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ORIGINAL PAPER

Novel nanoinsecticides based on essential oils to control the German cockroach

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Abstract The physicochemical characterization and residual insecticidal activity of poly(ethylene glycol) (PEG) nanoparticles containing essential oils (EOs) from geranium (*Geranium* sp.) and bergamot (*Citrus reticulata* L.) were evaluated against *Blatella germanica* for 1 year. The nanoparticles' size increased during the storage time from <235 to <450 nm; the EO content decreased approximately 50 %, and the abundance of the major components did not show any differences between pre- and post-formulation. The surface characteristics of nanoparticles were analyzed by transmission electronic microscopy. The EO nanoparticles produced a notable increase in the residual contact toxicity apparently because of the slow and persistent release of the active terpenes. In addition, the nanoformulation enhanced the EO contact toxicity. The results indicate that these novel systems could be developed as control agents against German cockroaches.

Keywords Nanoparticles · Geranium and bergamot essential oils · *Blatella germanica* · Residual contact toxicity

Key message

- There is a lack of information about the toxic activity of nanoparticles against *Blatella germanica*
- This study involves the development of essential oils-nanoparticles (EO-NPs) to control *B. germanica*
- EO-NPs enhance the toxic effects of the EO against *B. germanica*
- EO-NPs provide a novel tool for the German cocroach management.

Introduction

The German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae), is an important cosmopolite pest, commonly found in houses, restaurants, schools, hospitals, and other buildings (Schal and Hamilton 1990). This insect is a major public health concern because it is a mechanical vector of a number of human pathogenic microorganisms such as viruses, bacteria, protozoa, and helminthes (Fotedar et al. 1991; Pai et al. 2003), and it can cause allergic reactions in sensitive people (Gore and Schal 2007). *B. germanica* is also considered an important indicator of hygiene since it contaminates places with its excrement and exuviae (Yeom et al. 2012a).

Control of *B. germanica* is primarily dependent on continued applications with synthetic insecticides (Rust et al. 1993; Alzogaray et al. 2011; Yeom et al. 2012). The development of resistant populations to organochlorines,

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organophosphates, carbamates, and pyrethroid insecticides (Cochran 1989, 1995; Hemingway et al. 1993; Valles and Yu 1996; Wei et al. 2001; Casida and Durkin 2013) and concern about human safety and the environment (Casida and Durkin 2013; Köhler and Triebskorn 2013) have motivated the research in new and safe *B. germanica* control agents.

Biopesticides based on essential oils (EOs) appear to be a complementary or alternative method for integrated pest management (Tripathi et al. 2009; Werdin González et al. 2011, 2013; Athanassiou et al. 2013). EOs consist of mixtures of many bioactive compounds, such as alcohols, aldehydes, ketones, esters, aromatic phenols, and lactones as well as monoterpenes and sesquiterpenes (Regnault-Roger et al. 2012; Regnault-Roger 2013). Many essential oils from different families have diverse biological activities against *B. germanica*: the EOs from Lamiaceae produce contact toxicity and behavioral activity (Appel et al. 2001; Peterson et al. 2002; Tunaz et al. 2009), the EOs from Chenopodiaceae cause contact and fumigant toxicity (Zhu et al. 2012), those from Myrtaceae produce fumigant and contact toxicity and repellent activity (Alzogaray et al. 2011; Liu et al. 2011; Yeom et al. 2013), and those from Rutaceae, Cyperaceae, Anacardiaceae, Umbelliferae, and Zingiberaceae produce repellent activity (Sánchez Chopa et al. 2006; Yoon et al. 2009; Liu et al. 2011).

Despite these promising properties, problems related with EO volatility, poor water solubility, and a tendency to oxidation have to be resolved before they can be used as an alternative pest control system (Moretti et al. 2002). Nanoformulation of the EOs could resolve these problems, protecting EOs from degradation and losses by evaporation, achieving a controlled release of EOs and facilitating handling (Martín et al. 2010).

A nanoinsectide is defined as a formulation that intentionally includes elements in the nanometer size range and/or claims novel properties associated with this small size range (Kah et al. 2013). Some benefits of these nanoformulations are the improvement of efficacy due to the higher surface area, higher solubility, induction of systemic activity due to smaller particle size, and higher mobility and lower toxicity due to elimination of organic solvents in comparison to conventionally used pesticides and their formulations (Sasson et al. 2007; Kah et al. 2013). Nanotechnology applied to the development of new nanopesticides employs nanoparticles (NPs) having one or more dimensions in the order 10–1,000 nm (Soppimath et al. 2001). NPs can be classified on the basis of the type of material into metallic, semiconductor and polymeric nanoparticles (Liu 2006); the latter are the most promising for EO nanoformulation. In this work, poly(ethylene glycol) (PEG) was used as a coating or carrier material for NP formulation. It was selected because of its wide range of solubility, lack of toxicity, absence of antigenicity and immunotoxicty, and non-interference with enzymatic activities and conformations of polypeptides (Danprasert et al. 2003).

