embedded in soil or groundwater for years through volatilization and leaching. The long-term degradation period results in the accumulation of pesticides in the food chain, thus threatening the health of living organisms and the environment (Pérez-Lucas et al 2019). Furthermore, the difficulty of getting some pesticides to the site of action due to their hydrophobicity is a challenging problem. More than 70% of conventional pesticides are estimated to be inefficient due to their application repeatedly and the development of pesticide resistance (Hazra and Purkait 2019). Thus, more efficient new alternative techniques to conventional pesticides are needed.

Nowadays, non-chemical techniques that effectively reduce the harmfulness of pests are sought due to the growing popularity of organic farming and the green order policy (Jakubowska et al 2022). According to previous reports, essential oils (EOs) possess a wide range of chemicals that have been proven to be effective as pesticides and a safe alternative to synthetic pesticides (Koul et al 2008, Ben-Issa et al 2017, Raveau et al 2020, Ahmed et al 2021, Garrido-Miranda et al 2022, Sheasha et al 2023, Zhu et al 2024). However, field application of EOs in pest control is challenging because of their poor water solubility and rapidly degrade and volatility. Nanotechnologies are capable of overcoming these challenges. This technique can improve target surface coverage, stability and physical properties of essential oils (Modafferi et al 2024).

Nano-emulsion-based pesticides have proven to be one of the promising techniques in the field of pest management (Feng et al 2018). Moreover, the EOs or nano-emulsion oils demonstrated risk-free on natural enemies of pests (Giunti et al 2022, Modafferi et al 2024). These are formulations in which the nano-emulsion acts as a vector that carries and delivers the bioactive compounds to the target pests in plants. The effective release, accumulation and uptake of the active ingredients are due to the unique physicochemical properties of nano-sized materials, as their size ranges between 20–500 nm which enables them to be kinetically stable (Feng et al 2018, Pagar and Darekar 2019). Nano-emulsions mainly consist of oil and water phases separated by surfactants that act as emulsifiers to maintain the interfacial tension between the phases (Mustafa and Hussein 2020). Nano-emulsion pesticides are preferably formulated as oil in water emulsions which enhances the hydrophobicity and absorption of agrochemicals (Yang et al 2014).

The integration of agrochemicals in oil-based nano-emulsions protects them from photodegradation and increases their solubility and foliage adhesion (Nguyen et al 2013, Yang et al 2014). Oils are categorized either as essential oils or non-essential oils. Being rich in biological properties, nano-emulsion oil constituents could enhance the cytotoxicity, genotoxicity and antimicrobial activity against pathogens (Caputo et al 2020). This investigation focused on the evaluation of jojoba oil emulsion and nano-emulsion against two pests as an alternative to traditional control tools. Crude jojoba oil is extracted from the seeds (*Simmondasia chinensis*) by soaking them in solvents (Gad et al 2021). Jojoba oil has insecticidal bioactivity against pests in the traditional formulation and nano-formulation. Jojoba oil nano-emulsion (JONE) has an insecticidal effect against the rice weevil, *Sitophilus oryzae,* (Linnaeus) (Coleoptera: Curculionidae) that has been reported as one of the severest pests of cereal grains and their products (Sh et al 2015). The insecticidal efficiency of jojoba oil indicated a gradual increase in the mortality rate of *S. oryzae* adults with increasing oil concentrations and exposure period (Sh et al 2015). It has also been reported that the insecticidal effect increased with increasing the concentration and exposure time against the maize weevil, *Sitophilus zeamais,* (Motsch) (Coleoptera: Curculionidae). The current study investigated the efficacy of jojoba oil-based nano-emulsion as a novel approach of eco-friendly control of two sucking pests, *Tetranychus urticae* and *Myzus persicae*. In addition, their biosafety was assessed on the natural enemies, which included the eleven-spotted lady beetle, *Coccinella undecimpunctata* (Linnaeus) (Coleoptera: Coccinellidae), as a predator, and parasitized aphid mummies of *Diaeretiella rapae* (M'Intosh) (Hymenoptera: Braconidae).

2 Materials and Methods

2.1 Formulation of Emulsions

The jojoba oil-based nano-emulsion (JONE) was prepared in NanoTech Egypt for Photo-Electronics from Jojoba essential oil purchased from the National Research Center, Dokii, Egypt (Costa et al 2014). The final product of JONE contained 2% (w/w) Jojoba oil, 5% (w/w) polysorbate 80 as a hydrophilic emulsifier and 93% double distilled water. The emulsion was kept at room temperature (25 ± 2°C) until evaluation experiments.

The Jojoba oil bulk emulsion (JOBE) was prepared from Jojoba essential oil at a concentration of 10% (w/v) as stock. Triton X-100 (Oxford lab chem.) as an emulsifying agent was added at a rate of 0.5ml /100ml.

2.2 Physical properties of formulations

Size: Nano-emulsion droplet size was determined on the JEOL JEM-2100 high-resolution transmission electron microscope at an accelerating voltage of 200 kV.

Stability: Three tests of nano-emulsions and bulk emulsion forms were conducted, including zeta potential by using Zetasizer Nano ZS. (Malvern, UK), Model: ZEN 3600. Also, gravitational stress by centrifugation at 5000 rpm for 30 min and finally freezing of emulsions in tubes for two weeks at 0°C. Any separation phase is carefully noted and recorded (Metwally et al 2022, Ayllón-Gutiérrez et al 2023).

Foam volume: In a 250 ml cylinder, 100 ml of the formulations were poured. The cylinder was closed and inverted 30 times, then it was placed on a hard, straight surface and timing was started immediately. The foam volume formed was recorded after 10 s., 1 min and 10 min (Metwally et al 2022).

