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Insecticidal application of essential oils loaded polymeric nanoparticles to control German cockroach: Design, characterization and lethal/sublethal effects

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ABSTRACT

Essential oils (EO) from peppermint, palmarosa, geranium, lavender and rosemary were tested against the German cockroach, *Blatella germanica* L. (Blattaria: Blatellidae). Peppermint and palmarosa oils were the most effective and were included in a polyethylene glycol 6000 matrix to obtain EO loaded polymeric nanoparticles (EOPN). The physicochemical analyses indicated that, at 7 days postformulation, peppermint EOPN had sizes of 380 nm, the loading efficiency (LE) was 72.25% and the polydispersity index (PDI) was > 0.4 (polydisperse sample). Palmarosa EOPN had sizes of 191 nm; LE was 89.75% and PDI was < 0.25 (monodisperse sample). Peppermint and palmarosa EOPN enhanced the lethal and sublethal effects of the EO on *B. germanica*. These results suggest that the newly developed nanoinsecticides could be successfully used to control German cockroach.

1. Introduction

The German cockroach, *Blatella germanica* L. (Blattodea, Blattellidae), is a synanthropic and cosmopolitan pest, that causes allergic reactions in sensitive people (Yeom et al., 2018). These cockroaches also act as mechanical vectors of microorganism from the genus *Escherichia*, *Entamoeba*, *Giardias* and others (Dietrich et al., 2014; Werdin González et al., 2016). The overuse of synthetic insecticides like organophosphates and pyrethroids to control *B. germanica* populations has led to serious problems including impacts to non-target organisms, pest resistance and contamination of terrestrial and aquatic environments (Campos et al., 2018; Kah et al., 2018; Yeom et al., 2018). Development of eco-friendly strategies have been considered around the world to replace these conventional pesticides. Consequently, bioinsecticides based on essential oils (EO) appear to be a suitable method to insect pest control (Campolo et al., 2017; Mossa, 2016; Yeom et al., 2018). The EO and their active components are selective, biodegradable and they lessen the negative effects on animals and environment of synthetic insecticides (Bedini et al., 2018; Hashem et al., 2018; Karan et al., 2018). It has been postulated that these products could be used in a small-scale to treat cockroaches infesting human dwellings and workplaces (Alzogaray et al., 2013). In addition to the lethal effects (caused by contact, topical, fumigant or ingestion exposure), EO and their major components can affect insect's nutritional physiology and behavior. (Plata-Rueda et al., 2018; Taban et al., 2017; You et al., 2015). Several investigators have reported the lethal and sublethal effects of EO and their constituents against German cockroaches (Neupane et al., 2019; Ntalli et al., 2019, 2016; Yeom et al., 2018).

Factor such as low water solubility, high volatility and rapid oxidation of EO constituents affect their biological activity and its persistence, decreasing their potential to use in large-scale application (Campos et al., 2018; Mossa, 2016; Werdin González et al., 2017).

Polymeric nanoparticles design is a novel area for new bioinsecticides technologies. The incorporation of EO into a controlled release polymeric matrix will prevent the rapid degradation and evaporation of EO constituents, increase their toxicity and shelf life and enhance the handling (Athanassiou et al., 2018; Iavicoli et al., 2017; Prasad et al., 2017). Polymeric nanoparticles can be produced with natural, synthetic and semisynthetic polymers (Pascoli et al., 2018). In this work, polyethylene glycol was used as polymeric matrix due to its wide range of

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solubility, relatively non-toxic, biodegradability and readily producible (Athanassiou et al., 2018; Danprasert et al., 2003).

The goal of this work was to formulate and characterize EO loaded polymeric nanoparticles and to evaluate their lethal and sublethal effects against adult males of *B. germanica*.

2. Materials and methods

2.1. Insects

The insects were obtained from a susceptible strain maintained in the Laboratorio de Invertebrados II, Universidad Nacional del Sur, since 2002. *B. germanica* colonies were reared in glass jars (10 cm diameter × 14 cm high) covered by a fine mesh cloth for ventilation. The cockroaches were fed with dog food as kibble (Purina Dog Chow, Nestle Argentina S.A). The colonies were maintained in a growth chamber at 27 ± 1 °C, 45–50% RH (relative humidity), and 16:8 h L: D photoperiod. For all bioassays, adults male were used, since males and females of *B. germanica* showed different susceptibility ratio.

2.2. Chemicals

Essential oils of peppermint (*Mentha piperita* L.), geranium (*Geranium maculatum* L.), palmarosa (*Cymbopogon martinii* (Roxb.) Wats), lavender (*Lavandula angustifolia* Mill.) rosemary (*Rosmarinus officinalis* L.) were purchased from Swiss-Just (manufactured under supervision and control of Ulrich Justrich AG, Walzenhausen, Switzerland). Polyethylene glycol 6000 (PEG 6000) were procured from Merck KGaA, Germany. Analytical grade Hexane (Dorwill, Argentine) was used as solvent.

2.3. Preliminary bioassays

To evaluate the contact toxicity effects of the EO against adult males of *B. germanica*, filter papers (8.5 × 7.5 cm) were treated with 0.5 mL of EO or solvent alone (control). The concentrations ranged from 50 to 600 μg cm^{-2}. The filter papers were air dried for 20 min in order to evaporate the solvent completely and then were introduced in glass vials (2 cm diameter x 9 cm high). Six insects were added and the glass vials were covered with a fine mesh cloth for ventilation. Four replicates were performed. Insect mortality was determined after 24 h. When no legs and abdominal movements were observed, insects were considered dead.

