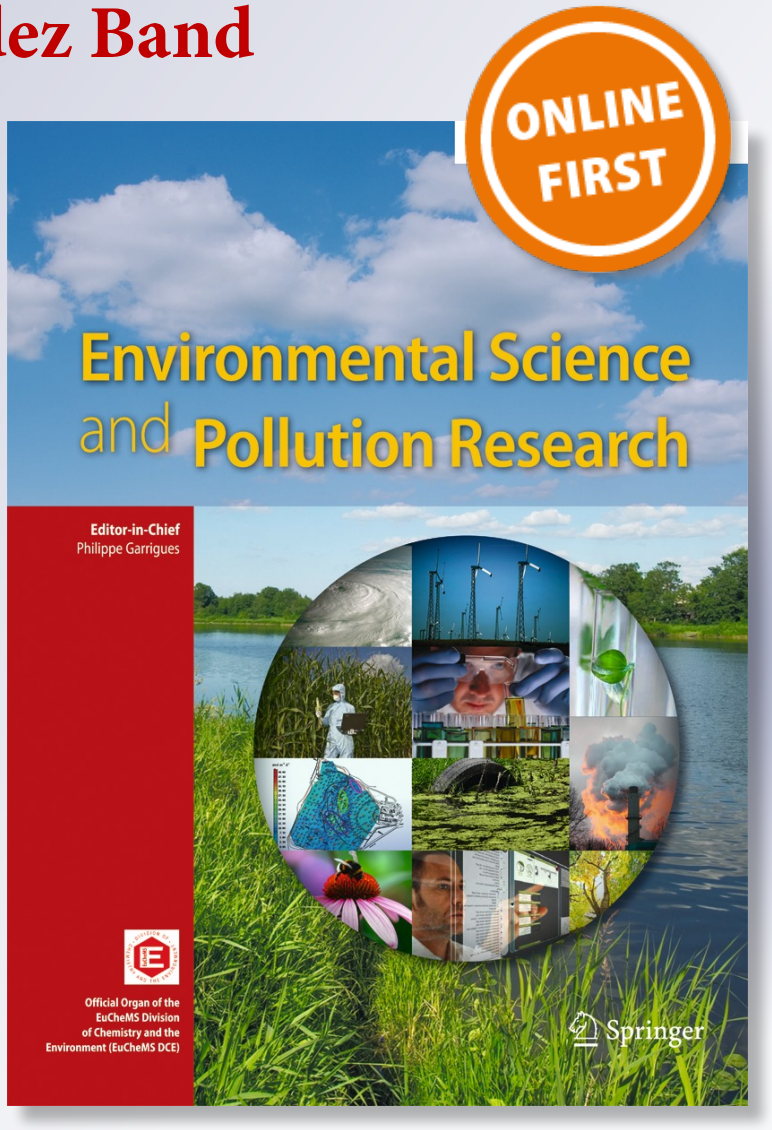
Polymer nanoparticles containing essential oils: new options for mosquito control

Jorge Omar Werdin González, Emiliano Nicolás Jesser, Cristhian Alan Yeguerman, Adriana Alicia Ferrero & Beatriz Fernández Band

Environmental Science and Pollution Research

ISSN 0944-1344

Environ Sci Pollut Res
DOI 10.1007/s11356-017-9327-4



Springer

Environ Sci Pollut Res
DOI 10.1007/s11356-017-9327-4

RESEARCH ARTICLE

Polymer nanoparticles containing essential oils: new options for mosquito control

Jorge Omar Werdin González[1,2] · **Emiliano Nicolás Jesser**[3] · **Cristhian Alan Yeguerman**[2] · **Adriana Alicia Ferrero**[3] · **Beatriz Fernández Band**[1]

Received: 28 February 2017 / Accepted: 19 May 2017

Abstract Mosquitoes (Diptera: Culicidae) are vectors of important parasites and pathogens causing death, poverty and social disability worldwide. The overuse of synthetic insecticides to control mosquito vectors lead to resistance, adverse environmental effects and high operational costs. Therefore, the development of eco-friendly control tools is an important public health challenge. In this study, two different essential oils (EO) (geranium, *Geranium maculatum*, and bergamot, *Citrus bergamia*) loaded polymeric nanoparticle (PN) were elaborated using polyethylene glycol (PEG) and chitosan (Qx) as the polymeric matrix/coating. In addition, the mosquito larvicidal acute and residual activity of the PN was evaluated on *Culex pipiens pipiens*. The physicochemical characterization of PN revealed that PEG-PN had sizes <255 nm and encapsulation efficiency between 68 and 77%; Qx-PN showed sizes <535 nm and encapsulation efficiency between 22 and 38%. From the toxicological test, it was observed that Qx-PN produced higher acute and residual activity than PEG-PN. Overall, this study highlights that polymer nanoparticles containing essential oil are a promising source of eco-friendly mosquito larvicidal products.

Responsible editor: Santiago V. Luis

✉ Jorge Omar Werdin González
jwerdin@hotmail.com

[1] FIA Laboratory, Analytical Chemistry Section, INQUISUR-CONICET, Universidad Nacional del Sur, Av. Alem 1253 (B8000CPB), Bahía Blanca, Buenos Aires, Argentina

[2] Laboratorio de Zoología de Invertebrados II, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, San Juan 670 (B8000CPB), Bahía Blanca, Buenos Aires, Argentina

[3] Laboratorio de Zoología de Invertebrados II, INBIOSUR-CONICET, San Juan 670 (B8000CPB), Bahía Blanca, Buenos Aires, Argentina

Keywords *Culex pipiens pipiens* · Polymer-based nanoparticles · Geranium and bergamot essential oils · Acute and residual larvicidal activity

Introduction

Mosquitoes (Diptera: Cullicidae) represent a key threat for millions of people worldwide, since they play a predominant role in the transmission of a variety devastating parasites and pathogens in tropical and subtropical area, including malaria, filariasis, yellow fever, dengue, Japanese encephalitis, chikungunya and recently the Zika virus which are today among the greatest health problems in the world (Benelli 2015; Govindarajan et al. 2016).

Mosquitoes belonging to Culex genus predominantly thrive near the human habitats. The *Culex pipiens* group included closely related mosquito with a wide geographical distribution. In Argentina, these mosquitoes are found from the south of Buenos Aires Province to the Santa Cruz Province (Almirón et al. 1995). *Cx. pipiens pipiens* L. has been incriminated as the primary vector of West Nile virus, St. Louis encephalitis and lymphatic filariasis caused by *Wuchereria bancrofti* (Nematoda: Filariodea) (Faraji and Gaugler 2015).