2.3 Pest Colony

Aphids: Green peach aphids were collected from naturally infected cabbage seedlings planted in a greenhouse at Fayoum governorate, Egypt. Infested seedlings were transferred to the laboratory. The aphids were reared for several generations on the small cabbage plants *Brassica oleracea* grown in plastic pots (17 cm height × 20 cm diameter) containing a mixture of soil and sand, under laboratory conditions at 20 ± 5 ºC, 55% ± 5 relative humidity (RH) and a photoperiod 14Light:10 Dark (Cantó-Tejero et al 2022). Individuals were identified according to (Blackman and Eastop 1984).

***Mites*:** Two-spotted spider mites were collected from naturally infected castor plants, *Ricinus communis* L. that were grown at Fayoum governorate. Mites were cultured for four months (many geneations) on the upper surface of *Acalypha wilkesiana* (Müll). To prevent mites from escaping, acalypha leaves were preserved in Petri dishes on a cotton pad that had been saturated with water. Adult females were transferred using a brush (No. 0) from gathered castor leaves to an acalypha leaf prepared as shown and left for egg-laying. To keep the cotton pad moist, the cotton discs were soaked in water each day to keep them fresh. The placed eggs were kept in a lab at 20–23 °C, 50 ± 5 % R.H. and 12:12 h day: night photoperiod until hatching. The acalypha leaves were changed weekly or as they became heavily damaged by the mites to provide a mite colony with fresh leaves. Mite rearing and classification key as shown in (Safar et al 2024).

2.4 Natural Enemies Colony

***Coccinellid beetle*:** The original *Coccinella undecimpunctata* were collected as larvae from fields unsprayed with pesticides in Fayoum Governorate. Lavae resided in large plastic containers covered with muslin cloth for ventilation under the previously described laboratory conditions and were fed on aphid nymphs reared on cabbage leaves. Newly emerged adults of *C. undecimpunctata* were identified (Bienkowski 2018) and used for the bioassay experiments.

Aphid Mummies: Mummies were obtained by collecting aphid-infected cabbage seedlings and parasitized aphids. The emerging parasites were examined under the binoculars and were of *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae) as described by (Singh and Singh 2015). These parasites were allowed to parasitize on aphid colonies raised in laboratory conditions at 20 ± 5 ºC, 55% ± 5 R.H., and 14L:10D. Then the bioassay experiments were carried out on these mummies.

2.5 Bio-pesticide Activity

The acute toxicity of JONE and JOBE against *M. persicae* and *T. urticae* was determined by spraying both forms directly. The residual effect was evaluated using the leaf dipping method.

Spraying method: A series of four dilutions of JONE (0.063, 0.125 and 0.25%), and JOBE (1, 2, and 4%), were prepared. An atomizer was utilized to spray 1 ml of each concentration upon the surface of the leaves. In each concentration, three replicates were used. The control was sprayed with 1 ml of double distilled water with Triton X-100 added (Lee et al 2023, Modafferi et al 2024).

For *M. persicae,* the test was conducted in plastic units (25 mm diameter and 15 mm height) and the substrate was a moist cotton pad coated in filter paper to keep aphids from being submerged. The unit was covered with muslin cloth tight with a rubber. After inserting one red pepper leaf, *Capsicum annuum* L. in each unit, and ten nymphs of *M. persicae* 2-day old were added.

For *T. urticae*, three discs (2 cm- diameter) of acalypha leaves were used and were set in a Petri dish (9 cm- diameter). Ten females of *T. urticae* at the same age /leaf disc were inserted.

Leaf dipping method: the leaves (acalypha discs or pepper leaves) were immersed in the previous concentrations for 10 seconds. After that, they remained at room temperature until they were fully dry. This experiment was designed as described in the above-mentioned experiment (Modafferi et al 2024). Mites and aphids that were not moving when touched by a fine brush were considered dead.

All experiments were conducted separately under 20 and 30 ºC temperatures. Numbers of live and dead mites and aphids were counted at 24, 48- and 72 hours post-treatment. The mortality percentages were corrected using Abbott's formula (Abbott 1925), and the toxicity line was calculated according to Finney's analysis (Finney 1980).

2.6 Biosafety of JONE and JOBE on Natural Enemies

Coccinellid beetle: JONE and JOBE were assessed using the spraying method on adults of *C. undecimpunctata*, under laboratory conditions at 20 ± 5 ºC, relative humidity of 55% ± 5 and 14L:10D. In addition to the untreated control, there were four concentrations, 0.063, 0.125, 0.25, and 0.5% for the nano-emulsion and 1, 2, 4, and 8 % for the bulk emulsion. Every treatment is divided into three replicates. Ten adult beetles were placed in each plastic unit (25 mm diameter and 15 mm height) as a replicate. One ml of each concentration was sprayed by an atomizer on coccinellid beetles. Twenty fresh 7-day-old aphid nymphs were added daily to provide the beetles with enough food. Dead beetles were counted for 72 hours (Mohammed 2020).

Aphid mummies: Mummies were sprayed in Petri dishes (9 cm- diameter) using the same technique as the coccinellid beetles. In addition to the control, the concentrations used were 1, 2, 4, and 8 % for bulk and 0.063, 0.125, 0.25 and 0.5 %for nano-emulsion. Each concentration was divided into three replicates, 10 mummies on cabbage leaf/replicate. After seven days of spraying, the rate of parasite emergency from the mummies was recorded. This experiment was conducted under laboratory conditions.

2.7 Statistical analysis

The mortality data were corrected using Abbott's formula (Abbott 1925) and The LC_{50}, and LC_{90} values were calculated by Ldp line according to Probit analysis of Finney (Finney 1980) and Chi Square test. To examine significant differences between means in the various treatments of JOBE and JONE the experimental design was a 4 concentrations × 2 application methods × 2 temperatures factorial in a randomized complete block with three replications carried out in an acarology laboratory, Plant Protection Department, Faculty of Agriculture, Fayoum University, Fayoum, Egypt. The collected results were statistically analyzed using the InfoStat computer software package (version 2012). For post-ANOVA mean separation, Duncan's multiple-range test (Gomez et al 1984) was used at a 0.05 probability level.