2.4. Essential oil loaded polymeric nanoparticles (EOPN): design and characterization

2.4.1. EOPN elaboration

EOPN were prepared using the melt-dispersion method according to Werdin-González et al. (2014) with modifications. Based on preliminary bioassays, peppermint and palmarosa oils were used to EOPN elaboration. 20 g of PEG 6000 were melted at 65 °C on a hotplate stirrer and then 2 g of each EO were added. The mixture was stirred using a Scilogex (D-500) Homogenizer for 10 min at 15,000 rpm. After that, the mixture was cooled at −4 °C for 45 min and completely ground in a refrigerated mortar box. Finally, the product was sieved using a stainless steel sieve (230 mesh), stored at 27 ± 2 °C in desiccators containing calcium chloride to prevent moisture absorption prior to testing. Each essential oil, was prepared in three batches daily.

2.4.2. EOPN size and loading efficiency

The EOPN powders were dispersed with distilled water and mean hydrodynamic diameter (Z-averages size) and polidispersity index (PDI) were determined using a dynamic light scattering (DLS) [Zetasizer nano-instrument ZEN 3690 model (Malvern, UK)] at 25 °C.

The loading efficiency (LE) was determined spectrophotometrically using EOPN powders dissolved in ethanol/water (3:1, v/v) [Shimadzu UV- 1203 photometer with the Ki netics-2-Program Pack P/N (206-62029-10; Shimadzu Corp., Kyoto, Japan)] (Werdin González et al., 2014, 2015). The LE was calculated using the equation:

LE (%) = (amount EO loading/ amount initial EO) x 100.

Each measure was repeated four times. The sizes, PDI and LE were recorded at 3 and 7 days post formulation.

2.4.3. Scanning electron microscopy

For the preparation of the samples, the EOPN dispersed in water were sonicated and a few drops of the samples were placed on a coverslip. Once the water evaporated, the samples were coated with a layer of gold using an Argon plasma metallizer. The images were visualized using a LEO EVO 40-XVP microscope, from CCT-CONICET-Bahía Blanca. The observation was made at a voltage of 10 kV and a magnification of 85.000x.

2.4.4. EOPN composition pre- and postformulation (3 and 7 days)

The chemical composition of each oil was determined by GC-MS. For oil extraction from the EOPN, 0.5 g of each sample were dissolved in 5 mL of distilled water and heated at 65 °C (palmarosa) or 75 °C (peppermint). When the mixture cooled, 4 mL of absolute ether were added to recollect the EO extracted. The compounds were identified comparing their retention indices (Kovats Indices) with those of known compounds and also comparing their mass spectra with those stored in the MS databases (NBS75K.L MS DATA). Relative percentage amounts were obtained directly from GC peak areas. GC-MS analyses were performed with a Hewlett-Packard 6890 chromatograph connected to a Hewlett-Packard 5972A mass spectrometer equipped with a capillary column (HP-5, 25 m × 0.25 mm, 0.25 μm film thickness). The carrier gas was helium with flow of 1 mL min^{-1}. The GC oven temperature was held at 50 °C for 2 min, programmed at 5 °C min^{-1} to 200 °C, then held at this temperature for 15 min. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35–350 amu. Ionization technique was EI. The temperature of the injection block was 280 °C. Each essential oil was diluted with ethyl ether to a concentration of 0.001 mg mL^{-1} (0.1% v/v) and 1 μL of that solution was injected in the GC-MS for the component analysis. A standard solution of n-alkanes was used in the same conditions to determine the Kovats Indices of each peak.

2.5. EOPN bioassays: lethal/sublethal effects on adult males of B. germanica

For all bioassays, EOPN from 7 days postformulation were used.

2.5.1. Contact toxicity assay

Similar method as 2.3 was used to evaluate the EOPN contact toxicity effects. The products were directly applied on the filter papers at equal concentration for the EO (no solvent was used). For control, the filter paper were treated with PEG 6000 processed as described in section 2.4.1 without EO addition. After 24 h exposure, the insect response was determined.

2.5.2. Nutritional physiology effects

In order to establish the activity of EOPN in the nutritional physiology, flour discs were elaborated by a mixture of flour, distilled water and EOPN (at doses of 2 and 4 mg EO $disc^{-1}$). Aliquots of 0.2 mL of this mixture were placed in glass plaque and dried for 18 h at 25 ± 1 °C, 60–70% RH.

To evaluate the physiological activity of EOPN, positive and negative controls were used. As positives controls two treatments were studied using the free EO. One, named residual, consisting of a flour disc (elaborated with flour and distilled water), which were treated with hexanic EO solution at doses of 2 and 4 mg EO $disc^{-1}$ and dried for 18 h. This control was used to compare the biological activity of EO

residues with EOPN. Another positive control, named acute, consisted of flour discs which were treated with hexanic EO solution at the same doses and dried for 15 min. This control was used to study the potentiation of biological activity caused by the EOPN. As the negative control two treatments were used; one consisted of flour discs treated with hexane alone (named hexanic) and another elaborated with PEG 6000 (named PEG 6000).

To perform the bioassay, two discs were weighed and placed in a plastic container. Six pre-weighed insects (after a 24 h starvation period) were added and maintained in controlled conditions. Each treatments and control were evaluated with four replicates.