The control of mosquitoes depends primarily on continued applications of organophosphates such as temephos and fenthion, insect growth regulators such as diflubenzuron and methoprene and bacterial larvicides such as *Bacillus thuringiensis* and *Bacillus sphaericus* (Lees et al. 2015). Although they are effective, their continuous use has disrupted natural biological control systems and has resulted in the widespread development of resistance. Moreover, these chemicals have negative effects on human health and the environment (Liu et al. 2013).

Published online: 03 June 2017

Eco-friendly control tools are urgently needed. In the latest years, extensive research has been carried out to investigate the efficacy of botanical products against mosquito vectors (Govindarajan et al. 2016; Pavela 2015). From this point of view, botanical pesticides are promising since they are effective, environmentally friendly, easily biodegradable and often inexpensive. Plant essential oils (EOs) have been suggested as alternative sources for insect control because some are selective, are biodegradable to non-toxic products and have few effects on non-target organisms and the environment (Regnault-Roger et al. 2012).

In this work, we use EOs from geranium, *Geranium maculatum* (L.) (Geraniaceae) and bergamot, *Citrus bergamia* (Risso) (Rutaceae). Geranium EO has been considered as an herbal medicine with astringent, tonic, analgesic and antidiabetic effects, among others (Lis-Balchin 2003). This EO also showed antimicrobial activity (Maudsley and Kerr 1999) and insecticidal effects against different insects pest, included *Pediculus humanus capitis*, *Blatella germanica*, *Tribolium castaneum* and *Rhizopertha dominica* (Gallardo et al. 2012; Werdin González et al. 2014, 2015). Bergamot EO is a common product employed in perfume, cosmetics, food and confections (Navarra et al. 2015). It has been reported that bergamot EO has both antibacterial and antifungal activity (Karaca et al. 2007) and anti-inflammatory, analgesic and antioxidant effects (Navarra et al. 2015; Sakurada et al. 2009; Tundis et al. 2012). This oil showed insecticidal activity against *Blatella germanica*, *Tribolium castaneum* and *Rhizopertha dominica* (Werdin González et al. 2014, 2015) and repellent effects on *Sitophilus zeamais*, *Cryptolestes ferrugineus* and *Tenebrio molitor* (Cosimi et al. 2009).

Although EOs have many useful properties, their use is limited due to their high volatility, rapid oxidation and degradation on exposure to air; in addition, EOs are poorly soluble in water (Sherry et al. 2013; Turek and Stintzing 2013).

Nanopesticides involve either very small particles of a pesticide active ingredient or other small engineered structures with useful pesticidal properties (Kookana et al. 2014). Nanoinsecticides based on essential oils represent an emerging technological development that could offer a range of benefits including increased potency, stability, durability, a reduction in the amounts of active ingredients that need to be used and safety to humans and the environment (de Oliveira et al. 2014; Khot et al. 2012). The nanoformulation of the EOs could enhance their solubility and bioavailability, protect them from the environmental agents and promote their control release, hence, improving their efficacy (Bilia et al. 2014; El Asbahani et al. 2015; Sherry et al. 2013).

A range of different formulations have been suggested as nanopesticides, including emulsions (nano- and microemulsion), inorganic nanomaterial (metal, metal oxides and nanoclays) and polymer-based nanoformulations (Buteler et al. 2015; Nuruzzaman et al. 2016; Shah et al. 2016).

Encapsulation of EOs using polymers has emerged as one of the most promising techniques (Kah and Hofmann 2014; Roy et al. 2014). A broad range of polymers have been used for the encapsulation of EO, and their selection depends on various parameters like their applicability, safety, biocompatibility, cost and availability (Vishwakarma et al. 2016). Different polymers have been consider for the synthesis of polymeric nanoparticle (PN) containing EO, including starch and its derivates (dextrins, maltodextrins, cyclodextrins), gums, alginates, chitin and polyesters (e.g. polyethylene glycol, poly-ε-caprolactone), among others (Alves et al. 2014; de Barros Fernandes et al. 2014; Kfoury et al. 2015; Vishwakarma et al. 2016; Varona et al. 2013; Werdin González et al. 2014).

The aim of this work was to prepare and to characterize two different polymeric nanoparticle (PN) containing EO (using polyethylene glycol (PEG) and chitosan (Qx) as the polymeric matrix/coating) and to evaluate the acute and residual toxicity of both PN against larvae of *Cx. pipiens pipiens*.

Materials and methods

Compounds

Chitosan (medium molecular weight, degree of deacetylation 75–85%), pentasodium tripolyphosphate (TPP) and Tween 80 were obtained from Sigma-Aldrich, Germany. 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). Polyethylene glycol 6000, acetic acid, hydrochloric acid and ethanol were purchased from Merck, Germany. The chemical composition of each EO determined by gas chromatography-mass spectrometry was previously informed (Werdin González et al. 2015, 2016) and showed in Table 1.

Preparation of nanoparticles using PEG 6000 as polymeric matrix/coating (PEG-PN)

PEG-PN was 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. Control samples were processed without EO addition. Then, the samples were 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

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-careen	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

desiccators containing calcium chloride to prevent moisture absorption prior to further experiments.

Preparation of nanoparticles using chitosan as polymeric matrix/coating (Qx-PN)

Qx-PN were prepared by ionic gelation according to a method modified from the ones described by Hosseini et al. (2013) and Woranuch and Yoksan (2013). Briefly, 20 ml of chitosan solution were prepared by agitating chitosan in an aqueous acetic acid solution (0.5% (*v*/*v*)) at ambient temperature (23–25 °C) overnight. Two chitosan concentrations were evaluated: 0.5% (*w*/*v*) and 1.0% (*w*/*v*). Tween 80 (0.200 g) was then added as a surfactant to the solution and stirred at 45 °C for 1 h to obtain a homogeneous mixture. Then, geranium or bergamot EO was gradually dropped into the stirred aqueous chitosan solution. The content of EO was varied (0.100, 0.200 and 0.400 g). To prepare Qx-PN, 20 ml of TPP solution was then added during homogenization (Teflon glass homogenizer—Glas-Col LLC, Terre-Haute, IN, USA) at a speed of 1200 rpm for 5 min. The concentration of TPP was also varied: 0.5% (*w*/*v*) and 1.0% (*w*/*v*). The mixture was then maintained into mid agitation for 40 min. Control samples were processed without EO addition.

The mixtures were kept at 4 °C during 24 h; then, the samples were observed in order to observe their macroscopic properties (turbidity, precipitation and viscosity).