3 Results and Discussion

3.1 Physical properties of formulations

The droplet size of JONE was 45±5 nm (**Fig 1**) as determined by a transmission electron microscope (TEM) at an accelerating voltage of 200 kV. The stability of the prepared concentrations of bulk emulsion 4% and nano-emulsion 0.5% was determined by centrifuging at 5000 rpm for 30 minutes and freezing at 0 °C for two weeks. The JONE was highly stable with no phase separation. However, the phase separation in both tests was observed by JOBE (**Fig 2**). The foam volume that formed in 100 ml of JOBE was 20, 12, and 10 ml, while in the same volume of JONE, it was 5, 3, and 1 ml after 10 seconds, 1 and 10 min, respectively (**Table 1**). The zeta potential for JOBE and JONE was appropriate, with values of -1.30 and -4.79 mV recorded, respectively **(Table 1** and **Fig 3).**

3.2 Acaricidal activity of JONE and JOBE

Using spraying and dipping methods, the acute toxicity both of jojoba oil nano-emulsion and bulk emulsion was assessed against *T. urticae* after 24, 48 and 72 hours at 20 and 30ºC. Data obtained showed that the spraying method was more effective in controlling *T. urticae* than leaf dipping under different temperatures. The results in **Table 2** illustrated that spraying JONE had a high impact on *T. urticae*. After 24 h of treatment, the LC_{50} values were 0.103 and 0.06% at temperatures of 20 and 30ºC, respectively. However, the LC_{50} of JOBE recorded 4.06 and 4.76% at the same temperatures. After 3 days, the LC_{50} values still decreased **(Table 2).** It was also observed in **Figs 4** and **5** that the increase in concentration led to a higher mortality percentage of *T. urticae* in both methods. The mortality percentage increased to 96.7% ±3.33 at the highest concentration (0.25%) in the nano-emulsion, when spraying at two temperatures. Bulk emulsion with the highest concentration (4%) resulted in a mortality percentage

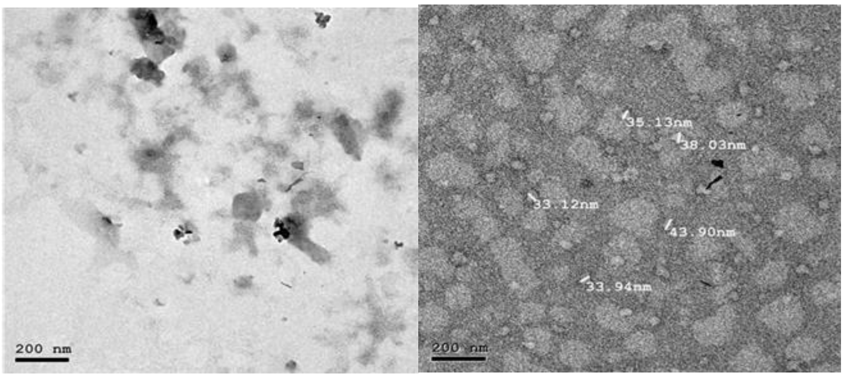

Fig 1. TEM of Droplet size of Jojoba oil-based nano-emulsion

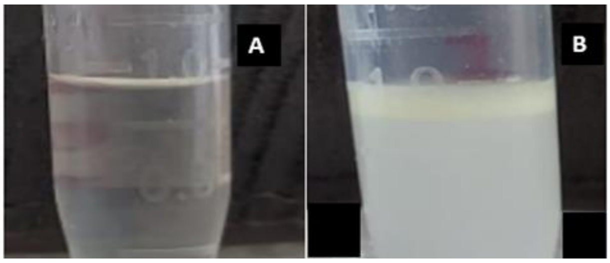

Fig 2. Stability of JONE (A) and JOBE (B)

Table 1. Physical properties of JONE and JOBE

Formulation	Stability			Foam volume (mL)		
	Zeta potential (mV)	phase separation		10 second	1 min	10 min
		Test Centrifuge	Test Freezing			
JONE 0.5%	-4.79	No	No	5	3	1
JOBE 4%	-1.30	Yes	Yes	20	12	10

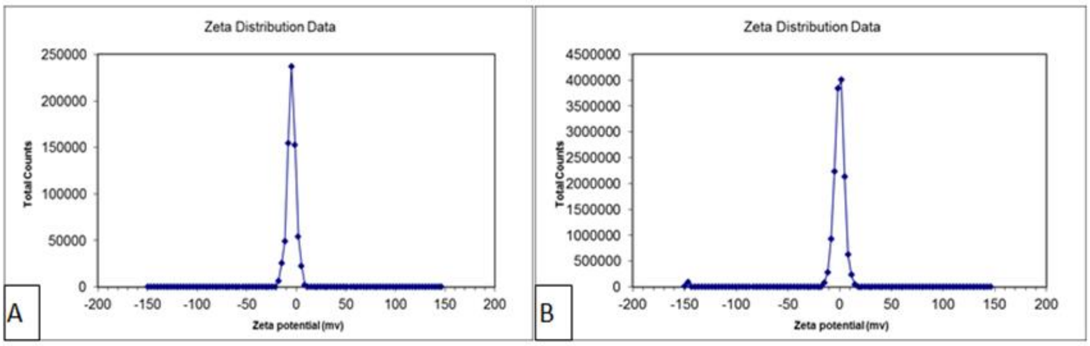

Fig 3. Zeta potential of JONE (A) and JOBE (B)

Table 2. Acaricidal activity of JONE and JOBE against *T. urticae* using spraying and dipping leaf methods at 20 and 30ºC