After 24 h, the weight of discs and surviving insects were recorded. Insect mortality was also recorded. Using this data, the nutritional indices and antifeedant/phagostimulant effect were calculated using the formulas:

$$\text{Relative growth rate (RGR) (mg mg}^{-1}\text{ day}^{-1}\text{)} = (A - B)/B \text{ x day}$$

$$\text{Relative consumption rate (RCR) (mg mg}^{-1}\text{ day}^{-1}\text{)} = D/B \text{ x day}$$

where A is the final mean biomass of surviving insects, B is the initial mean biomass of insects, D is the mean biomass ingested divided by the number of surviving insects after the bioassay.

$$\text{Efficiency of conversion of ingested food (ECI) (\%)} = (\text{RGR/RCR}) \text{ x } 100$$

$$\text{Feeding deterrence index (FDI) (\%)} = (T - C)/C \text{ x } 100$$

where T is the mean consumption of treated discs and C is the mean consumption of control discs.

2.5.3. Behavioral repellency effects

Behavioral bioassays were performed by half-treated arena (choice test). Half-filter paper discs (16 cm diameter) were treated with EO hexanic solution or with EOPN at LC_{50} concentration; the other half were untreated. The experimental arena was delimited with a PVC ring to prevent insect escape. Two cockroaches were left during 20 min in the experimental arena, where they were released in the untreated zone. The movement of each insect was recorded using a Panasonic video camcorder (SDR-S7P) and analyzed by video tracking system (described in Supplementary material S1). At 0, 3, 6, 12, 24, 36, 48, 60, 72, 84, the papers were used up to filming sessions. Controls were treated with hexane or with PEG 6000 processed as described in section 2.4.1 without EO addition. For each product and controls, four replicates were evaluated. The measurements taken with the tracking system included total time (s), walking speed (m s^{-1}), stop walking (times), moving time (s) and immobile time (s) in each half of the arena. Mortality was observed during the behavioral bioassay.

2.6. Statistical analyses and nutritional indices

Data for EOPN sizes, LE and behavioral measurements were analyzed by parametric Students t-test for independent samples. Nutritional indices (RGR, RCR and ECI) were analyzed by ANOVA and Least Significant Different (LSD).

To analyze the antifeedant/phagostimulant effect a series of FDI ranges were standardized. FDI values from −15 to 15: neutral; −15 > FDI > −45: slightly antifeedant effect; −45 > FDI > −75: moderate antifeedant effect; FDI < −75: highly antifeedant effect; 15 < FDI < 45: slightly phagostimulant effect; 45 < FDI < 75: moderate phagostimulant effect; FDI > 75: highly phagostimulant effect.

To determine the repellent effects of the products, a preference index (PI) was used: PI= (AI - AII)/(AI + AII), where AI is total time that insects remain in the untreated area and AII, in the treated area. Values higher than 0.1 indicate that the product generates repellency; values lower than −0.1, attractancy and values between −0.1 and 0.1 are included in the neutral zone (Benzi et al., 2009).

Table 1
Contact toxicity effect of EO against adult males of *B. germanica*.

Essential oil	LC_{50} (μg cm^{-2})[a]	C.I.	NOEC (μg cm^{-2})[b]
Peppermint	245.95 **a**	213.62–277.81	50
Palmarosa	246.00 **a**	207.61–284.11	50
Geranium	321.96 **b**	285.93–363.72	100
Lavender	433.00 **c**	363.92–496.81	200
Rosemary	Not calculated		600

[a] LC_{50} values showing different letter present significant differences (NSCI, $P < 0.05$).
[b] NOEC (No Observed Effect Concentration) values showing the highest tested concentration for which there are no significant statistical differences compared to the control group.

The lethal concentration (LC_{50}) was used to compare the lethal effects of the EO and EOPN in contact toxicity assays. LC_{50} values were calculated with their respective confidence intervals (CI) 95% using SPSS 15.0 statistical software and were considered significantly different if CI values did not overlap. After estimating the LC50 values, cockroach were exposed to a no observed effect concentration (NOEC) of EO (25–600 μg cm^{-2}). This bioassay was used to confirm that the concentrations were sublethal.

3. Results

3.1. Preliminary bioassays

The insecticidal activities of EO against adult males of *B. germanica* in contact bioassay are shown in Table 1. No mortality was observed in the controls. The dose mortality responses of each EO are shown in supplementary data (S2).

Peppermint and palmarosa oil demonstrated the strongest insecticidal activity in contact toxicity bioassay with LC_{50} value of 245.95 and 246.00 μg cm^{-2}, respectively. Significant differences were found between these EO and geranium (321.965 μg cm^{-2}) ($P > 0.05$). Lavender oil produced less insecticidal effect than peppermint, palmarosa and geranium oils ($P < 0.05$). Rosemary oil did not produce mortality even at the highest concentration, so its LC_{50} value was not calculated.

The toxicity order (toxic to least toxic) was peppermint = palmarosa > geranium > lavender > rosemary.

Based on these results, peppermint and palmarosa oils were further evaluate for EOPN elaboration.

3.2. EOPN design and characterization

EOPN were elaborated by the melt-dispersion method using polyethylene glycol 6000 (PEG 6000) as matrix system in PEG 6000: EO ratio 10: 1.