The formed particles were collected by centrifugation at 10,000 rpm for 10 min at 25 °C and subsequently washed with deionized water several times. The obtained wet particles were dispersed in distilled water (25 mL) and kept at 4 °C.

Characterization of polymeric nanoparticles

PN size

PEG-PN and Qx-PN 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)].

Encapsulation efficiency

The encapsulation efficiency was determined according to Werdin González et al. (2014), Hosseini et al. (2013) and Woranuch and Yoksan (2013). For PEG-PN, samples (0.1 g per part) were dissolved separately in 2 ml of absolute ethanol-H_2O (75:25). For Qx-PN, samples dispersions (in water, 1 mL) were mixed with hydrochloric acid solution (2 M, 8 mL) and boiled at 95 °C for 30 min; then, ethanol (4 mL) was added to the homogeneous mixture. PEG-PN and Qx-PN samples were centrifuged at 9000 rpm for 10 min. The supernatant was collected and analysed by UV–vis spectrophotometry [Shimadzu UV-1203 photometer with the Kinetics-2-Program Pack P/N (206-62029-10; Shimadzu Corp., Kyoto, Japan)] over a wavelength of 290 nm.

The amount of EO was calculated by appropriate calibration curve of free EOs in ethanol. Encapsulation efficiency (EE) was calculated from: EE (%) = (weight of loaded EO/weight of initial EO) × 100.

Insects

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

Acute larvicidal activity

Bioassays were performed using WHO (1996) with four instar larvae of *Cx. pipiens pipiens*. EOs alone, PEG-PN and Qx-PN and were added to plastic container with 80 ml of tap water. The concentration ranged from 10 to 150 ppm. Twenty larvae were placed in each container and were maintained at 27 ± 2 °C and 60–70% RH. After 24 h, the mortality was registered. Larvae were considered dead, when they did not react to touching with a needle. Two different controls were used; ones without treatments and others using the PN elaborated as 2.2 and 2.3 but without the addition of EO. Mortality

data were subjected to probit analysis in order to obtain LC_{50} and LC_{99} using the SPSS 15.0 statistical software. The LC_{50} values were considered significantly different if their 95% confidence intervals did not overlap.

Residual larvicidal activity

According to Prophiro et al. (2012), residual effect is defined as the ability to maintain larvicidal dosages lethal to a target organism for a certain period of time. Various stock dispersion of the EOs alone, PEG-PN and Qx-PN were prepared using the LC_{99} values and maintained in darkness at 27 ± 2 °C and 60–70% RH. During 28 days, the larvicidal effects against four instar larvae of *Cx. pipiens pipiens* was evaluated as above described. The mortality proportions (p) were recorded after 24 h. The values were transformed by Arcsen √ p. The effects of the different treatments (EO alone and PN) at each residual time were tested by using linear models by restrict maximum likelihood (REML) in the computer package INFOSTAT. The significance of each variable was tested by LSD. The residual time to obtain 50% mortality (RT_{50}) values was also calculated with their respective 95% confidence intervals (CI95%) (SPSS 15.0 statistical software).

Results and discussion

Characterization of polymeric nanoparticles

Nanoencapsulation of bioactive compounds is a method of providing a protective layers or matrices of single or mixture of polymers over the active ingredient. It represents a feasible and efficient approach to improve the physical stability of active substance (in this case, essential oils) and modulate its release, protect them from the interactions with environment, decrease their volatility and enhance their bioactivity because of the subcellular size (Bilia et al. 2014; Vishwakarma et al. 2016).

Researches on EOs nanoencapsulation were mainly focused on the synthesis of nanoemulsions, nanocapsules and nano/microparticles for their application in cosmetics, drugs, food packaging and preservatives (Acevedo-Fani et al. 2015; Manju et al. 2016). Even though, these technologies present great potential in the management of insect pest.

In this work, we synthesized two different polymer nanoparticles (PN) containing EOs (from geranium and bergamot) and we compare their biological activity on *Cx. pipiens pipiens* larvae.

According to the previous authors' work, the poly(ethylene glycol) (PEG) PN were prepared by melt dispersion method; PN prepared by this technique are of matrix type, where the active agent is dispersed over the carrier material in the form of relatively small droplets (Zuidam and Shimoni 2010). The chitosan (Qx) PN were prepared by a two-step process which involves the formation of EO droplets by an oil-in-water emulsion and the solidification of formed droplets by ionic gelation of the surrounding Qx with TPP (Esmaeili and Asgari 2015; Nagavarma et al. 2012); the PN formed by this technique are of reservoir type, where the active ingredient is present within a single coat (Zuidam and Shimoni 2010).

For Qx-PN we realized a preparation optimization of these formulations, varying Qx concentration (0.5 and 1% (*w*/*v*), EO content (0.100, 0.200 and 0.400 g) and TPP concentration (0.5 and 1% (*w*/*v*). For each sample, their macroscopic properties (turbidity, precipitation and viscosity) were observed. It is known that these parameters depend on compounds concentration and particles diameter (Montefuscoli et al. 2014). As a consequence of our observations, we concluded that the samples prepared with 0.5% Qx, 0.5% TPP and 0.200 g EO were optimal formulation for the present study and were selected to characterize the size, the EO content and to evaluate the biological activity.

The PN elaborated in the present work showed a different polymer/EO ratio: for PEG-PN was approximately 10:1 and for Qx-PN, 1:1. Table 2 summarizes the Z-average size, polydispersion index (PDI) and encapsulation efficiency (EE) of the PN.

PEGs are water-soluble synthetic polymers and were used as coating or carrier material for essential oil encapsulation. It was selected because its wide range of solubility, lack of toxicity, absence of antigenicity and inmunotoxicty, non-interference conformations of polypeptides and ease of excretion from living organisms (Khlebtsov and Dykman 2011; Werdin González et al. 2014).

For the PEG-PN elaborated in this work, the average sizes were <255 nm, the PDI < 0.270 and the EE were between 68 and 77%. Similar values were registered in previous works using the same EOs (Werdin González et al. 2014, 2016). Yang et al. (2009) used melt dispersion method to prepare garlic essential oil nanoparticles coated with PEG with <240 nm in the average diameter and 80% of encapsulation efficiency. When *Mentha piperita* was encapsulated by this technique, the sizes and the EE were 226–331 nm and 78–

Table 2 Z-average size, polydispersion index (PDI) and encapsulation efficiency of the polymeric nanoparticle containing EO

Nanoparticle	Size (m)	PDI	Encapsulation efficiency (%)
PEG geranium	253 ± 22	0.268 ± 0.025	77 ± 7
PEG bergamot	239 ± 29	0.198 ± 0.028	68 ± 5
PEG control	–	–	–
Qx geranium	439 ± 35	0.358 ± 0.062	38 ± 4
Qx bergamot	535 ± 42	0.379 ± 0.058	22 ± 3
Qx control	672 ± 75	0.647 ± 0.119	–

83%, respectively (Kumar et al. 2014). Varona et al. (2010) reported 14–66% variation in encapsulation efficiency of lavandin essential oil coated with PEG due to variation in oil load, environmental temperature and pressure during nanoparticles preparation. PEG control could not be characterized; probably the absence of EO in the preparation did not allow the nanoparticles formation.