The aim of this study was to characterize polymeric nanoparticles containing essential oils (EO-NPs) and to evaluate their insecticidal activity against first instars and adults of *B. germanica.*

Materials and methods

Compounds

Commercial essential oils from geranium (*Geranium* sp., Geraniaceae) and bergamot (*Citrus reticulata* L., Rutaceae) were purchased from Swiss-Just (manufactured under the supervision and control of Ulrich Justrich AG, Walzenhausen, Switzerland) and polyethylene glycol 6000 (PEG) (molecular mass 5,000–7,000) for synthesis from Merck (Hohenbrunn, Germany). The EOs were selected taking into account the biological activities produced in other insect pests (Werdin González et al. 2014).

Insects

One- to 4-day-old first instar and adult male *B. germanica* were obtained from a colony kept at the Laboratorio de Zoología de Invertebrados II (Universidad Nacional del Sur). The insects were provided from the Centro de Investigacions de Plagas e Insecticidas (CIPEIN-CITEDEF/CONICET) (Buenos Aires, Argentina) in 2002, maintained at 27 ± 2 °C and 65 ± 5 % RH with a 14L:10D photoperiod and reared with pellet rabbit food.

Essential oil-nanoparticle (EO-NP) preparation

EO-NPs were prepared using the melt dispersion method (Werdin González et al. 2014). Several parts of PEG 6000 (100 g per part) were heated separately at 65 °C in a magnetic stirring thermo-stated container in order to melt each one. Then, 10 g of geranium or bergamot EO was added to the PEG. To ensure the distribution of the EOs in the PEG matrix, the mixture was stirred intensely for 30 min. Next, the mixtures were cooled in a freezer at −4 °C for 2 h in order to allow the NPs to form spontaneously. The cooled mixtures were ground completely in a mortar box refrigerated at 0 °C and sieved using a sieve mesh 230 (standard sieve, stainless steel mesh, Cole-Parmer). The powders were placed in air-tight polyethylene pouches and stored at 27 ± 2 °C in desiccators containing calcium chloride to prevent moisture absorption prior to further experiments.

EO-NP characterization

EO content

Aliquots of the PEG 6000 EO mixtures were diluted in 75 % absolute ethanol-H_2O and heated at 50 °C for 30 min in a thermostatic water bath (model BMK2, Dalvo Instruments). A serial dilution was made in order to obtain a series of concentrations for each mixture. The colorimetric assay at 290 nm was carried out to determine absorbance of the different concentrations using a UV-visible spectrophotometer [Shimadzu UV-1203 photometer with the Kinetics-2-Program Pack P/N (206-62029-10; Shimadzu Corp., Kyoto, Japan)]. A standard curve of concentration versus absorbance of EO-PEG was determined.

EO-NP samples (0.1 g per part) stored for 0, 8, 16, 24, 32, 40, and 48 weeks were dissolved separately in 2 ml of 75 % absolute ethanol-H_2O. The mixtures were heated at 50 °C for 30 min in a thermostatic water bath until completely dissolved. The absorbance of the solution was then determined at 290 nm by a UV-visible spectrophotometer and compared to that of the standard curve. The EO content was calculated comparing these observations with the original quantity of EO incorporated. Each test was repeated four times.

EO-NP size

The average particle size and the particle size distribution for each stored sample were determined using dynamic light scattering (DLS), which analyzes fluctuations in the intensity of light scattering due to Brownian movement of the particles. EO-NP samples (0.2 g per part) were suspended in 10 ml distilled water for 30 min. Then, the dispersion was filtered using Wathman no. 1 filter paper. DLS was performed at 25 °C using a Zetasizer ZEN 3690 model nanoinstrument (Malvern, UK). Each test was repeated at least four times.

Electronic microscopy

A drop of each of the EO-NP suspension samples was transferred onto a carbon-coated copper grid, followed by negative staining with phosphotungstic acid solution for 1 min. After the replica had been dried at 25 °C, the image was visualized with a JEOL 100 CX-II electron microscope (JEOL, Akishima, Tokyo, Japan) at the Centro Científico y Tecnológico CONICET-Bahía Blanca (CCT-CONICET BBca).

EO composition pre-/post-nanoformulation

The chemical composition of each oil pre-/post-nanoformulation was determined by gas chromatography-mass spectrometry. For the extraction of the oil, 0.5 g of each formulation was dissolved in 5 ml distilled water and heated at 50 °C for 30 min; then, 4 ml of absolute ether was added to recollect the EO extracted from the particles.

The compounds were identified comparing their retention indices (Kovats indices) with those of known compounds and also comparing their mass spectra with those available from the MS databases (NBS75 K.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 1 ml/min flow. The GC oven temperature was held at 50 °C for 2 min, programmed at 5 °C/min 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. The temperature of the injection block was 280 °C.

Insecticidal activity of EOs and EO-NPs against B. germanica

Plastic containers (7 cm diameter × 5 cm height) were treated with EO hexanic solutions or with EO-NPs (in solid form). In the first case, the container's interior surface was coated with 1 ml of the hexanic solutions using a pipette; then, the solvent was allowed to dry for 10 min. In the second case, the EO-NPs were directly dispersed on the interior surface. For adults, the EO concentrations ranged from 0.125 to 0.75 mg/cm^2 and for EO-NPs from 1.25 to 7.5 mg/cm^2 (equal concentrations: 0.125 to 0.75 mg EO/cm^2). For first instars, the EO concentrations ranged from 0.025 to 0.25 mg/cm^2 and for EO-NPs from 0.25 to 2.5 mg/cm^2 (equal concentrations: 0.025 to 0.25 mg EO/cm^2). The samples were kept covered in darkness in a conditioned room at 27 ± 2 °C and 65 ± 5 %RH for 1 year. Plastic containers treated with hexane or PEG 6000 alone (processed as in EO-NP preparation) were used as controls.