Method	Formulation (Jojoba oil)	Time (hour)	20 ºC				30 ºC			
			LC_{50} % (95% CL)	LC_{90}% (95% CL)	Slope ± SE	χ^2	LC_{50}% (95% CL)	LC_{90}% (95% CL)	Slope ± SE	χ^2
Spraying	JONE	24	0.103 (0.09-0.12)	0.16 (0.15-0.24)	5.5 ± 0.95	81.48*	0.06 (0.02-0.08)	0.21 (0.15-0.65)	2.26 ± 0.69	59.53*
		48	0.08 (0.06-0.09)	0.17 (0.09-0.16)	6.68 ± 1.44	81.13*	0.03 (0.0-0.06)	0.14 (0.09-1.91)	1.76 ±0.79	73.50*
		72	0.07 (0.05-0.08)	0.12 (0.09-0.17)	5.78 ± 1.38	77.5*	0.03 (0.0-0.06)	0.11 (0.07-0.30)	2.41 ± 0.1	76.71*
	JOBE	24	4.06 (3.28-5.36)	11.61 (7.90-25.18)	2.81 ± 0.52	63.24*	4.76 (3.55-8.06)	21.11 (11.02-66.86)	1.98 ± 0.46	37.5*
		48	3.09 (2.40-4.09)	11.14 (7.23-27.38)	2.30 ± 0.45	53.94*	3.48 (2.46-5.18)	15.46 (8.67-71.31)	1.98 ± 0.49	40.34*
		72	1.52 (0.93-2.03)	5.53 (3.9-11.19)	2.28 ± 0.49	64.88*	2.09 (1.36-2.84)	9.29 (5.85-27.33)	1.98 ± 0.45	50.65*
Dipping leaf	JONE	24	0.19 (0.14- 0.37)	0.81 (0.40-9.09)	2.05 ±0.6	29.55*	0.29 (0.22-0.45)	1.35 (0.74-5.21)	1.93± 0.40	42.9*
		48	0.16 (0.12-0.28)	0.73 (0.37-8.6)	1.95± 0.58	32.2*	0.19 (0.13-0.28)	1.16 (0.61-5.68)	1.63± 0.37	44.3*
		72	0.12 (0.08-0.17)	0.46 (0.27-2.32)	2.19± 0.62	37.63*	0.08 (0.02-0.13)	0.81 (0.40-9.36)	1.28± 0.39	49.35*
	JOBE	24	3.54 (2.78- 4.77)	12.26 (7.87-31.05)	2.37± 0.46	48.92*	3.97 (3.0- 6.05)	17.43 (9.66-73.37)	1.99 ± 0.45	40.57*
		48	2.67 (1.94-3.34)	6.43 (4.93-10.64)	3.35± 0.68	65.17*	2.65 (1.63-4.07)	17.53 (8.57-68.23)	1.56± 0.43	37.66*
		72	1.71 (1.09-2.27)	5.88 (4.16-11.66)	2.39± 0.5	58.81*	1.64 (0.65-2.50)	13.93 (6.80-70.12)	1.38± 0.42	43.47*

JONE: Jojoba oil-based nano-emulsion, JOBE: Jojoba oil bulk emulsion, CL: Confidence limit at 95%, SE: Standard error, χ^2: Chi-Square test. * indicate signification Chi-Square test at p-value ≤ 0.05.

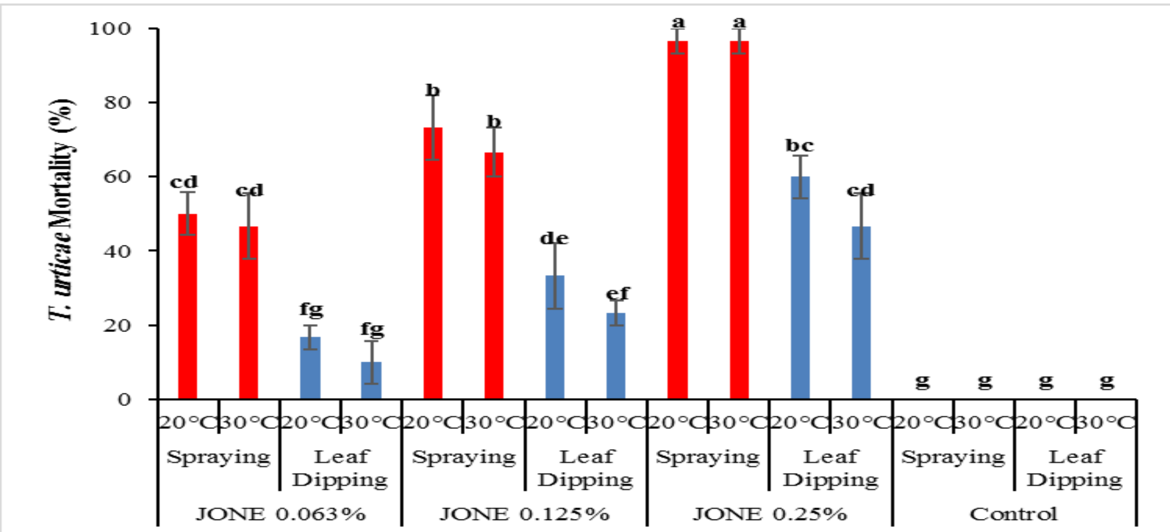

Fig 4. Effect of JONE on % *T. urtica* mortality after 24 hour of spraying and leaf dipping at 20 and 30°C

