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Ultrasound assisted formation of essential oil nanoemulsions: Emerging alternative for *Culex pipiens pipiens* Say (Diptera: Culicidae) and *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) management.

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ABSTRACT

Over the last years, nanotechnology has contributed to the development of new botanical insecticides formulations based on essential oils (EO), which are safe for the human health and the environment. Nanoemulsions (NEs) can enhance the bioactivity of the EO to prevent the premature volatility and degradation of the active ingredients. In our work, geranium EO (*Geranium maculatum* L.) was used to develop micro and nanoemulsions adding Tween 80 as surfactant. For NEs formulation, ultrasound was applied and the physicochemical and ultrasound parameters were optimized: oil: surfactant ratio = 1:2, ultrasound power = 65W, sonication time = 2 min, cycles = 30 on/ 20 off and ultrasonic probe distance = 3.7 cm. The NEs obtained had 13.58 nm and polydisperse index (PDI) values of 0.069. They were stored at 25°C and were stable for 60 days.

The present study also demonstrated the potential of NEs to enhance the toxicity of geranium EO against larvae of *Culex pipiens pipiens* (EO LC_{50}= 80.97 ppm, NEs LC_{50}= 48.27 ppm) and *Plodia interpunctella* (EO + β-cypermethrin LD_{50}= 0.16 µg $larvae^{-1}$, NEs + β-cypermethrin LD_{50}= 0.07 µg $larvae^{-1}$). Overall, our findings pointed out that NEs can increase twofold the insecticidal efficacy of EO, and thus, they can be considered further for the development of botanical insecticides.

Keywords

Indian meal moth; Mosquito; Ultrasound; Geranium EO; Nanoemulsion; Stability

1. INTRODUCTION

In plant–environment interactions, essential oils (EO) play important roles, such as plant–plant communication, self-defense, and attraction of pollinators (1). EO are liquid mixtures constituted by secondary metabolites, like terpenoids and phenylpropanoids as the main compounds. These major constituents are volatile, lipophilic and have a low molecular weight (2). Different works have demonstrated that EO show insecticidal, bactericidal, fungicidal, anti-inflammatory, antitumor and anti-carcinogenic properties, among others (3). EO are an excellent alternative to synthetic insecticides since they lessen the negative effects on human health and the environment. Consequently, they become a complementary method for integrated pest management strategies (4) Nevertheless, some characteristics present in EO, such as instability, volatility and low solubility in water, may limit their applications (5). Therefore, an applicable EO formulation that responds to these aspects is needed for commercial use (6).

One of the most auspicious innovations in food industry has been the encapsulation of different products (7). Encapsulation is a process in which active compound droplets are enclosed in a heterogeneous or homogeneous matrix to create small particles (8). In fact, this technique could be incorporated as a possible solution against EO problems, such as evaporation or degradation.

Different types of proteins, polysaccharide and synthetic emulsifiers have been used to nanoformulate the EO or their constituents (9). Over the last few years, the nanoformulation technology appears as one of the most advisable approaches, in particular for the development of Microemulsion (MEs) or Nanoemulsions (NEs) (10)

MEs are defined as homogeneous thermodynamically stable isotropic solutions with low viscosity (5). Surfactants and co-surfactants play an important role, since they provide MEs with stability. NEs are colloidal delivery systems, which can be produced from both low-energy and high-energy methods. High energy methods use a high pressure homogenizer, ultrasonicator or high shear homogenizer (11). Ultrasonication waves provide the energy to produce intense disruptive forces that break up the oil and water phases to produce tiny oil droplets (12). For this reason, the use of this technique is very frequent due to energy-efficiency, low production cost, ease of system manipulation and better control over formulation variables of ultrasound (13).

Accordingly, the scientific community focuses attention on the development of new strategies for pest control (14, 15, 16, 17). In order to evaluate the biological activity of EO and their nanoformulation, two insect pests were selected: *Culex pipiens pipiens* Say (Diptera: Culicidae), which is one of the main mosquito vectors of lymphatic filariasis caused by *Wuchereria bancrofti* and other important arbovirus (18), and *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae), which is a cosmopolite moth and one of the most serious pests affecting food industry. These larvae infest many different food products, such as cereal, seeds, nuts, dried fruits, and produce important economic losses (19). In previous studies in our laboratory, Jesser et al. (20), Mostefuscoli et al. (21) and Werdin González (22) showed that geranium EO was one of the most toxic products against *P. interpunctella* and *Cx p. pipiens.*

In the search to develop a new tool for these insects management, herein we present a method to elaborate eco-friendly nanoformulation based on geranium EO, tending to a)

reduce the surfactant concentration, b) increase the EO level and c) stabilize the nanoformulation over time. The ultrasonic technique could be an important contribution to achieve these goals.

