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Evaluation of sublethal effects of polymer-based essential oils nanoformulation on the german cockroach

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ABSTRACT

The German cockroach, *Blattella germanica* (L.), is a serious household and public health pest worldwide. The aim of the present study was to evaluate the sublethal activity of polymer-based essential oils (EOs) nanoparticles (NPs) on adults of *B. germanica.* The LC_{50} and LC_{25} for contact toxicity were determined. To evaluate the repellency of EOs and NPs at LC_{25}, a software was specially created in order to track multiple insects on just-recorded videos, and generate statistics using the obtained information. The effects of EOs and NPs at LC_{25} and LC_{50} on the nutritional physiology were also evaluated. The results showed that NPs exerted sublethal effects on the German cockroach, since these products enhance the repellent effects of the EOs and negatively affected the nutritional indices and the feeding deterrence index.

1. Introduction

Cockroaches are a major public health concern because they are mechanical vectors of a number of human pathogenic microorganisms and parasites (such as viruses, bacteria, protozoa, and helminthes) (Fotedar et al., 1991; Pai et al., 2003). They have been epidemiologically involved in toxoplasmosis, giardiasis, sarcocystosis, and intestinal amoebiasis (Graczyk et al., 2005; Tatfeng et al., 2005).

The German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae), is an important medical cosmopolitan pest, commonly found in houses, restaurants, schools, hospitals, and other large buildings (Schal and Hamilton, 1990). It is considered an important mechanical vector of many of parasites included those from the genus *Entamoeba*, *Giardia*, *Ascaris*, *Taenia*, *Trichuris*, and others (Hamu et al., 2014; Pai et al., 2003). The German cockroach can also cause allergic reactions in sensitive people (Gore and Schal, 2007) and they are considered important indicator of hygiene since they contaminate the places with their excrements and exuviae (Yeom et al., 2013).

Chemical control is a commonly used management tactic against *B. germanica*. Organchlorines, organophosphates, carbamates and pyrethroids and gel bait formulations of newer insecticides as fipronil and imidacloprid have been widely used to control *B. germanica* (Maiza et al., 2013). However, the development of resistant populations (Valles and Yu, 1996; Wei et al., 2001) and governmental restrictions on the availability and use of some conventional synthetic insecticide because their effects in human health and the environment (Casida and Durkin, 2013), have motivated the progress in new and safe German cockroach control agents, amongst which botanical alternatives, such as essential oils, are currently receiving particular attention.

Therefore, biopesticides based on essential oils (EOs) appear to be a harmless complementary or alternative method for integrated pest management with new modes of action and with benign ecotoxicological profile (Regnault-Roger et al., 2012; Tripathi et al., 2009). Essential oils are blends of approximately 20–80 different volatile plant metabolites but usually contain two or three major terpene or terpenoid components, which constitute up to 30% of the oil (Regnault-Roger et al., 2012). Its efficacy is often attributed to the oil's major component(s); however, there is also evidence that the various oil components may work in synergy (Ellse and Wall, 2014).This may occur because some oil components aid cellular accumulation and absorption of other toxic components

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(Regnault-Roger et al., 2012; Tripathi et al., 2009). Nevertheless, the mode of action of many essential oils or their components is largely unknown, although there is evidence of a toxic effect on the insect nervous system (Rattan, 2010). Alternatively, the hydrophobic nature of the oils may simultaneously exert mechanical effects on the insect such as by disrupting the cuticular waxes and blocking the spiracles, which leads to death by water stress or suffocation (Ellse and Wall, 2014). Many EOs and their constituents demonstrate lethal and sublethal effects against the German cockroach (Alzogaray et al., 2013, 2011; Phillips and Appel, 2010; Phillips et al., 2010; Yeom et al., 2013, 2012). Despite these promising properties, an important point is that EOs and its isolated active components frequently show high volatility and can easily decompose owing to direct exposure to heat, humidity, light and oxygen (Turek and Stintzing, 2013).

The development of nanotechnological botanical insecticide formulation has received great attention due to their ability to improve potency and stability, as well the safety of the nanosystems to humans and the environment (de Oliveira et al., 2014; Khot et al., 2012). The nanoformulation of the EOs could protect them from degradation and losses by evaporation, achieving a controlled release of these products and facilitating handling. Polymer-based nanoformulations have been shown the greatest potential for further development and practical applications (Kah and Hofmann, 2014). Polymers are versatile materials commonly used in medicine and pharmacy and with potential application in several other fields. Different polysaccharides (e.g., chitosan, alginates, starch), and polyesters (e.g., poly-ε-caprolactone, polyethylene glycol) have been considered for the synthesis of nanoinsecticides (Badawy et al., 2015; Christofoli et al., 2015; Kashyap et al., 2015; Ragaei and Sabry, 2014; Werdin González et al., 2014). Particular attention has recently been paid to chemistry of biocompatiable and biodegradable polymers, because they have an advantage of being readily hydrolysed into removable and non-toxic products, so they are friendly for the environment and safe for human health (Roy et al., 2014). On the other hand, the growing general trend of preferring polymeric nanoformulations by researchers can be correlated to the manifestation of higher efficacy in insecticidal property of the encapsulated ingredient compared to commercial formulations (De et al., 2014).