The bioassays were conducted periodically (0, 1, 3, 7, 14 days and monthly for 1 year). For each period, ten first instars or five adult males were introduced into each plastic container. Insect mortalities were recorded after 72-h exposure. Six independent replicates were performed.

To compare the insecticide magnitude of EOs vs. EO-NPs, a bioassay similar to those described above was conducted using minor concentrations, for the first instars the EOs alone or in the nanoparticle form ranging from 0.001 to 0.25 mg/cm^2, and for the adults, the values varied from 0.0125 to 0.75 mg/cm^2.

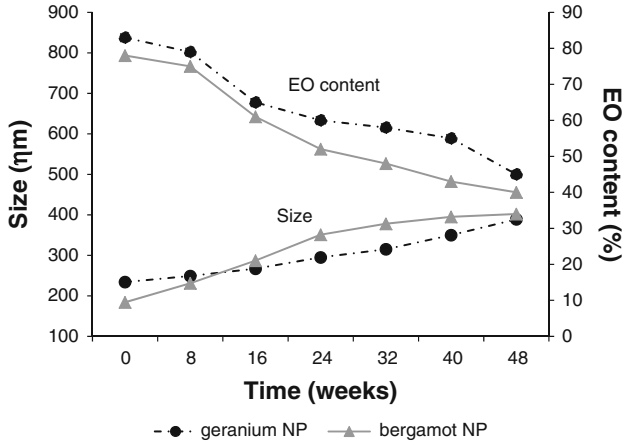

Fig. 1 EO-NP size and EO release profile during storage for 48 weeks

Statistical analysis

Data for EO loading efficiency and size were analyzed by ANOVA and LSD. The mortality data from residual contact toxicity were submitted to probit analysis using the statistical software SPSS 15.0; lethal concentration 50 % (LC50) and 95 % confidence intervals were estimated. The LC50 values were considered significantly different if their 95 % confidence intervals did not overlap. In order to compare the EO vs. EO-NP insecticidal activity, equal concentrations were calculated taking into account the EO content.

Results

EO-NP characterization

The geranium NP content decreased from 83 % (time = 0 week) to 45 % 48 weeks later, while the bergamot NP content decreased from 78 to 40 % (Fig. 1). This figure also shows that the average diameter increased throughout the experiment from 234 to 389 (geranium NP) and from 184 to 402 nm (bergamot NP). However, no significant differences were observed in particle size between time = 0 and time = 48 weeks ($P > 0.05$) (geranium NP: $f = 1.2753$, $df = 35$, $P = 0.2938$; bergamot NP: $f = 1.3821$, $df = 21$, $P = 0.26750$). The polydispersity index (PDI), which is a measure of the size distribution of NPs, was low at the beginning of the experiment (<0.2) but increased during the storage, reaching values >0.4 at the end.

Figures 2 and 3 show TEM images of EO-NPs deposited on the TEM grid. Geranium NP showed an irregular shape in good dispersion (Fig. 2). In contrast, the bergamot NP images demonstrated regular distributions and spherical shape with electrodensity zones, probably represented by the encapsulated EO (Fig. 3). The NP sizes were approximately the same as the results obtained using DLS. No significant differences were observed for EO-NP shape after different storage times.

The qualitative analyses of both pre- and post-formulation EOs were performed for 48 weeks using GC-MS. The results indicated that both commercial EOs are complex terpene (mono- and sesquiterpenes and derivates) mixtures naturally found in geranium and bergamot fruits and flowers. Before the nanoformulations were performed, a total of 11 components had been identified in geranium EO (Table 1) and 18 in bergamot EO (Table 2). For geranium EO, the major pre-formulation compounds were citronellol, geraniol, and linalool (26.1, 23.2, and 12.7 %, respectively). These monoterpenes were maintained as the principal EO components of NP during the 48 weeks of storage. For bergamot EO, the principal components found in the commercial product were linalyl acetate, limonene, and linalool, representing the 577, 16, and 11 %; these were the major ones found in the NP until the end of the experiment. In this case, two other compounds were maintained in the NPs during the entire storage time: α-caryophillene and β-pinene.

Insecticidal activity of EOs and EO-NPs against *B. germanica*

For first instars of *B. germanica*, a remarkable increase in the residual toxicity of the EO was achieved by its nanoformulation. At the highest concentration (0.25 mg EO/cm^2) (Fig. 4a), the NPs caused 100 % mortality in the course of 210 days in storage, while the EO alone caused 100 % mortality only in 1 day. The EO-NPs caused more than 85 % mortality after 360 days of storage (geranium NP = 87 ± 3 %; bergamot NP = 94 ± 4 %), whereas the EOs alone caused no mortality after just 7 days. At the lowest concentration (0.025 mg EO/cm^2) (Fig. 4b), the NPs caused 100 % mortality in the course of 90 days in storage, while the EO alone caused less than 30 % mortality on day 0 (geranium EO = 27 ± 3 %; bergamot EO = 16 ± 2 %). Geranium NPs produced more than 50 % mortality after 210 days in storage, while the bergamot NPs caused >50 % mortality after 270 days.