In the melt dispersion method, the molten PEG chains are randomly orientated. As the melt cools, nucleation can occur by either homogeneous or heterogeneous mechanisms (Chidavaenzi et al. 2001). The addition of a material (such as the EO) or the fast cooling of the melted PEG could act as an inhibitor of crystallization resulting in a higher percentage of amorphous and imperfectly crystalline material. The amorphous nature significantly improves the solubility of the nanoinsecticide in water (Liu et al. 2010), which increased the dissolution rate and bioavailability (Mishra et al. 2017). The amorphous state could also contribute to a higher EOs loading efficiency as has been previously informed in other systems (Chidavaenzi et al. 2001; Westesen et al. 1997).

Qx is a linear copolymer of β-(1–4)-linked 2-acetamido-2-deoxy-β-d-glucopyranose and 2-amino-2-deoxy-β-d-glycopyranose. It is obtained by deacetylation of its parent polymer chitin, a polysaccharide widely distributed in nature (e.g. crustaceans, insects and certain fungi) (Dash et al. 2011). Qx has been used for encapsulation of different compounds because it is a biocompatible and biodegradable polymer (Mendes et al. 2016; Zhaveh et al. 2015). Qx was used to synthesize nanogel loaded with thyme oil (Khalili et al. 2015) or *Cuminum cyminum* essential oil (Zhaveh et al. 2015), hydrogel of *Eucalyptus staigeriana* essential oil (Ribeiro et al. 2013), citronella oil and pimento oil microcapsules (Dima et al. 2014; Hsieh et al. 2006), chitosan nanoparticles and nanocapsules incorporated with lime essential oil (Sotelo-Boyás et al. 2017), chitosan/cashew gum beads loaded with *Lippia sidoides* essential oil (Paula et al. 2011), among others.

In this work, the Qx-PN showed higher sizes (439–535 nm) and PDI values (<0.380) than PEG-PN, and their EE were between 22 and 38%, values lower than nanoparticles made of PEG. Hosseini et al. (2013) produced chitosan nanoparticles loaded with oregano EO by ionic gelation and reported average sizes of 281 nm and EE from 5 to 25%. Esmaeili and Asgari (2015) using the same technique with *Caracum copticum* EO observed that nanoparticles possessed an average diameter in the range of 236–721 nm and EE < 40%. Eugenol and carvacrol, two components of EOs derived from various plant species, were also encapsulated in chitosan nanoparticles; eugenol nanoparticles exhibited average sizes from 80 to 100 nm and EE in the range of 2–21% (Woranuch and Yoksan 2013) and carvacrol nanoparticles, an average diameter of 40–80 nm and EE in the range of 14–31% (Keawchaoon and Yoksan 2011). In those cases, the Qx-NP were elaborated by ionic gelation technique. The higher size of the Qx-PN observed in this work could be due to the initial size of the EO droplets, which in this method is a key variable to determine the mean particle size. It is also known that the composition of the matrix/coating materials and/or their concentration also affect the final nanoparticles sizes (Fan et al. 2012; Hsieh et al. 2006). On the other hand, the dependency of encapsulation efficiency on oil load was explained by Maji et al. (2007), who postulated a complex interaction between oil load, stirrer efficiency and polymer as determining factor, governing the encapsulation efficiency of the system. Moreover, the temperature for the EO extraction from Qx nanoparticles could modify the real EO content of the PN.

Finally, Qx control showed sizes 672 nm and PDI of 0.647 indicating a broad particle size distribution within the suspension. Probably, the absence of EO droplets during nanoparticles formation promotes the aggregation of Qx-TPP coacervates affecting the size and PDI values.

Insecticidal activity of polymeric nanoparticles

One of the major drawbacks to the use of chemical insecticides for mosquitoes control is the potential risk of environmental contamination and indiscriminate effects of non-target organisms. Over the past few decades, many research focused on seeking new EOs that could become suitable active substances for the management of this insects pests (Isman and Grieneisen 2014; Pavela 2015; Pavela and Benelli 2016).

Actually, EOs are being used mainly as sources of active substances for various repellents (Benelli et al. 2013; Ghayempour and Montazer 2016; Nerio et al. 2010). At the same time, EOs have been sought as potential active compounds for safe botanical insecticides that could be applied against adult mosquitoes and their larvae (Dias and Moraes 2014; Govindarajan et al. 2016; Pavela 2009, 2015). Due to their lipophilic nature, larvicides based on EO have to be formulated to effectively apply in water.

In this work, we evaluated four PN containing active larvicidal principles (bergamot and geranium EO) which were dispersed on a water phase, in order to have a highly diluted stability upon application. PN could present a lower ecotoxicity due to elimination of organic solvents in comparison to conventional insecticides and their formulations (Kah et al. 2013).

The LC50 values for fourth-instar larvae of *Cx. pipiens pipiens* treated with the EO alone, PEG-PN and Qx-PN at the end of 24 h are shown in Table 3.

Pavela (2015) considered that EOs present potential as larvicide agent when it cause sufficient mortality in standard larvicidal tests, to achieve $LC_{50} \leq 100$ ppm. In our work, the EOs from geranium and bergamot fulfilled with this condition (57 and 81 ppm, respectively). The significant higher larvicidal effects of geranium EO could be attributed to its major constituents (citronellol and geraniol) since them different bioactivities towards insect pests have been recognized (Gallardo

Table 3 Comparative acute larvicidal activity effects between EO alone, PEG-PN and Qx-PN against *Culex pipiens pipiens*. LC_{50} and L_{99} values (ppm) obtained with data mortality after 24 h exposure

Product	LC_{50}[a,b]		LC_{99}[a,b]	
Geranium EO	57.28 (49.1–68.3)	c	101.47 (95.5–115.9)	b
Bergamot EO	81.45 (74.6–88.1)	d	153.1 (136.5–182.4)	c
PEG geranium	35.64 (30.3–45.5)	b	82.39 (62.9–105.1)	ab
PEG bergamot	72.24 (64.1–84.5)	cd	140.18 (121.1–167.5)	c
Qx geranium	22.63 (17.5–28.3)	a	81.96 (73.3–94.3) a	a
Qx bergamot	38.52 (26.1–49.4)	ab	87.58 (62.2–114.4)	ab

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

[b] Values followed by different letters within the same column are significantly different ($P < 0.05$)

et al. 2015; Guarino et al. 2015; Lucia et al. 2017). Moreover, recent studies demonstrated that citronellol and geraniol had larvicidal and knock-down effects on *Cx. pipiens* (Tabari et al. 2017).