When the average sizes of both EOPN were analyzed, no significant differences were observed between 3 and 7 days postformulation ($P > 0.05$) (Table 2). For each postformulation time, peppermint EOPN sizes were significantly higher than palmarosa EOPN ($P < 0.05$). DLS plot demonstrated that peppermint EOPN were polydisperse (PDI values > 0.61) whilst palmarosa EOPN were monodisperse (PDI values < 0.16) (Fig. 1 A and B). SEM images show that peppermint EOPN were irregular with heterogeneity sizes while palmarosa EOPN were circular with uniform sizes.

For peppermint EOPN, at 7 day postformulation, a significant drop in EO content was observed, from 94 to 72% ($P < 0.05$). For palmarosa EOPN, even a slight drop was observed, no significant differences were detected between samples ($P > 0.05$). When LE value were compared between peppermint and palmarosa EOPN, for each postformulation time, significant differences were observed only at 7 days ($P < 0.05$) (Table 2).

Table 2
Size (hydrodynamic diameter in nm), polydispersity index (PDI) and loading efficiency (LE in %) of the peppermint and palmarosa EOPN elaborated, at 3 and 7 days postformulation. N = 3 replicates.

EOPN	3 days postformulation			7 days postformulation		
	Size	PDI	LE	Size	PDI	LE
Peppermint	335 ± 38 **aA**	0.47 ± 0.015	93.75 ± 0.7 **aA**	310 ± 14 **aA**	0.61 ± 0.022	72.3 ± 1.6 **bA**
Palmarosa	167 ± 3 **aB**	0.21 ± 0.011	94.5 ± 3.1 **aA**	203 ± 8 **aB**	0.16 ± 0.012	89.7 ± 2.5 **aB**

Mean values ± SE differ at $P < 0.05$ (Students t-test). For the same EOPN, different minus letters in the same variable indicates significant differences between postformulation times. For the same postformulation time, different major letters in the same variable indicates significant differences between EOPN.

The chemical analysis showed that in peppermint EOPN, menthol was the major component in pre- and postformulation samples. At 3 days postformulation several minor components (such as α- and β-pinene and limonene) were not determined. After 7 days, eucalyptol, *iso*-pulegol and pulegone could not be registered. For palmarosa EOPN, all components were maintained equally at 3 and 7 days postformulation (Table 3).

Table 3
Chemical composition and percentage content of the peppermint and palmarosa EOPN (preformulation, 3 and 7 days postformulation).

RT (min)	Component	Preformulation	Postformulation	
			3 days	7 days
Peppermint				
7.32	α- pinene	1.42	–	–
8.60	β -pinene	1.52	–	–
10.21	Limonene	3.26	–	–
10.28	Eucalyptol	5.69	0.52	–
19.80	Isopulegol	0.69	1.05	–
14.03	Isomenthone	16.90	5.29	6.95
14.35	p-menten-3-ona	10.03	7.69	7.57
14.60	Menthol	52.51	81.85	81.37
16.61	Pulegone	0.83	2.00	–
18.04	Menthol acetate	7.15	1.60	4.11
Palmarosa				
12.49	Linalool	2.55	3.08	2.69
16.41	β -citronellol	9.94	9.68	12.42
17.11	Geraniol	77.07	77.50	76.38
20.49	Geranyl acetate	4.58	5.58	6.30
21.41	Cariophyllene	5.86	4.16	2.30

3.3. EOPN bioassays: lethal/sublethal effects on adult males of *B. germanica*

3.3.1. Contact toxicity assay

When the most toxic oils, peppermint and palmarosa EO (Table 1), were encapsulated in a polymeric matrix, an enhanced insecticidal activity was registered. Peppermint EOPN increased 8 times the EO insecticidal activity with LC_{50} values of 31.43 μg cm^{-2} (24.222–38.998). On the other hand, palmarosa EOPN enhanced 10 times the EO activity with LC_{50} values of 25.41 μg cm^{-2} (20.669–30.196). Even though, no significant differences were detected between EOPN insecticidal activities ($P > 0.05$).

3.3.2. Nutritional physiology effects

Table 4 shows the effects of EO and its EOPN in the nutritional physiology of *B. germanica*. No mortality was registered after 24 h of exposure.

At doses of 2 mg $disc^{-1}$, palmarosa EOPN significantly decreased both RGR and RCR; however, acute peppermint oil significantly increased both indexes ($P < 0.05$). Palmarosa EOPN and acute peppermint EO produced slightly antifeedant and phagostimulant effects, respectively.