2. Materials and Methods

2.1. Materials

The geranium essential oil was purchased from Swiss-Just (manufactured under supervision and control of Ulrich Justrich AG, Walzenhausen, Switzerland). Emulsifiers (Tween 80 and Triton x-100) and β-cypermethrin were procured from Sigma-Aldrich Commercial. Analytical grade Acetone and Ethanol (Dorwill, Argentina) were used as solvent and co-surfactant respectively.

2.2. Insects

The mosquito larvae were collected from a water stagnated area. The species identification was conducted at the Laboratorio de Zoología Invertebrados II, UNS, Argentina, and it was identified as *Cx p. pipiens*. The larvae were maintained under suitable temperature for acclimatization

Colonies of *P. interpunctella* were kept without exposure to any insecticide in a growth chamber at 27±1 °C, 45-50% r.h. (relative humidity) and 16:8 h L:D photoperiod. Colonies grew in plastic containers (13 cm diameter × 30 cm high), covered by a fine mesh cloth for ventilation. Each one contained a mixture of maize flour, wheat flour, powdered milk, honey and glycerin of analytical grade (2:1:1:1:1 w/w).

2.3. Preparation of micro and nanoemulsion (MEs / NEs)

The MEs were formulated by adding 25 mL of double distilled water to a mixture of geranium EO, emulsifier (Tween 80) and ethanol in different ratios. The mixture was stirred at 700 rpm for 30 min (conventional method).

For the NEs elaboration, different ratios of geranium EO and emulsifier (Tween 80) were mixed, and 25 mL of double distilled water were added. The coarse emulsion was stirred at 700 rpm for 2 min and put into a plastic container (3 cm diameter x 50 cm high) (Fig. 1). Thereafter, it was sonicated using a Sonics Vibra cell, VCX 130 with a titanium probe tip (9.5 mm diameter, 130 W nominal power, 20 kHz frequency). The ultrasound was maintained at 65 W, the cycles were 30 on/20 off and the sonication time was 2 min.

2.4. Droplets sizes

The average droplets sizes, polydispersity index (PDI) of the MEs and NEs were measured by dynamic light scattering (DLS) using a Zetasizer nano instrument ZEN 3690 model (Malvern, UK). Each measurement was accomplished at room temperature three times.

2.5. Emulsion stability

To study the stability of the emulsion during the first 72 h, changes in the droplet size and the visual appearance were observed. However, to evaluate the stability over time, the same variables were considered adding potential zeta variable. During this test, the samples were maintained at 25 °C and were checked one time a week. Three replicates of each measurement were carried out.

2.6. Larvicidal assays

***2.6.1. Cx p. pipiens* bioassays**

Bioassays were performed according to WHO (23) with fourth instar larvae of *Cx p. pipiens*. All experiments were performed in quadruplicate with 20 fourth instar larvae in

each replicate (n= 80). Geranium essential oil was mixed in tap water containing Tween 80 (1% p/v). The NEs were diluted in tap water alone. The concentration ranged from 10 to 250 ppm. Two different controls were used, one without treatments and another using the surfactant alone (in the corresponding ratio). The biological assays were performed at 27±2 °C, 45-50% r.h. and 16:8 h L:D photoperiod. After a 24 h exposure, the mortality level was recorded in order to obtain LC_{50} using SPSS 25.0 statistical software. The LC_{50} values were considered significantly different if their 95 % confidence intervals did not overlap.

2.6.2. P. interpunctella bioassays

To evaluate the combined effect of geranium EO or NEs and β-cypermethrin, aliquots of each product were topically applied to batches of 10 *P. interpunctella* larvae (early fourth instar). A pre-treatment was carried out using a sublethal dose of the EO (20 µg $larva^{-1}$) or the NEs at an equal dose. Previous studies elucidated that when the water evaporates, the nanoemulsion retains its properties (24, 25). After a 2 h pre-treatment, acetonic solutions of β-cypermethrin were applied in doses ranging from 0.006 to 4 µg $larva^{-1}$. Acetone or surfactant alone (in the corresponding ratio) were topically applied as control. All experiments were performed in quadruplicate and the larvae were maintained at 27±2 °C, 45-50% r.h. and 16:8 h L:D photoperiod. Insect mortality was recorded after a 72 h exposure in order to calculate LD_{50} using the SPSS 25.0 program. The LD_{50} values were considered significantly different if their 95 % confidence intervals did not overlap.