In this study, we also observed that PEG-PN containing geranium EO were more toxic than those from bergamot EO ($P < 0.05$). No significant differences were observed between Qx-PN loaded with geranium or bergamot EOs ($P > 0.05$). The concentration needed to achieve mortality depends on many factors, such as the chemical composition of the EO, the interaction of the active substance with the environmental factors and the capacity of the substances to penetrate the cuticle and the mechanism of action (Pavela 2009; Rattan 2010).

For geranium PN, the LC_{50} values of Qx-PN was close to 23 ppm; PEG-PN present significantly higher values which were superior to 35 ppm ($P < 0.05$). As a result, it is concluded that both geranium PN (based on PEG and Qx) enhanced the insecticidal activity of the EO showing $LC_{50} < 50$ ppm.

For bergamot PN, a similar response was observed, being Qx-PN significantly more toxic than PEG-PN ($P < 0.05$) (LC_{50} Qx-PN was close to 37 ppm and PEG-PN, 73 ppm). But in this case, just the formulation based on Qx significantly enhanced the biological activity of the EO ($P < 0.05$).

The high efficacy of the PN compared with the EO alone could be due to their nanometric size which increased surface area, enabling a better penetration into the larval body and an effective distribution of the active ingredient, enhancing the larvicidal potency (Balaji et al. 2015; Werdin González et al. 2016). This effect would be achieved either by faster penetration by direct contact through the insect's cuticle or by ingestion and penetration through the digestive tract (Benelli 2016; Margulis-Goshen and Magdassi 2013). Balaji et al. (2017), studying the larvicidal effect of nanoformulation of diethylphenylacetamide on *Culex quinquefasciatus*, proposed that the polymeric carrier plays a major role in protecting the active compound from the encounter of detoxifying enzymes in the larval gut region.

The improvement of the efficacy of an insecticide nanoformulation (included botanical) was generally proposed due to a variety of mechanism: (A) a modification in the toxicokinetic processes of the active ingredient (modification of penetration pattern, bioavailability and detoxification mechanisms), (B) a release of the active ingredient in a slow/targeted manner and/or (C) a protection of the insecticide from premature degradation/volatilization (de Oliveira et al. 2014; Kookana et al. 2014; Werdin González et al. 2016, Werdin González et al. 2015).

In this work, we also observed that, for each EO loaded, the Qx-PN were significantly more toxic than PEG-PN. For geranium PN, CL_{50} values were Qx-PN: 23 ppm < PEG-PN: 36 ppm; for bergamot PN, CL_{50} values were Qx-PN: 39 ppm < PEG-PN: 73 ppm. Even chitosan is widely regarded as being a non-toxic and biologically compatible polymer, it is important to consider that the formulation of chitosan with an active ingredient may alter the toxicokinetic and biodistribution profiles (Kean and Thanou 2010). In general, it has been proposed that the larger surface area of nanoinsecticides tends to provide easy penetration into cells and has enhanced efficacy as an insecticide (Mishra et al. 2017). In this sense. Nam et al. (2009) reported that chitosan nanoparticles showed an enhanced distribution in the mammals cells (compared to the parent hydrophilic polymers) and that several distinct uptake pathways (e.g. clathrin-mediated endocytosis, caveolae-mediated endocytosis and macropinocytosis) are involved in their internalization. It is known that the nature of the polymer used for the encapsulation also with the particles size and the surface charge of the nanoparticles formed also affect the cellular uptake and biodistribution, modifying the general toxicological profile (He et al. 2010).

In the present work, it was also studied the residual effects of the EO alone and the PN (Table 4).

Table 4 Residual effects of EO alone and PN analysed by linear models REML and residual time 50 values (RT_{50} in days) against four instar larvae of *Culex pipiens pipiens*

Product	Mean mortality proportion (p)[a,b,c]		RT_{50}[b,c]	
EO geranium	0.613 (0.584–0.642)	a	15.50 (14.3–17.1)	a
EO bergamot	0.098 (0.081–0.117)	d	2.35 (1.7–2.8)	c
PEG geranium	0.318 (0.291–0.347)	c	6.11 (3.2–9.8)	b
PEG bergamot	0.111 (0.093–0.130)	d	2.24 (1.6–2.9)	c
Qx geranium	0.474 (0.444–0.504)	b	8.89 (4.7–12.6)	b
Qx bergamot	0.375 (0.347–0.405)	c	5.83 (5.5–6.2)	b

[a] Data were retransformed from Arcsen $\sqrt{p}$

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

[c] Values followed by different letters within the same column are significantly different ($P < 0.05$)

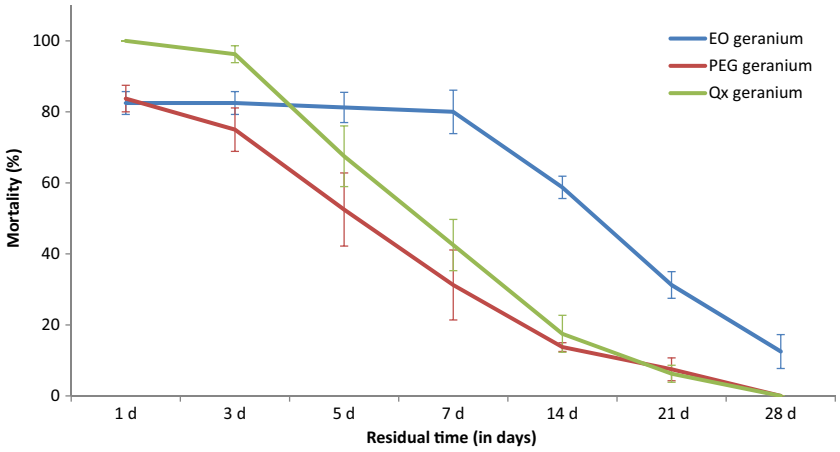


Fig. 1 Residual larvicidal activity of geranium EO alone and its PN against four instar larvae of *Culex pipiens pipiens*

The linear models REML analysis showed that geranium EO had the higher residual effect followed by the Qx-PN loaded with this EO; moreover, the residual activity of PEG geranium was significantly lower than the EO alone and Qx-PN. The Qx bergamot effect did not differ from those of PEG geranium. Finally, the EO from bergamot alone and in the form of PEG-PN had the lowest residual activity, with no significantly differences between them (Table 4).