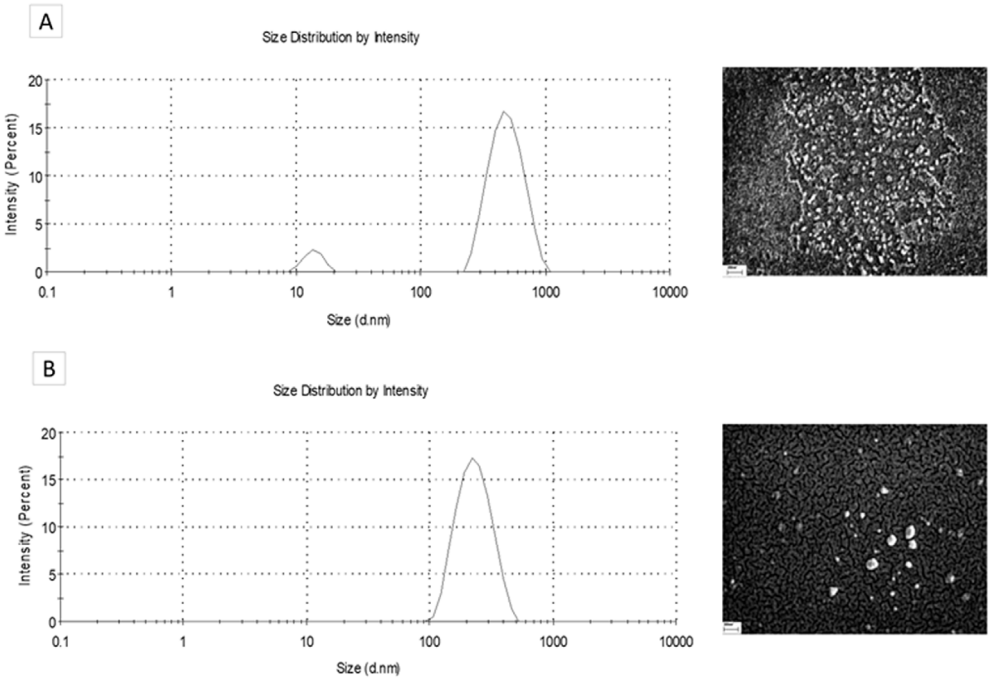

Fig. 1. DLS curves and SEM image (magnification: 85.000x.) of peppermint (A) and palmarosa (B) EOPN at 7 days postformulation.

Table 4
Nutritional indices and antifeedant/phagostimulant effect of peppermint and palmarosa oils and their EOPN on adult males of *B. germanica* (acute EO: evaporation time 15 min; residual EO: evaporation time 18 h; EOPN: evaporation time 18 h).

Dose	Treatment	RGR[a]		RCR[b]		ECI[c] %		FDI[d]	
2 mg disk^{-1}	Palmarosa EOPN	0.006 ± 0.002	a	0.007 ± 0.001	a	75.0 ± 15.337	a	−18.3	slightly AF
	Peppermint EOPN	0.055 ± 0.003	bc	0.033 ± 0.006	bc	164.3 ± 33.610	a	2.7	neutral
	Acute palmarosa (+control)	0.081 ± 0.005	cd	0.062 ± 0.012	de	132.1 ± 27.007	a	6.5	neutral
	Acute peppermint (+control)	0.104 ± 0.007	d	0.103 ± 0.021	f	101.2 ± 20.697	a	30.1	slightly PS
	Residual palmarosa (+control)	0.050 ± 0.003	b	0.030 ± 0.006	bc	163.8 ± 33.508	a	−5.1	neutral
	Residual peppermint (+control)	0.079 ± 0.002	cd	0.065 ± 0.015	de	125.1 ± 25.564	a	−9.5	neutral
	Hexanic (- control)	0.053 ± 0.003	bc	0.054 ± 0.011	cd	165.2 ± 33.794	a		
	PEG 6000 (- control)	0.047 ± 0.004	b	0.036 ± 0.007	bc	155.3 ± 31.768	a		
4 mg disk^{-1}	Palmarosa EOPN	0.007 ± 0.001	ab	0.007 ± 0.001	a	62.5 ± 12.781	b	−76.9	highly AF
	Peppermint EOPN	−0.016 ± 0.003	a	0.005 ± 0.001	a	−437.5 ± 89.46	a	−76.5	highly AF
	Acute palmarosa (+control)	−0.007 ± 0.001	a	0.006 ± 0.001	a	−318.7 ± 65.184	a	−88.2	highly AF
	Acute peppermint (+control)	0.086 ± 0.017	d	0.071 ± 0.014	d	120.1 ± 24.552	b	17.7	slightly PS
	Residual palmarosa (+control)	0.035 ± 0.007	bc	0.035 ± 0.007	b	200.0 ± 40.899	b	−24.3	slightly AF
	Residual peppermint (+control)	0.071 ± 0.013	cd	0.057 ± 0.011	d	120.3 ± 24.609	b	10.4	neutral
	Hexanic (- control)	0.053 ± 0.010	cd	0.040 ± 0.008	bc	106.8 ± 21.850	b		
	PEG 6000 (- control)	0.047 ± 0.009	c	0.036 ± 0.007	bc	155.3 ± 31.768	b		

[a] RGR: relative growth rate.
[b] RCR: relative rate of consumption.
[c] ECI: efficiency of conversion of ingested food.
[d] FDI: feeding deterrence index. Mean values ± SE with different letters within the same column indicate significant differences (LSD, $P < 0.05$). AF: antifeedant effect; PS: phagostimulant effect. – or + control: negative and positive control. N = 4 replicates of six insects.

At 4 mg disc^{-1}, peppermint and palmarosa EOPN significantly decreased RGR and RCR values; peppermint EOPN also decreased ECI value ($P < 0.05$). Both EOPN produced highly antifeedant effect. In addition, acute palmarosa oil significantly decreased RGR, RCR and ECI values ($P < 0.05$) and generated highly antifeedant effect. Residual palmarosa oil just produced a slightly antifeedant effect.

The acute and peppermint oil significantly increased RCR values ($P < 0.05$), but just acute exposure produced slightly phagostimulant effect.

3.3.3. Behavioral repellency effects

Behavioral bioassays that peppermint EO was repellent for 12 h while palmarosa oil extended this effect for 36 h. The EOPN enhanced the biological activity of EO extending the repellent effects for 36 h (peppermint EOPN) and 72 h (palmarosa EOPN) (Fig. 2 A and B, respectively).