For adults of *B. germanica*, the EO nanoformulation also greatly increased the residual toxicity of the EO. At the highest concentration (0.75 mg EO/cm^2) (Fig. 5a), geranium NPs caused 100 % mortality in the course of 210 days in storage, while bergamot NP in the course of 300 days; both EOs alone produced 100 % mortality only in 1 day. The EO-NPs caused more than 50 % mortality after 360 days of storage (geranium NP = 53 ± 6 %; bergamot NP = 86 ± 7 %), whereas the EOs alone produced mortality after just 7 days. At the lowest

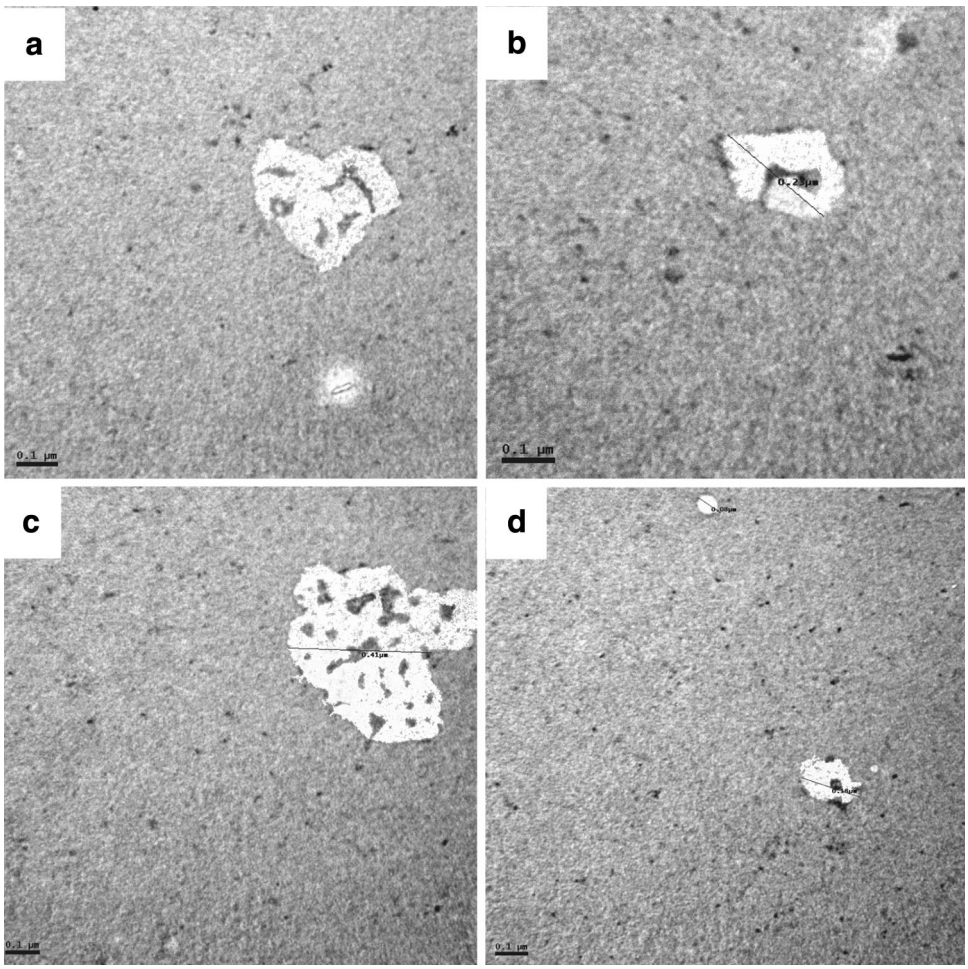

Fig. 2 TEM image of geranium EO nanoparticles

concentration (0.125 mg EO/cm^2) (Fig. 5b), geranium NPs caused more than 50 % mortality until 180 days in storage, while bergamot NP until 240 days; the EO alone caused less than 30 % at day 0 (geranium EO = 20 ± 4 %; bergamot EO = 26 ± 4 %).

NPs are known to exhibit unique properties compared with their bulk counterparts, including higher toxicity (Anjali et al. 2010). Thus, we compared the biological efficacy of the EOs alone and in nanoparticle form (Table 3). The nanoformulation enhanced the EO contact activity against first instars and adults of *B. germanica*; on day 0 and 1 of storage time, EO-NPs produced significantly lower LC50 values than the EOs alone. In first instars, both EO-NPs potentiated the toxicity effects by more than 12 times. In adults, both EO-NPs potentiated the toxicity effects by more than 10 times.

Discussion

The application of essential oils is being increasingly considered for pest control as they are generally perceived to be less toxic to humans and the environment than synthetic neurotoxic insecticides (Talbert and Wall 2012). To our knowledge, this is the first report describing the use of nanoinsecticides based on essential oils to control *B. germanica*, a frequent cockroach pest in urban environments and food production facilities.