The larvicidal effects of geranium EO and its PN are shown in Fig. 1. One- and 3-day-old residues from Qx-PN showed the highest residual activity producing more than 96% mortality. From the 5th day, geranium EO alone showed the highest activity producing more than 50% mortality until the 14th day. The residues from Qx and PEG-PN produced more than 50% mortality until the 5th day of storage. Based on RT_{50} values, the toxicity order was geranium EO > Qx-PN = PEG-PN ($F = 91.98$, df = 5, $P < 0.0001$) (Table 4).

The larvicidal effects of bergamot EO and its PN are shown in Fig. 2. During all the bioassay, Qx-PN showed the highest residual activity, reaching more than 50% mortality until the 5th day. Just 1-day-old residues from bergamot EO and PEG-PN produced more than 50% mortality. Based on RT_{50} values, the toxicity order was QX-PN > bergamot EO = PEG-PN (Table 4).

Considering the previews results, different behaviours of the nanosystems occurred showing distinct residual toxicological profiles. These variations could depend on the mechanism of release of the EO from the PN, but other variables (such as the nanoparticles suspension stability after dilution, the nanoparticle size and PDI, the interaction between the PN and biological systems, among other) could also influence.

There are different possible methods for active ingredients release from a PN: the direct desorption of drug bound to the surface PN, diffusion through the polymer wall coating, erosion of the matrix PN or a combined erosion–diffusion process (Kumari et al. 2010).

For geranium PN, just 1- and 3-day Qx-PN residues presented high residual toxicity; then, the EO alone showed the maximum residual activity. Probably, a rapid initial release or burst occurred on the PN: the PEG-PN during the first day of storage and Qx-PN possibly until the 3rd day. This could reduce the toxicological effects expected to the nanoformulation. In consequence, the mortality could be related to the total amount of EO loaded: geranium EO (101 ppm) > Qx-PN (81 ppm) = PEG-PN (82 ppm). Similar result were observed working with emulsion and microemulsion loaded with geraniol, one of the main component of

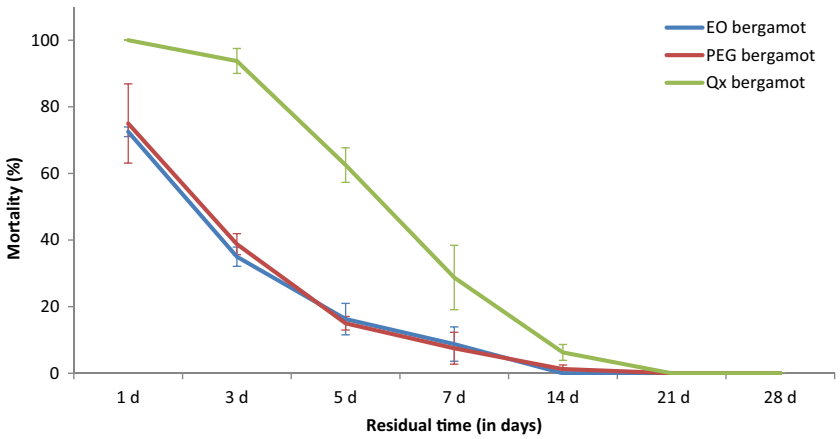


Fig. 2 Residual larvicidal activity of bergamot EO alone and its PN against four instar larvae of *Culex pipiens pipiens*

geranium EO, on four instar larvae of *Cx. pipiens pipiens* (Montefuscoli et al. 2014).

On the other hand, Qx-PN loaded with bergamot EO showed the highest residual effects. Since the PN formed by ionic gelation is expected to be of reservoir type, it is possibly that control release of the bergamot EO occurred from the PN by diffusion or the combined diffusion-erosion process, thus enhancing the residual larvicidal activity of the EO. The differences observed between Qx and PEG-PN containing bergamot EO could also be influenced by PN type (probably PEG-PN are of the matrix type), the nature of the polymer used for nanoencapsulation and the size and encapsulation efficiency, among others.

Further studies are necessary in order to understand the release mechanism followed by the polymeric nanoparticles and its influence on the mosquitoes larvae.

Conclusion

The use of essential oils can be considered as an important alternative insecticide for the control of mosquito larvae. This is the first time that incorporation of geranium and bergamot EO into Qx-PN is reported in the literature. Overall, our results showed that the Qx-PN present great perspectives for practical application in mosquito control. Even these PN had higher sizes and lower EE than PEG-PN, Qx-PN had a lower polymer/EO ratio and higher acute and residual larvicidal activity. This research adds knowledge to the development of newer and safer nanoinsecticides based on essential oils for mosquito control.

Acknowledgements The authors gratefully acknowledge Universidad Nacional del Sur and CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas) for the financial support.

Compliance with ethical standards All applicable international and national guidelines for the care and use of animals were followed.

Conflict of interest The authors declare that they have no conflicts of interest.

References

Acevedo-Fani A, Salvia-Trujillo L, Rojas-Graü MA, Martín-Belloso O (2015) Edible films from essential-oil-loaded nanoemulsions: physicochemical characterization and antimicrobial properties. Food Hydrocoll 47:168–177

Almirón WR, Humeres SG, Gardenal CN (1995) Distribution and hybridization between *Culex pipiens* and *Culex quinquefasciatus* (Diptera: Culicidae) in Argentina. Mem Inst Oswaldo Cruz 90: 469–473

Alves SF, Borges LL, dos Santos TO, de Paula JR, Conceição EC, Bara MT (2014) Microencapsulation of essential oil from fruits of *Pterodon emarginatus* using gum arabic and maltodextrin as wall materials: composition and stability. Dry Technol 32:96–105

Balaji APB, Mishra P, Suresh Kumar RS, Mukherjee A, Chandrasekaran N (2015) Nanoformulation of poly(ethylene glycol) polymerized organic insect repellent by PIT emulsification method and its application for Japanese encephalitis vector control. Colloids Surf B 128: 370–378