The behavioral responses of *B. germanica* to EO and EOPN were also analyzed by the tracking system for 86 h. Depending on the product assayed; behavioral variables were modified just for 24 h. After that variable were unaffected by EO or EOPN.

At the beginning of the bioassays (time 0) peppermint and palmarosa oil significantly decreased variables stop walking, immobile time and moving time in treated zone ($P < 0.05$). At 12 and 24, these measurements were unmodified. Walking speed was unchanged at any time (Fig. 3).

At the beginning of the bioassays both EOPN significantly decreased stop walking, immobile time and moving time ($P < 0.05$). At 12 h, peppermint and palmarosa EOPN significantly decreased stop walking and immobile time ($P < 0.05$). At 24 h, just palmarosa EOPN decreased stop walking and increased immobile time, whilst peppermint EOPN increased moving time ($P < 0.05$). Walking speed was unchanged at any time (Fig. 3).

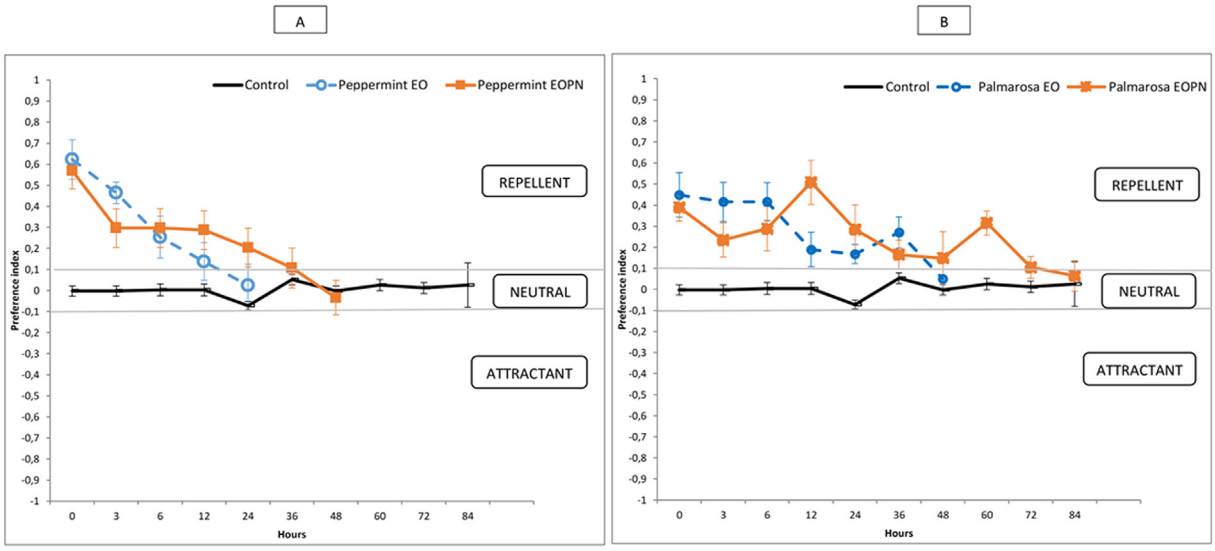

Fig. 2. A. Repellent effect of peppermint (A) or palmarosa EO (B) and their EOPN at LC_{50} on adult males of *B. germanica*.