3. Results and Discussion

3.1. Screening studies: Type of Surfactant and Microemulsions

Triton x-100 and Tween 80 were evaluated as surfactant in order to obtain geranium EO MEs using low energy methods. Different EO: surfactant ranges (from 5:1 to 1:5) were analysed. The addition of ethanol as co-surfactant was also studied. From this screening study, Triton x-100 was ineffective to form a stable nanosystem, while Tween 80 was capable of producing MEs only with the addition of ethanol (fig 2). These MEs with 1:4:0.001 (v/v) and 1:3:0.001 (v/v) ratios of oil: Tween 80: ethanol had a droplet diameter of 113 nm and 122.1 nm and PDI values of 0.388 and 0.397, respectively (table 1), and the macroscopic aspect was translucent (Figure 2 C). In recent works, some authors formulated EO such as geranium, eucalyptus, *Trachyspermum ammi, Crithmum maritimum* and *Pimpinella anisum* as MEs using Tween 80 and obtained similar results as in our study (5, 21, 26).

After these initial experiments, and in connection with the aims of this work, we have used ultrasound to reduce the surfactant concentration, maintaining or enhancing the stability of the system.

3.2. Nanoemulsion

3.2.1 Effect of ultrasonic energy

In order to determine the minimum possible droplet size (MPDS) of geranium NEs, the following ultrasonic parameters were studied: optimum power, sonication time, cycle and ultrasonic probe distance.

The determination of the optimum power is an important parameter in order to determine the MPDS, minimizing the energy loss and production cost at an industrial scale (27). To understand the effect of input power density on droplet size, the ultrasonic power settings were from 26W to 117W. The results showed that the optimum power value was 65W (table 2), which is a lower value than those reported in the literature (13, 28, 29). Typically, when the power applied is over the optimum one, with the aim of reducing the droplet size beyond the MPDS, an "over-processing" may be produced (in this instance, the droplet size was determined by the composition of the system, such as type of EO or surfactant, concentration of EO or surfactant, ratios, water concentration, in others)(30). Accordingly, applying more power beyond this point becomes unnecessary and unproductive since it consumes extra energy. Therefore, the maximum power for further experiments was fixed at 65W.

A longer sonication time produces a smaller droplet size (31). Nevertheless, after reaching the MPDS at the optimal time, the size does not change. Moreover, the over-processing may degrade the bioactive compounds (27). In this work, the time range analysed was from 2 to 5 min, and our results indicated that the optimal sonication time was 2 min (table 2).

Another important factor was the cycles, since the "on/off" pulse change the droplet size. During the pulse ''on'' time, cavitation bubbles grow, but during the pulse ''off'' time, they reduce (32). The effect of the cycles in the production of geranium EO nanoemulsion was evaluated using pulses from 20 on/30 off to 59 on/1 off (table 2). The assay showed that 30 on/20 off pulses was the optimum cycle for reaching the MPDS.

However, beyond this cycle value, the droplet size increases and the bioactive compounds may be degraded (32). Hence, for the rest of the experiments, the ultrasonic cycle was fixed at 30 on/20 off.

The optimum ultrasonic probe distance is the one between the bottom of the container and the ultrasonic probe at which MPDS could be achieved. In this work, the ultrasonic probe distance was varied from 4.7 cm to 3.7 cm. The experiments showed that 3.7 cm was the optimum ultrasonic probe distance (Fig. 1).

3.3. Effect of surfactant concentration

Table 3 shows the effect of the surfactant concentration on the NEs size. At all ratios, when the emulsions were formed using ethanol, the sizes were higher than 500 nm with a narrow size distribution (PDI <0.1), and the macroscopic aspect was white (Fig. 2 E.). The visual appearance depends, basically, on the droplet size and their interaction with visible light. It is known that when the dispersive phase diameter is lower than 20 nm, the system is transparent and there is no interaction. When diameter is higher, the particles can interact with the light producing optical effects (30).