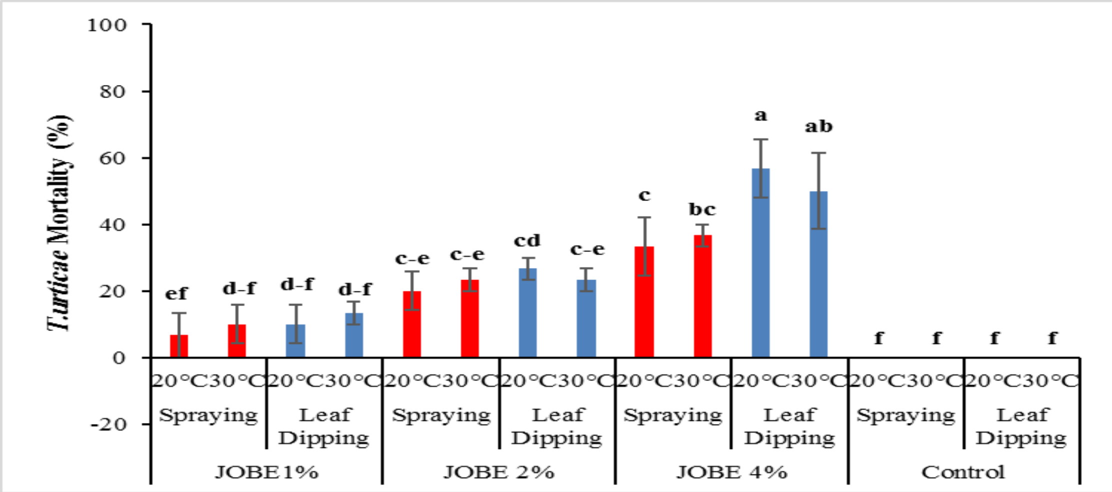

Fig 5. Effect of JOBE on % *T. urtica* mortality after 24 hour of spraying and leaf dipping at 20 and 30°C

of 56.7%±8.8 at 30°C after 24 h of spraying. The temperatures at the highest concentration in nano form and bulk form were not significantly different.

The Dipping Leaf method was effective in controlling mites by evaluating the residual effects of emulsions. After 24 hours of treatment, JONE had LC_{50} of 0.19 and 0.29%, while JOBE had an LC_{50} of 3.54 and 3.97%, at 20 and 30C, respectively. The mortality rate of *T. urticae* in JONE was (60±5.77 and 46.7±8.82%), and (56.7±8.8 and 50±11.5 %) in JOBE at the highest concentration at both temperatures **(Table 2** and **Figs 4** and **5).**

3.3 Insecticidal activity of JONE and JOBE

Results in **Table 3** demonstrated that JONE possesses insecticidal activity against *M. persicae*, using both methods. At an LC_{50} level and 20°C, the spraying method had a more toxic effect on *M. persicae* than that of the leaf dipping method **(Table 3).** JONE LC_{50} values were (0.23, 0.19, and 0.18 %) when applied by spraying at 20ºC, 24, 48, and 72 h post-treatment. On the contrary, the dipping leaf method resulted in JONE LC_{50} values of 0.53, 0.23 and 0.19 % respectively. According to JONE and JOBE LC_{50} values after 24 h of treatment, nano-emulsion had a toxic effect on nymph aphids 22.35 and 10.31 times greater than bulk emulsion in spraying and 14.6 and 20.1 times greater in leaf dipping, both at 20 and 30°C, respectively. Furthermore, it was noticeable that the slope value for the nano-emulsion on aphids and mites was extremely high and steeper. Thus, the mortality rate increases rapidly with any slight increase in concentration. Our results revealed that *T. urticae* and *M. persicae* were more susceptible to JONE than JOBE and it had persistence on the leaves.

On the other hand, the highest mortality % of aphid individuals were recorded at 53.3±6.67 and 50±5.77 %) at the highest concentration of 0.25 % of JONE after 24 hours (**Fig 6**). While the JOBE showed the highest mortality rate of (40±5.8 and 46.6±3.3 %) % at the highest concentration 4% (**Fig 7**).

Consequently, JONE possesses insecticidal and acaricidal effects toward *T. urticae* and *M. persicae* as an alternative to synthetic pesticides.

3.4 Biosafety of JONE and JOBE on the non-target organism

The toxicity of JONE and JOBE towards *Coccinella undecimpunctata* adults and the emergence rate of *Diaeretiella rapae* parasite from aphid mummies was determined, using the direct spray method.

For *C. undecimpunctata* adults, the impact of different concentrations of JONE and JOBE on the survival of the predator was shown in **Table 4**. After 24 h of spraying, the rate of predator survival was 80.0% ± 5.77 at a concentration of JONE 0.5% and 93.33% ± 3.33 at 8% JOBE. Therefore, JONE and JOBE were slightly toxic to the coccinellid beetles. Survival for *C. undecimpunctata* seems similar in treatments of JONE and JOBE when applied at concentrations of 0.063 and 0.125% in nano-emulsion and 1, 2, and 4% in bulk emulsion, where it recorded 100%. Thus, there was no side effect of JONE and JOBE on *C. undecimpunctata* adults when applied to control mites and aphids. The LC_{50} values of both formulations tested affect pests and do not affect their natural enemies as the relative

Table 3. Insecticidal activity of JONE and JOBE against *M. persicae* using spraying and dipping leaf methods at 20ºC and 30ºC