Nanoinsecticides can consist of inorganic (metal oxides, for example) and/or organic ingredients (polymers and EOs, as in this case) in various forms (nanoparticles, micro- and nanoemulsions) (Montefuscoli et al. 2014). Properties such as composition, size, shape, and structure vary greatly according the type of nanopesticide and are also expected to vary with the storage time for any given product (Kah et al. 2013).

We observed that the EO content decreased approximately 50 % after 1 year of storage. As the NPs were prepared using melt dispersion, the fast cooling of the melted PEG 6000 and the addition of the EO could act as an inhibitor of crystallization resulting in a higher percentage of amorphous and imperfectly crystalline material; the amorphous character is common for polymeric molecules used as carriers. This state could contribute to a

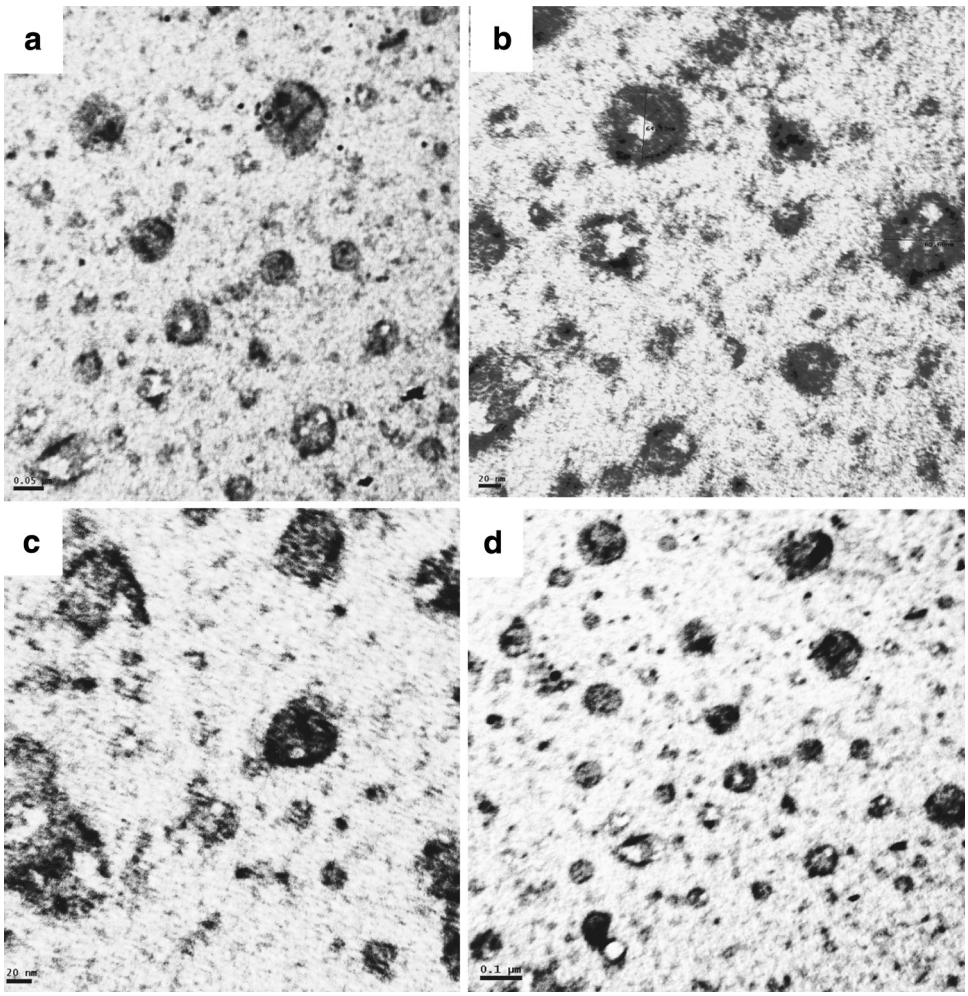

Fig. 3 TEM image of geranium EO nanoparticles

Table 1 Chemical composition of the geranium EO pre- and post-nanoformulation (after 1, 12, 24, 36, and 48 weeks) and percentage content of each component

Retention time (min)	Compound	Pre-formulation (%)	Post-formulation (%)				
			1 week	12 weeks	24 weeks	36 weeks	48 weeks
13.06	Linalool[a]	12.7	8.4	12.5	8.7	8.5	9.9
14.64	Menthone[a]	11.1	4.2	3.7	–	–	–
16.74	Citronellol[a]	26.1	36.1	31.3	35.3	36.3	36.6
17.48	Geraniol	23.2	43.7	47.4	55.0	55.2	53.5
17.98	Citronellyl formate[a]	10.3	2.1	3.5	1.0	–	–
18.70	Geranyl formate[a]	7.9	1.8	1.6	–	–	–
20.83	Geranyl acetate	1.5	0.7	–	–	–	–
21.88	α-Caryophillene	2.0	0.5	–	–	–	–
23.07	Neryl acetate	2.8	0.9	–	–	–	–
24.36	Citronellyll butyrate[a]	0.8	1.1	–	–	–	–
25.13	Geranyl butyrate	1.6	0.5	–	–	–	–

[a] Terpene can be in (+), (−), or (±) form

Table 2 Chemical composition of the bergamot EO pre- and post-nanoformulation (after 1, 12, 24, 36, and 48 weeks) and percentage content of each component