In the case of systems elaborated without ethanol, the droplet size increased when the surfactant concentration was reduced, affecting its macroscopic aspect. Saberi et al. (33) demonstrated that when emulsion are elaborated, the ethanol concentration play an important role in formation of NEs, since increase of ethanol concentration produce an increase in droplet diameter size and PDI value.

Whitish emulsions were formed using ratios from 0.5:1 to 1:1 oil-surfactant. These emulsions showed dispersed phase diameters in the range of 79.41 to 106.4 nm, and had high PDI values (>0.2) (Fig. 2 D). The emulsions formulated with 1:1.5 oil-surfactant ratio have a translucent macroscopic aspect (Fig. 2 B), with an average size of 55.9 nm, and were found to be bimodal (peak 1 at 316.9 nm and peak 2 at 15.20 nm), with a broad droplet size distribution (PDI = 1). All these emulsions were not stable after three days.

Finally, transparent NEs were achieved using 1:2 oil-surfactant ratios. These nanosystems measured 13.58 nm, their PDI values were 0.069 and were stable after three days (Fig. 2 A).

During emulsification, the surfactant concentration plays a critical role in the reduction of the droplet size, since the surfactant reduces the interfacial tension at oil/water interface (30). This idea could explain our observation where the increase in the oil-surfactant ratio from 1:0.5 to 1:2 reduces the droplet diameter size. Moreover, the change in the colour of the emulsions from white to transparent is also due to a reduction in the droplet diameter size, which is smaller than visible light (34). Concerning the PDI values, the NEs elaborated using oil-surfactant ratio from 1:2 had low PDI values (<0.2). These measures indicate the uniformity of the droplets within the formulations that would resemble monodisperse stable systems (35).

3.4. Emulsion stability

The NEs (oil-surfactant ratio from 1:2) were analysed by monitoring the droplet size, the PDI and the potential Z values at 1, 30, 60 and 120 days of storage at 25°C. Figure 3 represents the NEs stability over time. As showed, for 60 days, no changes were observed in the particle size and the PDI, demonstrating excellent NEs stability with peak sizes around 14 nm and PDI values lower than 0.2. Furthermore, the percentage change in mean particle diameter was less than 10% for all samples. In addition, the negative potential Z values increased over 60 days of storage. This small enhancement of the negative charges could stabilize the NEs by electrostatic repulsion (36). At 120 days of storage, it was observed an increase in mean droplet size. The NEs showed two peaks at 14.41 and 247.1 nm, and a PDI value of 0.486. At this time, the potential Z showed a small decrease. Chang et al. (37) formulated NEs using thyme oil, water and tween 80, which were stable for 30 days. Furthermore, Duarte et al. (38) elaborated NEs with larvicidal effects using water, tween 20 and *Rosemarinus officinalis* oil. In this work, after 30 days of preparation, it was observed an increase in mean droplet size from 50.15 nm to 180.0 nm. These results represent a change in the mean particle diameter around 250%. The NEs produced in our work, maintained approximately the mean particle diameter for 60 days. Moreover, after 120 days of storage, NEs do not showed phase separation which is an important factor for most commercial applications. In this regards, larvicides based on EOs have been proposed as relative low coast insecticides compared with other botanical pesticides, such as pyrethrum and neem since their active ingredients are commonly used in cosmetic and food industry (39). In order to

obtain an EO nanoformulation for commercial use, further researches are needed to increase NEs stability.

3.5. Bioassays

Over the last years, advancements in nanotechnology have offered new tools for the use of EO as more environmentally friendly insecticides (40). Consequently, the insecticidal effects of the NEs elaborated in this work (oil-surfactant ratio from 1:2) were tested on *Cx p. pipiens* and *P. interpunctella* larvae.

The LC_{50} values for fourth instar larvae of *Cx p. pipiens* exposure to geranium EO and their NEs are shown in figure 4. For geranium EO, the LC_{50} value was 80.97 ppm (75.49 - 86.29) and for their NEs it was 48.27 ppm (44.09 - 53.39). Consequently, the NEs enhanced the insecticidal activity 1.67 times.