Method	Formulation (Jojoba oil)	Time (hour	20 ºC				30 ºC			
			LC_{50} (%) (95% CL)	LC_{90} (%) (95% CL)	Slope ± SE	χ^2	LC_{50} (%) (95% CL)	LC_{90} (%) (95% CL)	Slope ± SE	χ^2
Spraying	JONE	24	0.23 (0.17-0.32)	1.01 (0.59-3.08)	1.98 ± 0.39	48.45*	0.35 (0.27-0.51)	1.12 (0.70 -2.93)	2.53 ± 0.48	50.68*
		48	0.19 (0.14- 0.26)	0.81 (0.50 -2.19)	2.02± 0.39	65.06*	0.28 (0.21-0.39)	0.92 (0.59 -2.49)	2.49 ± 0.54	48.73*
		72	0.18 (0.12-0.25)	0.74 (0.46 -2.04)	2.08 ± 0.45	47.46*	0.15 (0.1-0.23)	0.94 (0.51 -4.50)	1.63 ± 0.39	44.4*
	JOBE	24	5.14 (3.59-10.1)	23.79 (11.41-28.66)	1.93 ± 0.55	29.96*	3.61 (2.65-5.60)	18.71 (9.74- 57.98)	1.79 ± 0.42	39.3*
		48	2.88 (1.55-5.55)	30.93 (11.05-40.65)	1.24 ± 0.42	33.23*	2.29 (1.59-3.09)	10.99 (6.64-36.11)	1.88± 0.42	49.68*
		72	2.25 (1.55-2.99)	8.81 (5.78 -21.97)	2.16± 0.46	47.70*	2.07 (1.47 -2.71)	8.54 (5.62 -20.95)	2.08 ± 0.43	56.2*
Dipping leaf	JONE	24	0.53 (0.33-1.81)	2.73 (1.05-59.19)	1.46 ± 0.40	26.85*	0.29 (0.22-0.47)	1.46 (0.77-6.46)	1.84 ± 0.4	41.3*
		48	0.23 (0.16-0.36)	1.55 (0.74-11.08)	1.54 ±0.37	40.73*	0.24 (0.18 -0.35)	1.14 (0.65-3.98)	1.90± 0.39	47.2*
		72	0.19 (0.13-0.28)	1.08 (0.59-4.57)	1.748 ± 0.38	46.27*	0.21 (0.15-0.31)	1.22 (0.64 -5.80)	1.67 ± 0.37	43.64*
	JOBE	24	7.74 (5.38-19.94)	30.26 (13.98-32.05)	2.17 ± 0.58	27.98*	5.83 (4.33-10.61)	22.36 (11.78-74.08)	2.2 ± 0.52	36.79*
		48	5.19 (3.4 -16.88)	48.01 (15.52-52.31)	1.33 ± 0.42	28.56*	3.45 (2.46-5.56)	20.83 (10.09-83.98)	1.64 ± 0.42	36.97*
		72	3.53 (2.13-7.84)	34.35 (12.12-38.08)	1.3 ± 0.43	30.32*	1.98 (1.04 -2.92)	13.78 (7.09-50.1)	1.52 ± 0.43	42.09*

JONE: Jojoba oil-based nano-emulsion, JOBE: Jojoba oil bulk emulsion, CL: Confidence limit at 95%, SE: Standard error, $\chi 2$: Chi-Square test. * indicate signification Chi-Square test at p-value ≤ 0.05

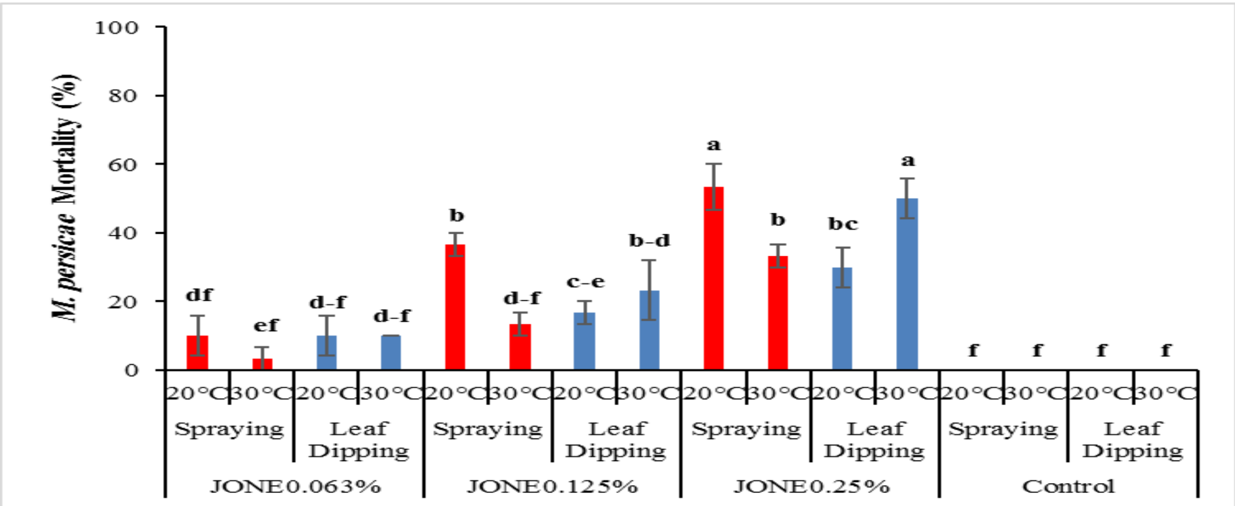

Fig 6. Effect of JONE on % *M. persicae* mortality after 24 hour of spraying and leaf dipping at 20 and 30°C