Retention time (min)	Compound	Pre-formulation (%)	Post-formulation (%)				
			1 week	12 weeks	24 weeks	36 weeks	48 weeks
8.23	β-pinene[a]	2.4	3.0	3.1	3.2	2.7	1.4
8.64	β-myrcene[a]	1.9	1.8	–	–	–	–
9.37	3-carene[a]	1.8	–	–	–	–	–
9.75	Limonene[a]	16.0	10.9	8.0	8.6	7.0	6.5
10.61	γ-Terpinene	2.3	0.9	–	–	–	–
13.06	Linalool[a]	11.0	11.3	14.1	14.8	22.3	30.2
17.57	Linalyl acetate[a]	57.0	62.6	66.2	68.1	62.2	56.8
21.88	α-Caryophillene	6.2	8.4	5.8	5.0	5.3	4.9
	Others	1.4	1.1	2.8	0.3	0.5	0.2

[a] Terpene can be in (+), (−), or (±) form

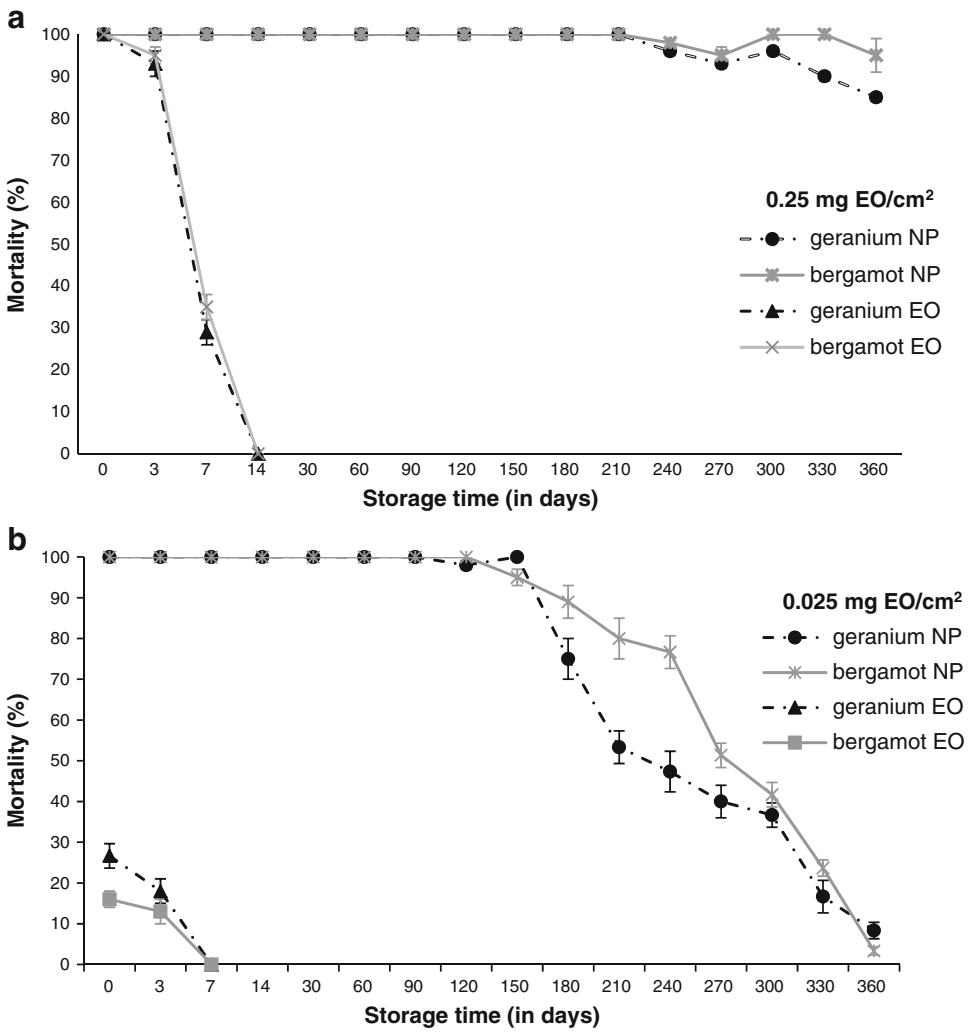

Fig. 4 Residual contact toxicity of EO alone and EO-NPs after 72-h exposure against first instars of *B. germanica*. **a** At highest concentration: 0.25 mg EO/cm^2; **b** at lowest concentration: 0.025 mg EO/cm^2 EO

higher EO loading efficiency and to the storage stability, as has previously been determined in other systems based on PEG (Westesen et al. 1997; Chidavaenzi et al. 2001; Yang et al. 2009).