On the other hand, figure 5 show the insecticidal activity on *P. interpunctella* larvae using β-cypermethrin alone or EO or NEs combined with β-cypermethrin. LD_{50} value for β-cypermethrin was 0.89 μg $larvae^{-1}$ (0.72 - 1.08). In case of the EO + β-cypermethrin, the LD_{50} value was 0.16 μg $larvae^{-1}$ (0.12 - 0.19). The insecticidal activity of the EO + β-cypermethrin was 5.62 times higher than the β-cypermethrin alone. Finally, NEs + β-cypermethrin had a LD_{50} value of 0.07 μg $larvae^{-1}$ (0.02 - 0.11). In particular, NEs + β-cypermethrin showed a strong insecticidal activity with respect to the β-cypermethrin alone (12.23 times). In addition, NEs enhanced 2.17 times the insecticidal activity of geranium EO.

Several EO have demonstrated to affect metabolism, physiology and behaviour of insects (40). The EO produce neurotoxic effects and their mode of action include the inhibition of acetylcholinesterase (AChE) and the block of cholinergic system and octopaminergic and GABAergic system (42, 43, 44, 45). Different works showed that EO have insecticidal activity against *Cx p. pipiens* (21, 46, 47) and *P. interpunctella* (20).

Recent studies demonstrated that EO enhances the toxicity of various synthetic insecticides (48, 49). The enhancement could be possible due to different mechanism of neurotoxic action, penetration increase of synthetic insecticides and inhibition of detoxified enzymatic systems (48). Norris et al. (50) showed that geranium EO interferes with detoxification processes on *Aedes aegypti*, leading to higher bioavailability of the topically applied pyrethroid. Hence it is possible that EO screened in this study act in a similar manner.

Accordingly to our observations, the NEs developed by ultrasound containing geranium EO and Tween 80 as emulsifier enhanced the biological activity of EO. Likely, the nanosize of the droplet in the NEs led to a higher EO penetration rate through the cuticle, which is a critical step for the insecticidal activity of any product (39). Moreover, NEs have other several advantages such as solubility and chemical stability. Besides NEs increase the affinity between EO particles and target organism and enhance the longevity (51). In addition, Hashem et al. (52) demonstrated that NEs could be producing an extensive damage in the cuticle and triggering histological damage.

As previously mentioned, the use of EOs in insect control is an alternative pest management method for minimizing the harmful effects of some synthetic insecticides

on the environment (53). EOs appear in nature and different works have demonstrated that they have only very low toxicities for non-target organisms and are safe for the environment (5, 43, 54, 55). Moreover, due to high volatility of EOs, it suggests that residue issues will be minimal when they are applied in the soil and aquatic ecosystem (39). On the other hand, to improve EOs solubility, solvents are needed to add on the formulation (56). Nevertheless the NEs elaborated in our work, are solvent free and based on surfactant with not ecotoxicity effects due to their high levels of biodegradability (57).

4. Conclusion

Nowadays, nanotechnology play a critical role in the development of more sustainable and ecofriendly insecticidal formulations. The present work firstly focused on the optimization process of ultrasound-assisted geranium oil nanoemulsions. The smallest and more stable NEs (average size = 13.58 nm, PDI = 0.069, transparent, stability = 60 days) were obtained when coarse emulsions (oil: surfactant ratio = 1:2) were treated at 65W for 2 min (30 on/ 20 off), and with an ultrasonic probe distance of 3.7 cm. This study also demonstrated that these NEs have the ability to enhance almost twofold the insecticidal activity of EO on *Culex pipiens pipiens* and *Plodia interpunctella* larvae. Lastly, the ultrasonic technique could be successfully used for the elaboration of geranium NEs with a high EO: surfactant ratio and high stability over time. These NEs can also be considered as highly promising formulation for the development of botanical

insecticides. Further studies are still needed to evaluate the ecotoxicological effects of these nanoemulsions.

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Table 1. Characterization of geranium EO formulations elaborated by conventional technique (N =3).

Oil : Surfactant	Co-surfactant	Diam. Size (nm) ± SE [a]	PDI ± SE [a]	Stability after 72h	General properties
1:4	0.1% Ethanol	113.0 ± 37.39 **a**	0.388 ± 0.015 **a**	No	Translucent
1:3	0.1% Ethanol	122.1 ± 38.76 **a**	0.397 ± 0.018 **a**	No	Translucent

[a] SE = standard error

For each column, same letters indicate no significant statistical differences ($P < 0.05$)

Table 2. Characterization of ultrasound parameters studied for elaboration of geranium EO formulations.

Variables	Studied range	Optimum value
Ultrasound power	26W – 117W	65W
Cycles	20 on/30 off - 59 on/1 off	30 on/ 20 off
Time	2 min – 5 min	2 min
Ultrasound probe distance	3.5 cm – 5 cm	3.7 cm

Table 3. Characterization of geranium EO formulations elaborated by ultrasonic technique (N=3).