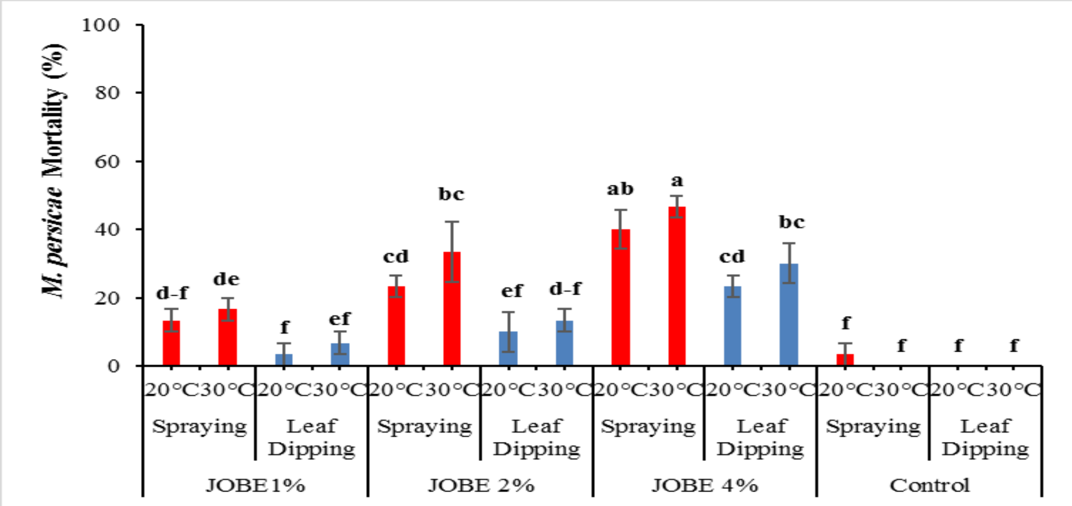

Fig 7. Effect of JOBE on % *M. persicae* mortality.after 24 hour of spraying and leaf dipping at 20 and 30°C The same letters shown on the column indicate that there are not significantly at according Duncan's multiple-range test, at a 0.05 probability level.

Table 4. Effect of JONE and JOBE on *C. undecimpunctata* adults and emergence *D. rapae* from aphid mummies using spraying under laboratory conditions

Formulations	Treatments	*C. undecimpunctata* adults Survival % (Mean ± SE)			Emergence *D. rapae* from aphid mummies Emergency % (Mean ± SE)
		1 DAY	2 DAY	3 DAY	7 DAY
JONE	0.063	100±0.0	100±0.0	100±0.0	100±0.0
	0.125	100±0.0	100±0.0	100±0.0	90±5.77
	0.25	93.33±3.33	90.0 ±0	86.67±3.33	86.67±3.33
	0.5	80.0±5.77	73.33±8.82	56.67±6.67	76.67±3.33
	Control	100±0.0	100±0.0	100±0.0	100±0.0
JOBE	1%	100±0.0	100±0.0	100±0.0	100±0.0
	2%	100±0.0	100±0.0	100±0.0	93.33±3.33
	4%	100±0.0	100±0.0	100±0.0	83.33±3.33
	8%	93.33±3.33	90± 5.77	83.33±6.67	76.67±3.33
	Control	100±0.0	100±0.0	100±0.0	100±0.0

Mean of three replications, SE: Standard Error, DAY after treatment.

toxicity to mites and aphids were (6.12, 2.74) and (2.25, 1.78) for JONE and JOBE, respectively after 24 h of exposure **(Table 5).** Finally, these results demonstrated the risk-free of jojoba oil-based nano-emulsion and bulk emulsion on coccinellid beetles. Hence, it was evident from the results that it is safe to spray plants infested with mites and aphids with JONE and JOBE without the loss of the natural enemy, *C. undecimpunctata* populations.

For aphid mummies, after 7 days of spraying, the effect of jojoba oil bulk emulsion (JOBE) and jojoba oil nano-emulsion (JONE) on the rate of *D. rapae* parasite emergence from aphid mummies was noticed under laboratory conditions. (**Table 4**). The concentrations of 0.063 and 1 % of JONE and JOBE showed the highest emergence 100% as in untreated control. While 0.5 and 8 % exhibited the lowest one 76.67 % emergence of the parasite. JONE and JOBE were slightly harmful to aphid mummies. Therefore, the two-form emulsions were considered safe formulations for biological control agents. To summarize the results we obtained, JONE was considered a promising eco-friendly bio-pesticide against aphids and mites.

Table 5. The LC_{50}, LC_{90} values (%) and relative toxicity of JONE and JOBE on *C. undecimpunctata* adults using spraying

Formulations	Time (hour)	LC_{50} (%) (95% CL)	LC_{90} (%) (95% CL)	Slope ± SE	χ^2	Relative Toxicity (of LC_{50} mite)	Relative Toxicity (of LC_{50} Aphid)
JONE	24	0.63 (0.47-1.29)	1.58 (0.94-25.05)	3.20±1.15	19.09*	6.12	2.74
	48	0.51 (0.38-0.74)	1.16 (0.79-4.72)	3.62±1.12	22.2*	6.38	2.68
	72	0.39 (0.31-0.48)	0.67 (0.54-1.05)	5.55±1.29	37.37*	5.57	2.17
JOBE	24	9.15 (7.81- 10.09)	12.33 (10.93-18.28)	9.89±3.02	27.68*	2.25	1.78
	48	8.23 (6.71-8.98)	10.45 (9.53-13.6)	12.35±3.85	35.02*	2.66	2.86
	72	6.89 (0.11-8.15)	9.78 (8.39-38)	8.43±4.04	39.49*	4.53	3.06

JONE: Jojoba oil-based nano-emulsion, JOBE: Jojoba oil bulk emulsion, CL: Confidence limit at 95%, SE: Standard error, Relative Toxicity= LC_{50} of Coccinellid beetles / LC_{50} Mites or Aphids, χ^2: Chi Square test. * indicate signification Chi-Square test at p-value ≤ 0.05

The efficacy of JONE as bio-pesticides against the sucking pests, *M. persica*e and *T. urticae* was investigated. Jojoba oil-based nano-emulsion exhibited high effectiveness against these pests compared to that of bulk EOs, besides being safe for natural enemies. The physical characteristics of JONE indicate a better nano-emulsion as a good bio-pesticide for the commercial market. Physical properties contribute to the widespread and penetration of pests' cuticles and plant leaves. It has been reported that a more negative zeta potential gave a better nano-emulsion for pest control (Heydari et al 2020, Modafferi et al 2024). The size of the droplets is affected by the surfactant-to-oil ratio (Pavoni et al 2019, Perumal et al 2021). Nano formulations chemicals have the ability to increase the solubility of components and improve stability, leading to greater efficacy for pest control (Mustafa and Hussein 2020).