The information on NP size is particularly important for understanding the behavior of these nanosystems. Moreover, in addition to composition, the bioviability of the system is also influenced by the particle size. The

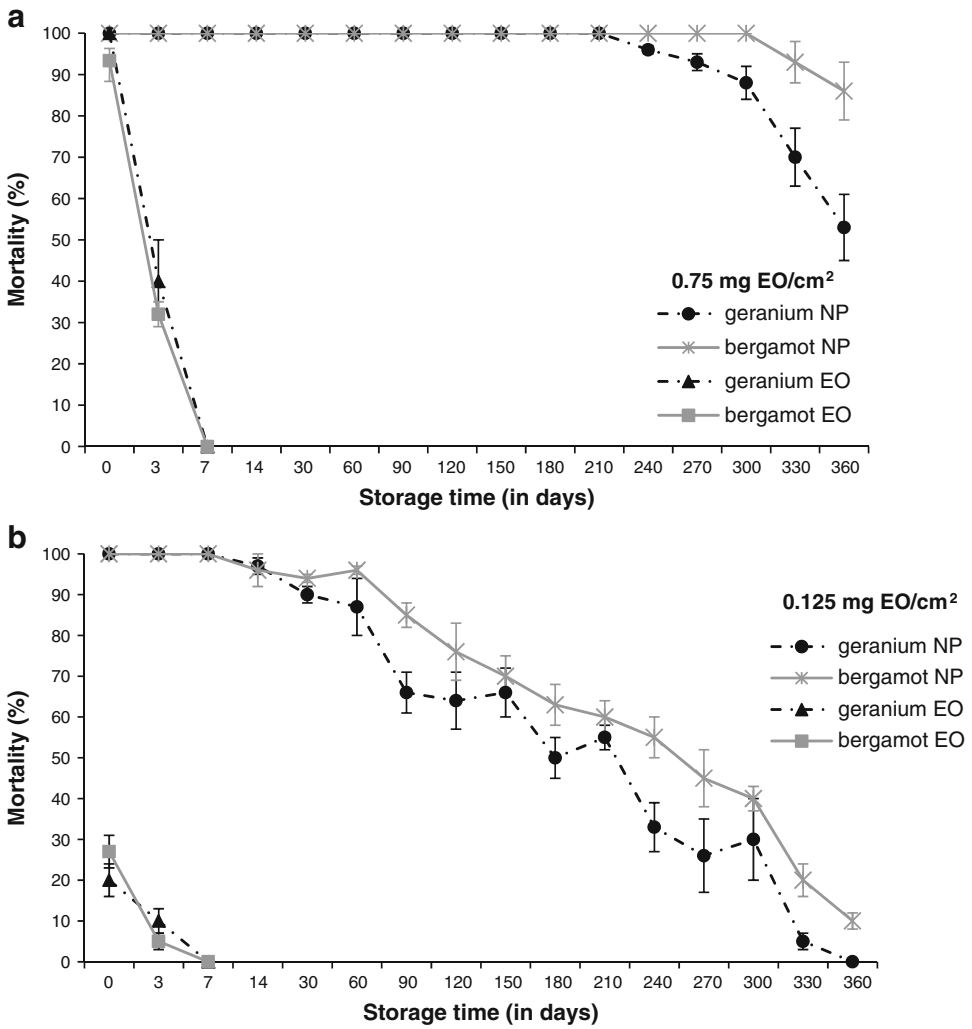

Fig. 5 Residual contact toxicity of EO alone and EO-NPs after 72-h exposure against adults of *B. germanica*. **a** At highest concentration: 0.75 mg EO/cm^2; **b** at lowest concentration: 0.125 mg EO/cm^2)

Table 3 Comparative contact toxicity effects between EO alone and EOs in the NP form against *B. germanica* at 0 and 1 day in storage. LC50 values (mg EO/cm^2) obtained with data mortality after 72-h exposure

State	Product	Day 0[a]	Day 1[a]
First instars	Geranium EO	0.062 (0.047–0.077)	0.091 (0.069–0.115)
	Geranium NP	0.005 (0.004–0.008)	0.006 (0.004–0.011)
	LC50 geranium EO/LC50 geranium NP	12.40	15.16
	Bergamot EO	0.145 (0.118–0.166)	0.165 (0.134–0.189)
	Bergamot NP	0.012 (0.006–0.019)	0.013 (0.09–0.018)
	LC50 bergamot EO/LC50 bergamot NP	12.09	12.69
Adults	Geranium EO	0.222 (0.172 – 0.278)	0.476 (0.389 – 0.582)
	Geranium NP	0.021 (0.014–0.029)	0.043 (0.036–0.046)
	LC50 geranium EO/LC50 geranium NP	10.57	11.07
	Bergamot EO	0.419 (0.356–0.488)	0.475 (0.395–0.545)
	Bergamot NP	0.026 (0.015–0.039)	0.044 (0.21–0.053)
	LC50 bergamot EO/LC50 bergamot NP	16.11	10.79

[a] The 95 % lower and upper confidence intervals are shown in parentheses

DLS technique was applied to investigate the average size of the particles, indicating that this parameter increased during the storage time from <235 to <450 nm. According to these results, it is possible to suppose that an agglomeration process occurred during the storage, which promoted the increase in NPs size, with a correlative PDI.

Otherwise, no problems were detected when the EO-NPs were solubilized in distilled water. This could be a consequence of the particle nanosize and the amorphous state of PEG achieved during the nanoparticle formation process.

The TEM images revealed nanoparticles in good dispersion, in the nanometric range (according with DLS results), appearing round (bergamot NP) or with an irregular morphology (geranium NP).