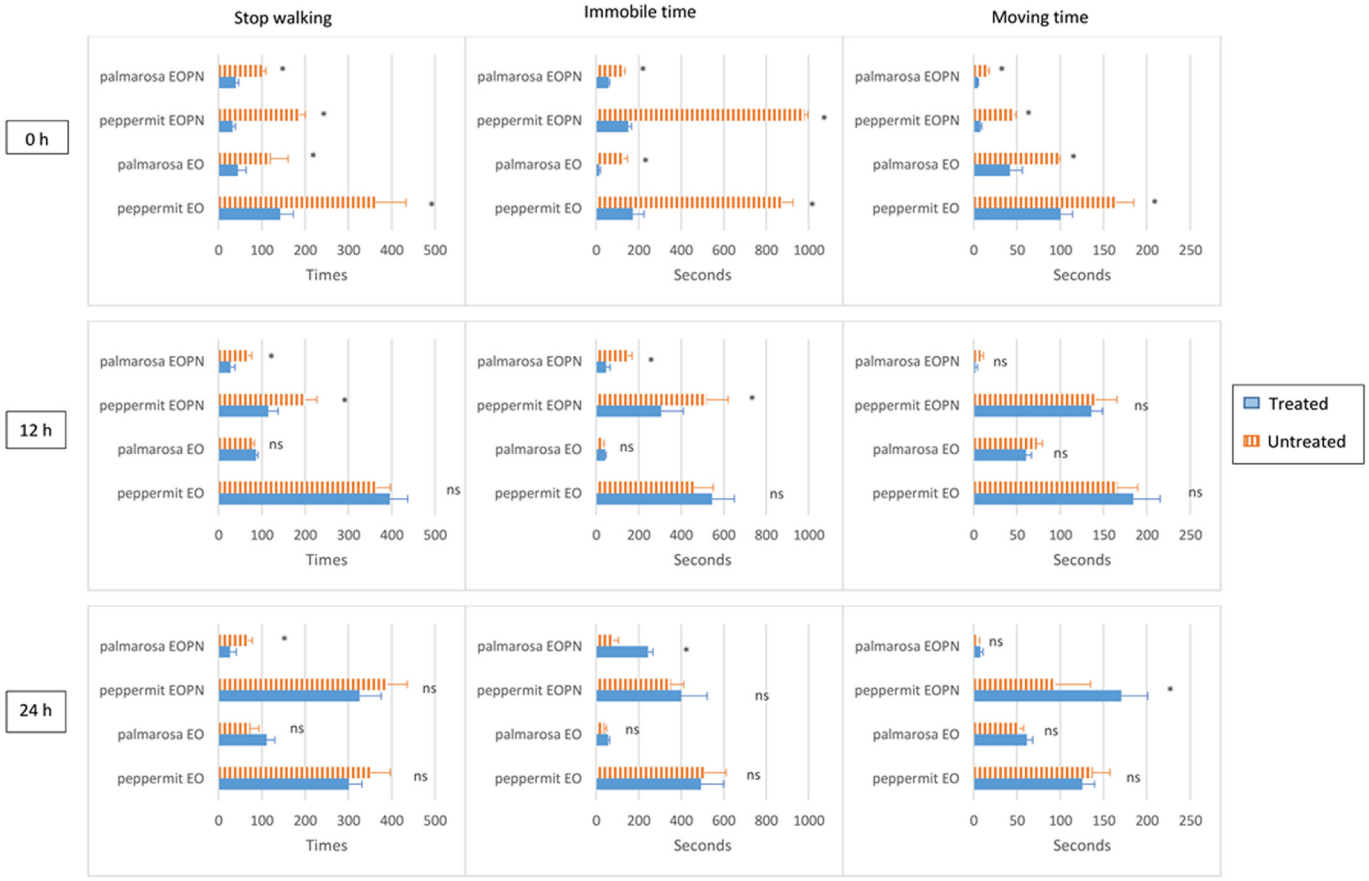

Fig. 3. Stop walking, immobile time and moving time at 0,12,24 h of peppermint and palmarosa EO and their EOPN at LC_{50} on adult males of *B. germonica.* Treatments (Mean ± SEM) differ from control at $P < 0.05$ (Students t-test).

4. Discussion

4.1. Exploratory bioassays

The EO were shown to possess contact toxicity to *B. germanica* and the toxicity order was: peppermint = palmarosa > geranium > lavender > rosemary.

Previous work demonstrated the contact toxicity activity of peppermint EO to *Plodia interpunctella*, *Aedes aegypti*, *Culex quinquefasciatus* and *Culex pipiens* (Ansari et al., 2000; Jesser et al., 2017). Moreover, Yeom et al. (2018) informed that *Mentha spicata* produced high insecticidal activity to *B. germanica* males by topical exposure. Palmarosa EO showed a high contact insecticidal activity to *P. interpunctella* (Jesser et al., 2017); however, this oil produced low contact toxicity to *Tribolium castaneum* (Caballero Gallardo et al., 2012). The toxicological effects could be due to the inhibition of acetylcholinesterase, optopaminergic receptors or total adenosine triphosphatases activity (Burčul et al., 2019; Neupane et al., 2019; Ntalli et al., 2019).

4.2. Physicochemical characterization of polymeric nanoparticles

PEG polymers are usually employed for pharmaceutical preparations and their properties also allow them to use as a nanocarrier for different insecticides products (Pascoli et al., 2018).

It has been established that an association between polymeric nanoparticles size and PDI and the degree of encapsulation or release of EO from nanosystem, which impacts based on LE (Gomes et al., 2011; Zuidam and Shimoni, 2010). High PDI values, such as observed with peppermint EOPN, indicates irregular shapes and wide range of sizes and could result in a low encapsulation or quick release of EO during the postformulation period. Consequently, peppermint EOPN showed a high decrease of EO content from 3 to 7 day postformulation. Moreover, at 7 day postformulation minor components (which in the original sample represents < 6%) were not detected. Palmarosa EOPN, with low PDI values, would have higher levels of encapsulation or a slower oil release rate. This EOPN showed a more controlled release of EO from the polymeric matrix and all components were maintained at 7 day postformulation. Finally, the differences observed between peppermint and palmarosa EOPN could be due to the chemical profile of EO to be encapsulated (Da Rosa et al., 2020).

Previous work reported that EOPN elaborated with *M. piperita* oil and PEG 6000 using melt-dispersion technique in equal ratio as the present study, had sizes of 331 ± 12.84 nm, PDI = 0.547 ± 0.015 and LE of about 85% (Kumar et al., 2011). These values are similar to those obtained in this work with EOPN at 3 days postformulation. EOPN elaborated with EO of *Allium sativum* presented sizes of 233 ± 108 nm with a monodisperse distribution and LE of 80% (Yang et al., 2009). Campolo et al. (2017) used melt-dispersion technique to obtain PEG 6000 nanoparticles loaded with citrus essential oils. These nanoparticles had sizes from 212 to 240 nm, LE > 88% and PDI between 0.23 and 0.34. In other recent work, we characterized PEG 6000 polymeric nanoparticles loaded with geranium EO (*Geranium maculatum*) and bergamot EO (*Citrus bergamia*). The geranium EOPN sizes were from 234 to 253 nm, PDI approximately 0.25 and LE between 77 and 83%, whilst for the bergamot EOPN sizes were from 184 to 236 nm, PDI 0.25 and LE between 68 and 78% (Werdin González et al., 2014, 2017).