Balaji APB, Ashu A, Manigandan S, Sastry TP, Mukherjee A, Chandrasekaran N (2017) Polymeric nanoencapsulation of insect repellent: evaluation of its bioefficacy on *Culex quinquefasciatus* mosquito population and effective impregnation onto cotton fabrics for insect repellent clothing. Journal of King Saud University-Science. doi:10.1016/j.jksus.2016.12.005

de Barros Fernandes RV, Borges SV, Botrel DA (2014) Gum arabic/starch/maltodextrin/inulin as wall materials on the microencapsulation of rosemary essential oil. Carbohydr Polym 101:524–532

Benelli G (2015) Plant-borne ovicides in the fight against mosquito vectors of medical and veteranary importance: a systematic review. Parasitol Res 114:3201–3212

Benelli G (2016) Plant-mediated biosynthesis of nanoparticles as an emerging tool against mosquitoes of medical and veterinary importance: a review. Parasitol Res 115:23–34

Benelli G, Flamini G, Fiore G, Cioni PL, Conti B (2013) Larvicidal and repellent activity of the essential oil of *Coriandrum sativum* L. (Apiaceae) fruits against the filariasis vector *Aedes albopictus* Skuse (Diptera: Culicidae). Parasitol Res 112:1155–1161

Bilia AR, Guccione C, Isacchi B, Righeschi C, Firenzuoli F, Bergonzi MC (2014) Essential oils loaded in nanosystems: a developing strategy for a successful therapeutic approach. Evid Based Complement Alternat Med. doi:10.1155/2014/651593

Buteler M, Sofie SW, Weaver DK, Driscoll D, Muretta J, Stadler T (2015) Development of nanoalumina dust as insecticide against *Sitophilus oryzae* and *Rhyzopertha dominica*. International J Pest Manag 61: 80–89

Chidavaenzi OC, Buckton G, Koosha F (2001) The effect of co-spray drying with polyethylene glycol 4000 on the crystallinity and physical form of lactose. Int J Pharm 216:43–49

Cosimi S, Rossi E, Cioni PL, Canale A (2009) Bioactivity and qualitative analysis of some essential oils from Mediterranean plants against stored-product pests: evaluation of repellency against *Sitophilus zeamais* Motschulsky, *Cryptolestes ferrugineus* (Stephens) and *Tenebrio molitor* (L.) J Stored Prod Res 45:125–132

Dash M, Chiellini F, Ottenbrite RM, Chiellini E (2011) Chitosan—a versatile semi-synthetic polymer in biomedical applications. Prog Polym Sci 36:981–1014

Dias N, Moraes DFC (2014) Essential oils and their compounds as *Aedes aegypti* L. (Diptera: Culicidae) larvicides: review. Parasitol Res 113: 565–592

Dima C, Cotârlet M, Alexe P, Dima S (2014) Microencapsulation of essential oil of pimento [*Pimenta dioica* (L) Merr.] by chitosan/k-carrageenan complex coacervation method. Innov Food Sci Emerg Technol 22:203–211

El Asbahani A, Miladi K, Badri W, Sala M, Addi EA, Casabianca H, Elaissari A (2015) Essential oils: from extraction to encapsulation. Int J Pharm 483:220–243

Esmaeili A, Asgari A (2015) In vitro release and biological activities of *Carum copticum* essential oil (CEO) loaded chitosan nanoparticles. Int J Biol Macromolec 81:283–290

Fan W, Yan W, Xu Z, Ni H (2012) Formation mechanism of monodisperse, low molecular weight chitosan nanoparticles by ionic gelation technique. Colloids Surf B 90:21–27

Faraji A, Gaugler R (2015) Experimental host preference of diapause and non-diapause induced *Culex pipiens pipiens* (Diptera: Culicidae). Parasit Vectors 8:1

Gallardo A, Picollo MI, González-Audino P, Mougabure-Cueto G (2012) Insecticidal activity of individual and mixed monoterpenoids of

geranium essential oil against *Pediculus humanus capitis* (Phthiraptera: Pediculidae). J medical entomol 49:332–335

Gallardo A, Picollo MI, Mougabure-Cueto G (2015) Lethal activity of individual and mixed monoterpenoids of geranium essential oil on *Musca domestica*. Parasitol Res 114:1229–1232

Ghayempour S, Montazer M (2016) Micro/nanoencapsulation of essential oils and fragrances: focus on perfumed, antimicrobial, mosquito-repellent and medical textiles. J Microencapsul 33:497–510

Govindarajan M, Rajeswary M, Benelli G (2016) Chemical composition, toxicity and non-target effects of *Pinus kesiya* essential oil: an eco-friendly and novel larvicide against malaria, dengue and lymphatic filariasis mosquito vectors. Ecotox Environ Safe 129:85–90

Guarino S, Colazza S, Peri E, Bue PL, Germanà MP, Kuznetsova T, Soroker V (2015) Behaviour-modifying compounds for management of the red palm weevil (*Rhynchophorus ferrugineus* Oliver). Pest Manag Sci 71:1605–1610

He C, Hu Y, Yin L, Tang C, Yin C (2010) Effects of particle size and surface charge on cellular uptake and biodistribution of polymeric nanoparticles. Biomaterials 31:3657–3666

Hosseini SF, Zandi M, Rezaei M, Farahmandghavi F (2013) Two-step method for encapsulation of oregano essential oil in chitosan nanoparticles: preparation, characterization and in vitro release study. Carbohydr Polym 95:50–56

Hsieh WC, Chang CP, Gao YL (2006) Controlled release properties of chitosan encapsulated volatile citronella oil microcapsules by thermal treatments. Colloids Surf B 53:209–214

Isman MB, Grieneisen ML (2014) Botanical insecticide research: many publications, limited useful data. Trends Plant Sci 19:140–145

Kah M, Hofmann T (2014) Nanopesticide research: current trends and future priorities. Environ Inter 63:224–235

Kah M, Beulke S, Tiede K, Hofmann T (2013) Nanopesticides: state of knowledge, environmental fate, and exposure modeling. Crit Rev Env Sci Tec 43:1823–1867

Karaca M, Özbek H, Him A, Tütüncü M, Akkan HA, Kaplanoğlu V (2007) Investigation of anti-inflammatory activity of bergamot oil. Eur J Gen Med 4:176–179

Kean T, Thanou M (2010) Biodegradation, biodistribution and toxicity of chitosan. Adv Drug Deliv Rev 62:3–11

Keawchaoon L, Yoksan R (2011) Preparation, characterization and in vitro release study of carvacrol-loaded chitosan nanoparticles. Colloids Surf B 84:163–171

Kfoury M, Auezova L, Ruellan S, Greige-Gerges H, Fourmentin S (2015) Complexation of estragole as pure compound and as main component of basil and tarragon essential oils with cyclodextrins. Carbohydr Polym 118:156–164