The real impact of the size and shape of NPs on their toxicological effects was at the beginning of the investigation, and its understanding is crucial for designing novel nanoinsecticides. Some studies have focused on these topics; for example, Mendes et al. (2014) found that NPs' cellular uptake is size dependent, so the toxicological process is influenced by this parameter. Besides, it has been established that wire-shaped silver NPs cause higher cytoxicity than spherical ones on alveolar epithelial cells (Stoehr et al. 2011). Another recent study with silica NPs showed that variations in their shape and size can trigger different cellular responses and even influence cell migration on surfaces (Huang et al. 2010).

The GC-MS results showed that the abundance of the major components did not show any differences between pre- and post-formulations. We may assume that the PEG 6000 could stabilize the EO in a polymeric matrix, enabling significant reduction of the volatility of the terpene constituents. Even when the EO content varied with the storage time (mainly on the minor components), we also found that the chemical nature of the EO components in the nanoformulation was not modified during the storage time, so no oxidized or hydrolytic derivates from the original compound were found. This indicates that no breakdown of active components had occurred, enhancing the effectiveness of the EOs. Previous reports showed that the main monoterpenes loaded in our nanoparticles produced lethal and sublethal effects in *B. germanica* (Jang et al. 2005; Phillips and Appel 2010; Phillips et al. 2010; Alzogaray et al. 2013; Yeom et al. 2013). It has been suggested that in some cases one compound within the oil is particularly toxic, while in others compounds have been shown to act synergistically.

The EO nanoparticles produced a notable increase in the residual contact toxicity against first instar and adults of *B. germanica* apparently because of the slow and persistent release of the active terpenes achieved by the nanoformulation. At present, the nanoformulation pesticide aims toward measured releases of necessary and sufficient amounts of these products for a period of time to obtain the fullest biological efficacy (Ghormade et al. 2011). A controlled release formulation is defined as a combination of a biologically active agent and a polymer arranged to allow the delivery of the agent to the target at controlled rates over a specified period (Hack et al. 2012). Isman et al. (2011) pointed out that a principal disadvantage of EOs used as pesticides is their lack of persistence, which requires two or more applications to exert a satisfactory management of pests. The EO-NPs evaluated in this work will provide an alternative method for EO application: on the one hand, the frequency may be reduced because of its sustained controlled release pattern; on the other hand, an aqueous application could be done, because, as mentioned above, the NPs are soluble in water; therefore, no auxiliary organic solvents are required, which are commonly used in chemical insecticide application and potentiate the ecotoxicological effects of these harmful products.

In addition, the nanoformulation enhances the toxicological activity of the EOs against *B. germanica*. The penetration pattern, bioavailability of the NPs and detoxification mechanism involved are potential explanations for the enhancement of the bioactivity of EO-NPs.

The EO compounds may enter through the insect cuticle, in a similar manner to conventional insecticides, but due to their highly lipophilic composition, entry into the hemolymph may be slow and limited (Veal 1996). More commonly, toxicity is considered a result from penetration of volatile components through the tracheal system, although the precise mode of action remains unclear.

Nanoparticles are also much more mobile than their bulk counterparts, enabling better penetration into insect tissues and enhancing insecticidal activity (Nel et al. 2006, 2009). This can be achieved either by faster penetration by direct contact through the insect's cuticle or by ingestion and penetration through the digestive tract (Margulis-Goshen and Magdassi 2012). Moreover, as nanoparticles exhibit large specific surfaces, they can potentially cause higher adhesiveness of EO-NPs to the insect's body, increasing the exposure time to the biological active molecules.

In relation to their bioavailability, PEGylated nanoparticles can produce enhancement of bioactivity of different biomolecules in insects (Jeffers and Roe 2008). For example, when the decapeptide trypsin-modulating oostatic factor (TMOF) was PEGlyated, it caused an increase of TMOF toxicity to the mosquito larvae *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) and to the lepidopterans neonates of *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae). Furthermore, in *H. virescens* larvae the conjugated peptide-PEG was accumulated in the insect hemolymph (Jeffers et al. 2012). Nachman et al. (2012) demonstrated that pyrokinin analogs (multifunctional

neuropeptides) conjugated with two PEG polymers promote their biostability when the pea aphid *Acyrthosiphon pisum* (Homoptera: Aphididae) is fed on a basal diet with the peptide analogs increasing their antifeedant activity.

Finally, various studies reported the ability of insects to detoxify EO compounds. Many terpenes found in the geranium and bergamot EOs are detoxified by different intracellular biochemical pathways, reaching substrates that are more hydrophilic and thus readily excretable by the insect (Hendry 1996; Miyazawa et al. 1998; Davoudi et al. 2011; Rossi and Palacios 2013). Moreover, the P450 oxidizing system is part of the detoxifying process (Rossi et al. 2012).

When the insects were exposed to the EO-NPs, a decreased detoxifying ratio (compared with terpenes alone) could occur because the NPs kept in the extracellular media were not available to the detoxifying systems. Thus, more bioactive products could reach the site(s) of action (Isman 2000; Regnault-Roger et al. 2012), enhancing the toxic effects of the EOs.

Conclusion

The benefits of the EO-NPs include the enhancement of efficacy due to the greater surface area, sustained controlled release, and induction of systemic activity due to smaller particle size and greater mobility. Consequently, the possibility of employing these nanoinsecticides to control *B. germanica* may warrant further investigation.

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