In conclusion, it is obvious that the melt-dispersion method is a simple, convenient, and low-cost technique for EO encapsulation, which allows EOPN to be obtained with sizes > 100 nm, PDI variable

and high loading efficiency. Moreover, the scalability of this technique at commercial level is easy to achieve by the pesticide industry in order to obtain EOPN.

4.3. EOPN bioassays: lethal/sublethal effects on adult males of B. germanica

We found that the incorporation of peppermint or palmarosa oils into a solid polymeric nanoparticles, using PEG 6000 as a coating material, prevented rapid evaporation and enhanced lethal and sublethal oil activity. The small size of EOPN increased the contact surface, improving absorption and interaction with biological tissues (Rocha et al., 2018).

Peppermint and palmarosa EOPN had LC_{50} values of 31.43 and 25.41 μg cm^{-2} at 24 h of exposure to *B. germanica*, and enhanced the insecticidal activity of the oil 8 times and 10 time, respectively. In previous studies we reported similar LC_{50} values produced geranium and bergamot EOPN to *B. germanica,* even up to 72 h of exposure time. Both EOPN enhanced the toxic effects of EO, about 10 times with the geranium EOPN and 16 times for bergamot EOPN (Werdin González et al., 2015). Kumar et al. (2011) reported that peppermint EOPN significantly increased insecticidal activity in larvae of *M. domestica* when compared to EO. Similarly, garlic EOPN enhanced the insecticidal activity against *T. castaneum* (Yang et al., 2009).

Our results clearly showed that EOPN produced sublethal effects to *B. germanica*, since nanoparticles negative affected nutritional indices and FDI and enhance the repellent effects. Different studies evaluated the effects of synthetic and natural insecticides on nutrition and behavior of *B. germanica* (Alzogaray et al., 2013; Liu et al., 2011; Peterson et al., 2002). However, little information is available about the sublethal effects of EOPN on these insects. Recent studies reported that geranium and bergamot EOPN produced higher nutritional physiological effects than free EO to *T. castaneum*, *R. dominica* and *B. germanica* (Werdin González et al., 2014, 2016). Kumar et al. (2011) reported an increase in the sublethal or toxic effect of peppermint EOPN applied to the diet to *M. domestica*.

Peppermint and palmarosa EO and their polymeric nanoparticles modify the nutritional physiology to *B. germanica*. Acute exposure of peppermint EO at maximum dose increased food consumption, since the oil had a slightly phagostimulant effects. However, their EOPN reduced food consumption, since the nanoparticles had a highly antifeedant effects. These EOPN also reduced the efficiency of conversion of ingested food and the growth rate. This nutritional affection could be due to the alteration of the enzymatic systems involved in the digestion or food absorption promoted by the polymeric nanoparticles (Parra et al., 2012).

Acute exposure of palmarosa EO at maximum dose had antifeedant effects, reducing food consumption and growth rate. Residual exposure of palmarosa EO just produced a slightly antifeedant effects. Palmarosa EOPN, (evaporation time equivalent to residual EO exposure) had highly antifeedant effects and reduced some nutritional indices as acute exposure oil. This could be due to the slow and controlled release of EO promoted by the nanosystem.

Changes in insect behavioral toxicity may occur due to actions of the active compounds effects on the nervous system, stimulating or decreasing insect's mobility (Plata-Rueda et al., 2018). Rocha et al. (2018) described repellent effects of *Pogostemon cablin* oil and its nanoformulation to leaf-cutting ants.

Peppermint oil produced repellents effect for 12 h, whilst palmarosa EO for 36. Moreover, the behavioral variables (stop walking, immobile time and mobile time) were decreased by the oils just at 0 time. Werdin González et al. (2016) found that geranium and bergamot EO produce repellent effects for 6 h of adults of *B. germanica*. Plata-Rueda et al. (2018) reported that resting period was shorter in terpenoids exposure with LC50 and LC90 values than control. The low persistence of the biological activity of EO are likely due to the high volatility of active compound. Peppermint and palmarosa EOPN showed repellent effects for 36 and 72 h, respectively. Behavioral variables were modified by EOPN for 24 h. Werdin González et al. (2016) reported the improved repellent effects of the geranium and bergamot EOPN to *B. germanica*. Likely, EOPN can prevent the fast evaporation of active compounds so the repellence was enhanced.

Olfactory cues play an important role in the behavior cockroaches (Kaufman, 2019). Consequently the EOPN designed in our work could be useful to eliminate harborages areas, to deter the insects from clean surfaces and food preparation or to prevent of the establishment of pest population.

5. Conclusion

The present study suggests that peppermint and palmarosa EOPN could be a novel alternative method for German cockroach control. Also the results revealed that the EOPN increased the lethal activity the oils enhanced the EO repellent and behavioral effects and modified negatively the nutritional indices on the German cockroaches. Furthermore, these products can also be considered as highly promising formulation for the development of new effective and safety insecticides.

Author Contribution Statment

Cristhian Yeguerman: Conceptualization, Methodology, Investigation, Writing – Original Draft, Visualization. Emiliano Jesser: Conceptualization, Methodology, Investigation, Writing – Review and Editing. Manlio Massiris: Software, Writing – Original Draft. Claudio Delrieux: Software, Supervision. Ana Murray: Methodology, Supervision. Jorge Werdin González: Conceptualization, Writing – Review and Editing, Supervision, Project administration.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2019.110047.

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