Oil : Surfactant	Co-surfactant	Diam. Size (nm) ± SE [a]		PDI ± SE [a]		Stability after 72h	Visual appearance
1:2	0.1% Ethanol	545.6 ± 13.00	**c**	0.102 ± 0.023	**d**	No	White
1:2	-	13.58 ± 2.30	**a**	0.069 ± 0.007	**b**	Yes	Transparent
1:1.5	0.1% Ethanol	562.4 ± 4.75	**c**	0.113 ± 0.012	**d**	No	White
1:1.5	-	55.92 ± 33.62	**b**	1 ± 0.0	**g**	No	Translucent
1:1	0.1% Ethanol	523.7 ± 53.63	**c**	0.020 ± 0.004	**a**	No	White
1:1	-	106.4 ± 44.53	**b**	0.277 ± 0.017	**e**	No	whitish
1:0.5	0.1% Ethanol	588.1 ± 73.323	**c**	0.097 ± 0.006	**c**	No	White
1:0.5	-	79.41 ± 44.30	**b**	0.386 ± 0.015	**f**	No	whitish

[a] SE = standard error.
For each column, different letters indicate significant statistical differences ($P < 0.05$)

Table 4. Average Diameter, Polydispersity Index (PDI) and Zeta Potential for Geranium nanoemulsions for 120 days of storage at 25°C. For bimodal samples the relative importance of the different component of the size distribution is also expressed (N=3).

Days	Average Diameter (nm) ± SE [a]		PDI ± SE [a]	Zeta Potential (mV) ± SE [a]
1	13.58 ± 2.30 **a**		0.069 ± 0.007 **a**	-3.98 ± 1.37 **a**
30	14.85 ± 3.54 **a**		0.089 ± 0.011 **a**	-4.49 ± 1.63 **a**
60	14.91 ± 4.75 **a**		0.109 ± 0.019 **a**	-4.76 ± 1.74 **a**
120	Peak 1= 14.41 ± 1.32 **a**	Peak 2= 247.1 ±21.74 **b**	0.486 ± 0.027 **b**	-4.36 ± 1.88 **a**

[a] SE = standard error.

For each column, different letters indicate significant statistical differences ($P < 0.05$)

Figure 1. Schematic representation of ultrasonication components.

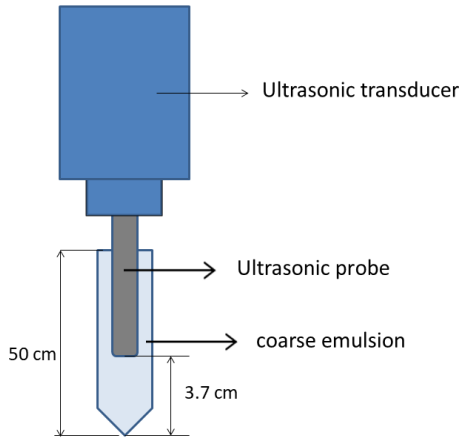


Figure 2. Visual appearance (column 1), size distribution (column 2) and auto-correlation function (column 3) of geranium EO micro-nanoemulsions (N=3).