In a recent work, we demonstrated the lethal activity of polymeric nanoparticles based on EOs against *B. germanica*. The nanoparticles showed an average diameter <235 nm (PDI <0.280) and a loading efficacy $>75\%$. These polymer-based nanoformulations produced a notable increase of the residual contact toxicity during 1 year, apparently due to the slow and persistent release of the active terpenes. In addition, the nanoparticles enhanced the EO contact toxicity (Werdin González et al., 2015).

In general, the evaluation of nanopesticides activities against many insect pests is centered on acute toxicity by fumigant, contact or oral exposure (Kah and Hofmann, 2014; Kah et al., 2013; Perlatti et al., 2013). In addition to the direct induced mortality, sublethal effects of nanopesticides on arthropod physiology and behaviour must be considered for a complete analysis of their impact.

Sublethal effects are defined as effects on individuals that survive exposure to a pesticide (Desneux et al., 2007). Sublethal effects may impair many various physiological and behavioural traits on the exposed organism, e.g. reproductive, longevity, orientation (attractanty and repellency) and nutritional and feeding activity (Biondi et al., 2013; Planes et al., 2013; Werdin González et al., 2013).

Therefore, the present study was carried out to determine the sublethal activity of polymer-based EO nanoformulation on *Blatella germanica*, an insect pest of medical importance.

2. Materials and methods

2.1. Compounds

Essential oils namely geranium, *Geranium maculatum* (L.) and bergamot, *Citrus bergamia* (Risso) were purchased from Swiss-Just (manufactured under supervision and control of Ulrich Justrich AG, Walzenhausen, Switzerland) and polyethylene glycol 6000 (PEG) (molecular mass 5000–7000) for synthesis from Merck (Hohenbrunn, Germany). Analytical grade Hexane (Dorwill, Argentine) was used as solvent. The chemical composition of each EO determined by gas chromatography-mass spectrometry was previously informed (Werdin González et al., 2015) and showed in Table 1.

2.2. Insects

Adult males 1–3 days old from *Blatella germanica* were obtained from a colony kept at the Laboratorio de Zoología de Invertebrados II (Universidad Nacional del Sur). The insects were maintained at 27 ± 2 °C, 60–70% RH and a 14: 10 hL: D photoperiod. Adult males had an average weight of 49.44 mg (N = 100).

2.3. Essential oils - nanoparticles (NPS) preparation and characterization

EOs-NPs were prepared using the melt dispersion method. Briefly, several parts of PEG 6000 (100 g per part) were heated separately at 65 °C in a magnetic stirring thermo-stated container. After being melted, 10 g of geranium or bergamot EOs were separately mixed with PEG. To ensure the distribution of the EO in the PEG matrix, the mixture was stirred heavily for 30 min. Next, the mixture was cooled at -4 °C for 2 h in order to form the NPs spontaneously. Then, it was ground completely in a mortar box refrigerated at 0 °C and sieved using a sieve mesh 230. The powders were placed in airtight polyethylene pouches and stored at 27 ± 2 °C in desiccators containing calcium chloride to prevent moisture absorption prior to further experiments.

For the characterization, the NPs powders were dispersed with distilled water and its mean hydrodynamic diameter (Z-averages size) and Polydispersity Index (PDI) were assessed by Dynamic Light Scattering (DLS) [Zetasizer nano instrument ZEN 3690 model (Malvern, UK)]. The loading efficiency was determined spectrophotometrically [Shimadzu UV- 1203 photometer with the Kinetics-2-Program Pack P/N (206-62029-10; Shimadzu Corp., Kyoto, Japan)] (Werdin González et al., 2015, 2014). The Z-average size, polydispersion index and loading efficiency of the PEG-based EO

Table 1
Chemical composition of EOs and percentage content of each component.

Retention time (min)	Compound	*Citrus bergamia*	*Geranium maculatum*
8.36	β-pinene	2.38	
9.87	Limonene	17.49	
10.59	3-carene	4.77	
13.06	Linalool	9.46	12.67
13.85	Menthone		11.14
16.14	Citronellol		26.14
16.48	Geraniol		23.19
16.57	Linalyl acetate	58.27	
16.98	Citronellyl formate		10.27
17.70	Geranyl formate		7.94
20.85	Geranyl acetate		1.51
20.86	Caryophillene	7.63	2.00
23.70	Neryl acetate		2.78
24.36	Citronellyll butyrate		0.78
25.13	Geranyl butyrate		1.58