The application of conventional pesticides is no longer the best option for aphids and mites control due to many of the problems associated with it, such as pest resistance, the side effects on non-target organisms, and environmental toxicity (Pavoni et al 2019). Nowadays, botanical pesticides have become a promising and an alternative strategy to conventional essential oils (EOs) have a variety of biological effects against different pests and are environmentally friendly. They can have insecticidal, acaricidal, anti-feeding and repellants activities (Moretti et al 1998, Nerio et al 2010, Sertkaya et al 2010, Elhalawany and Dewidar 2017, Lazarević et al 2020). The jojoba oil contains different active components such as; retinol, gibberellic acid, carvyl acetate, igernellin, linolenic acid and retinal (Shawer et al 2022). The Jojoba oil formulation showed effectiveness against the rice weevil, *Sitophilus oryzae* (Linnaeus) (Coleoptera: Curculionidae) and the red flour beetle, *Tribolium castaneum* (Herbst) (Tenebrionidae) and the grasshoppers (Soltan 2020, Shawer et al 2022). In contrast, these essential oils degrade rapidly when exposed to temperature and light. Not only it is unstable, but it is also weakly soluble in water (Regnault-Roger and Philogène 2008Martín et al 2010, Metwally et al 2022). However, the use of nano-emulsions is considered an excellent strategy, it increases the potency of essential oils and uses it as commercial formulations. Nano-emulsion-based essential oil improves the solubility of compounds in water, mobility, and decreases volatility. Besides having great stability within a wide temperature range (Jasrotia et al 2022, Martín et al 2010, Pavoni et al 2019).

In recent years, nano-emulsions of essential oil have been employed to control pests. Results revealed that JONE is highly effective against *M. persicae* and *T. urticae* compared to JOBE. The current study confirmed the efficacy of jojoba nano-emulsion against *M. persicae* and *T. urticae*. In addition, the nano-formulation of jojoba oil was more toxic than the conventional form against adults of *T. castaneum*. Besides, the toxicity of the nano-emulsion of jojoba oil against the rice weevil, *Sitophilus oryzae* has been investigated under laboratory conditions using wheat grain treated with the formulation (Sh et al 2015). Moreover, spraying jojoba oil-based nano-emulsion against *M. persicae* had a higher toxicity than leaf dipping. In the same context, direct contact with garlic oil-based nano-emulsion exhibited more efficacy against the 3rd instars of *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) than residual contact. The LC_{50} values were 0.248% and 0.782% for direct contact and residual, respectively (Modafferi et al 2024).

The present results are also in line with those of El-Shewy (2018) confirming that the nano-emulsion jojoba oil (LC_{50} of 0.381%) had a greater effect compared to crude jojoba oil (LC_{50} of 2.853%), on the 4th instar larvae of the black cutworm, *Agrotis ipsilon* (Hufnagel), that had been fed on treated leaves. Larval and pupal weight and number of deposited eggs/females of *A. ipsilon* decreased as well. Likewise, camphor oil nano-emulsion against 2nd instar larvae of the cotton leafworm, *Spodoptera littoralis* (Boisd.) was observed (Marouf et al 2021). Nano-emulsion of camphor oil was more effective against this pest, with an LC_{50} recorded at 88.67 ppm compared with 1699.85 ppm for bulk camphor oil. Fumigation by 1,8-cineole nano-emulsion had more toxicity against adult *T. urticae*, corn leaf aphid, *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae) and silver leaf whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), than those of free monoterpene treatments (Ayllón-Gutiérrez et al 2023). Another study conducted by Nuryanti et al (2020) demonstrated the toxicity of nano-emulsion of the java chili, *Piper retrofractum* essential oil against the brown plant hopper, *Nilaparvata lugens* (Stal) (Hemiptera: Delphacidae).

Interestingly, pest control chemicals must be selective towards non-target organisms. Conventional pesticides have a negative impact on natural enemies. The adverse side effects of pesticides on natural enemies involve mortality, lowered predation capability, and lowered parasitism rates (Passos et al 2022). The current study showed that JONE was harmless for both adults of *C. undecimpunctata* predator and parasitized aphid mummies. Consequently, JONE can be utilized for control as an alternative to the synthetic pesticide. Where it was appropriate with integrated pest management. In a similar finding, garlic oil-based nano-emulsion was effective against the *Planococcus citri*, (Risso) (Hemiptera: Pseudococcidae), it had no harmful effect on the predatory ladybird, *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae) (Modafferi et al 2024). Hexanal nano-emulsion did not exhibit harmful effects on the parasite *Trichogramma chilonis* (Ishii) and the predator *Chrysoperla carnea* (Stephens) at 0.04 %. The parasitization rate was 96.53 %, with an emergence rate of 85.05 % in the predator (Karthika et al 2015).

As such, nano-emulsions of essential oils are excellent promising alternatives that will be used in the pest control program in the future. They are considered a new generation of bio-pesticides that respect the environment.

4 Conclusion

Jojoba oil-based nano-emulsion was highly effective against *M. persicae* and *T. urticae.* Its unique physical characteristics make it an excellent natural pesticide as a commercial product for the market. Nano-emulsion of jojoba oil was proved to be risk-free on natural enemies. Thus, JONE is considered a safe alternative to conventional pesticides. However, the safety of these nano-emulsions for non-target organisms must be confirmed through further studies in addition to evaluating their efficiency on many other pests of economic importance under field conditions. Trials using green surfactants rather than Tween 80 and Triton X- 100 are considered in an approach to making an entirely green pesticide.

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