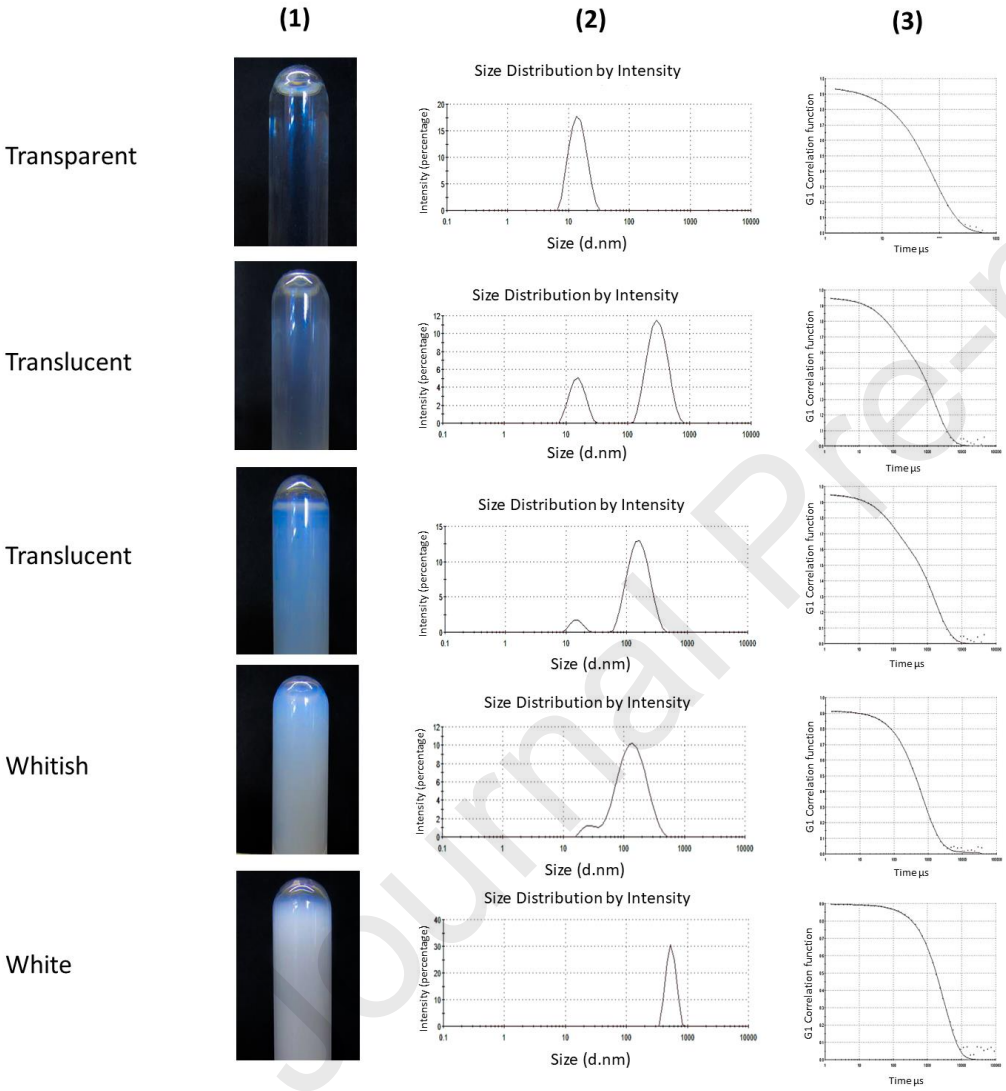
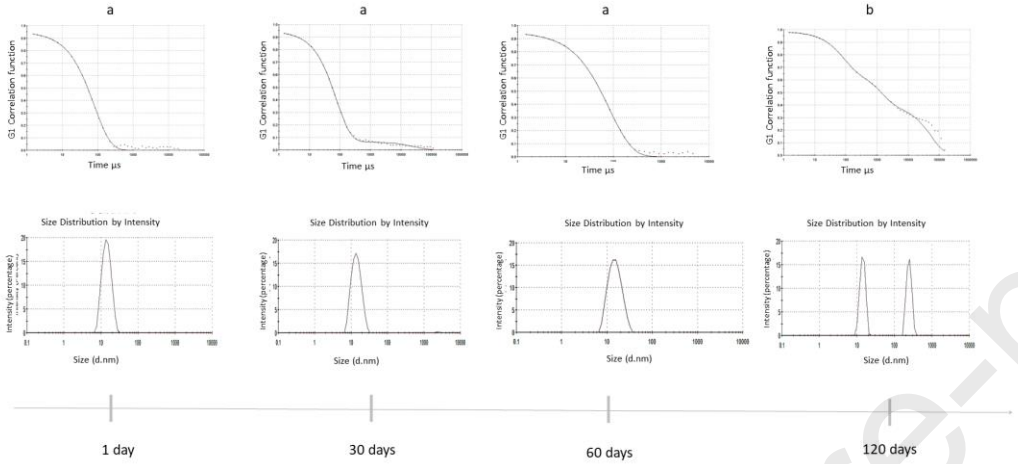
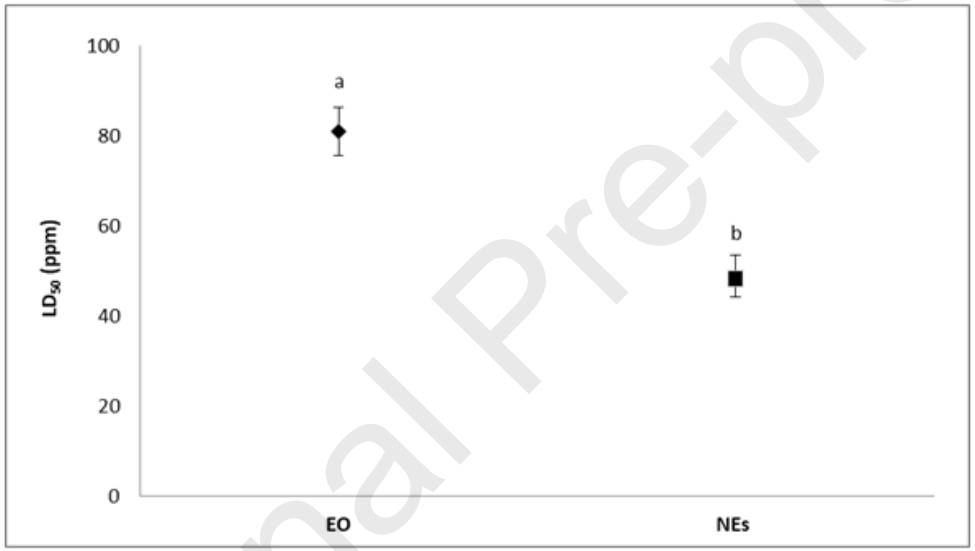


Figure 3: Geranium EO nanoemulsions stability during 120 days of storage at 25 °C expressed as function of autocorrelation function and size distribution (N=3).



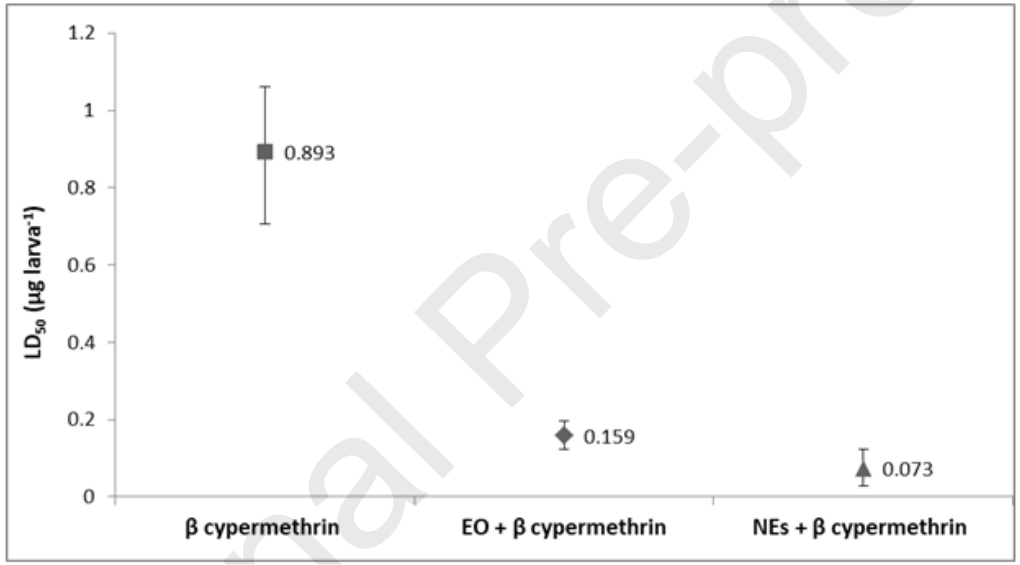
Different letters indicate statistical differences ($P < 0.05$)

Figure 4. Biological activity of geranium essential oil (EO) and their nanoemulsions (NEs) against fourth instar larvae of *Cx p. pipiens. (*N=4 replicates of 20 insects)



Different letters indicate significant differences between treatments (Cl overlap, $P < 0.05$).

Figure 5. Biological activity of β cypermethrin alone or combine with geranium essential oil (EO) and their nanoemulsions (NEs) against fourth instar larvae of *P. interpunctella.* (N=5 replicates of 10 insects)



Different letters indicate significant differences between treatments (CI overlap, $P < 0.05$).

Highlights

- Geranium essential oil nanoemulsions (NEs) were prepared using ultrasonication.
- Physicochemical and ultrasonic parameters were optimized.
- Geranium NEs were stable for 60 days at 25° C.
- Geranium NEs were effective to control mosquitoes and Indian meal moth.