Khalili ST, Mohsenifar A, Beyki M, Zhaveh S, Rahmani-Cherati T, Abdollahi A, Tabatabaei M (2015) Encapsulation of thyme essential oils in chitosan-benzoic acid nanogel with enhanced antimicrobial activity against *Aspergillus flavus*. LWT-Food Sci Technol 60:502–508

Khlebtsov N, Dykman L (2011) Biodistribution and toxicity of engineered gold nanoparticles: a review of in vitro and in vivo studies. Chem Soc Rev 40:1647–1671

Khot LR, Sankaran S, Maja JM, Ehsani R, Schuster EW (2012) Applications of nanomaterials in agricultural production and crop protection: a review. Crop Prot 35:64–70

Kookana RS, Boxall AB, Reeves PT, Ashauer R, Beulke S, Chaudhry Q, Lynch I (2014) Nanopesticides: guiding principles for regulatory evaluation of environmental risks. J Agric Food Chem 62:4227–4240

Kumar P, Mishra S, Malik A, Satya S (2014) Preparation and characterization of PEG-Mentha oil nanoparticles for housefly control. Colloids Surf B 116:707–713

Kumari A, Yadav SK, Yadav SC (2010) Biodegradable polymeric nanoparticles based drug delivery systems. Colloids Surf B 75:1–18

Lees RS, Gilles JRL, Hendrichs J, Vreysen MJB, Bourtzis K (2015) Back to the future: the sterile insect technique against mosquito disease vectors. Curr Opin Insect Sci 10:156–162

Lis-Balchin M (2003) Geranium oil and its use in aromatherapy. In: Lis-Balchin M (ed) Geranium and pelargonium: history of nomenclature, usage and cultivation. CRC Press, London, pp 234–246

Liu Y, Sun C, Hao Y, Jiang T, Zheng L, Wang S (2010) Mechanism of dissolution enhancement and bioavailability of poorly water soluble celecoxib by preparing stable amorphous nanoparticles. J Pharm Pharmacol Sci 13:589–606

Liu XC, Dong HW, Zhou L, Du SS, Liu ZL (2013) Essential oil composition and larvicidal activity of *Toddalia asiatica* roots against the mosquito *Aedes albopictus* (Diptera: Culicidae). Parasitol Res 112:1197–1203

Lucia A, Toloza AC, Guzmán E, Ortega F, Rubio RG, (2017) Novel polymeric micelles for insect pest control: encapsulation of essential oil monoterpenes inside a triblock copolymer shell for head lice control. doi:10.7717/peerj.3171

Maji TK, Baruah I, Dube S, Hussain MR (2007) Microencapsulation of *Zanthoxylum limonella* oil (ZLO) in glutaraldehyde crosslinked gelatin for mosquito repellent application. Bioresour Technol 98:840–844

Manju S, Malaikozhundan B, Vijayakumar S, Shanthi S, Jaishabanu A, Ekambaram P, Vaseeharan B (2016) Antibacterial, antibiofilm and cytotoxic effects of *Nigella sativa* essential oil coated gold nanoparticles. Microb Pathog 91:129–135

Margulis-Goshen K, Magdassi S (2013) Nanotechnology: an advanced approach to the development of potent insecticides. In: Ishaaya I, Palli SR, Horowitz (eds) Advanced technologies for managing insect pests. Springer, New York, pp 295–314

Maudsley F, Kerr KG (1999) Microbiological safety of essential oils used in complementary therapies and the activity of these compounds against bacterial and fungal pathogens. Support Care Cancer 7:100–102

Mendes J, Paschoalin R, Carmona V, Sena A, Marques A, Marconcini J et al (2016) Biodegradable polymer blends based on corn starch and thermoplastic chitosan processed by extrusion. Carbohydr Polym 137:452–458

Mishra P, Balaji APB, Dhal PK, Kumar RS, Magdassi S, Margulis K, Chandrasekaran N (2017). Stability of nano-sized permethrin in its colloidal state and its effect on the physiological and biochemical profile of Culex tritaeniorhynchus larvae. Bulletin Entomol Res 1–13. doi:10.1017/S0007485317000165

Montefuscoli A, Werdin González JO, Palma S, Ferrero AA, Fernández Band BS (2014) Design and development of aqueous nanoformulations for mosquito control. Parasitol Res 113:793–800

Nagavarma BVN, Yadav HK, Ayaz A, Vasudha LS, Shivakumar HG (2012) Different techniques for preparation of polymeric nanoparticles—a review. Asian J Pharm Clin Res 5:16–23

Nam HY, Kwon SM, Chung H, Lee SY, Kwon SH, Jeon H, Oh YK (2009) Cellular uptake mechanism and intracellular fate of hydrophobically modified glycol chitosan nanoparticles. J Control Release 135:259–267

Navarra M, Mannucci C, Delbò M, Calapai G (2015) *Citrus bergamia* Essential oil: from basic research to clinical application. Front in pharmacol 6:36

Nerio LS, Olivero-Verbel J, Stashenko E (2010) Repellent activity of essential oils: a review. Bioresour Technol 101:372–378

Nuruzzaman M, Rahman MM, Liu Y, Naidu R (2016) Nanoencapsulation, nano-guard for pesticides: a new window for safe application. J Agric Food Chem 64:1447–1483

de Oliveira JL, Campos EVR, Bakshi M, Abhilash PC, Fraceto LF (2014) Application of nanotechnology for the encapsulation of botanical insecticides for sustainable agriculture: prospects and promises. Biotechnol Adv 32:1550–1561

Paula HC, Sombra FM, de Freitas CR, Abreu FO, de Paula RC (2011) Preparation and characterization of chitosan/cashew gum beads loaded with *Lippia sidoides* essential oil. Mater Sci Eng C 31: 173–178

Pavela R (2009) Larvicidal property of essential oils against *Culex quinquefasciatus* Say (Diptera: Culicidae). Ind Crop Prod 30:311–315

Pavela R (2015) Essential oils for the development of eco-friendly mosquito larvicides: a review. Ind Crop Prod 76:174–187

Pavela R, Benelli G (2016) Essential oils as ecofriendly biopesticides? Challenges and constraints. Trends Plant Sci 21:1000–1007

Prophiro JS, da Silva MAN, Kanis LA, da Silva BM, Duque-Luna JE, da Silva, OS (2012) Evaluation of time toxicity, residual effect, and growth-inhibiting property of Carapa guianensis and Copaifera sp. in Aedes aegypti. Parasitol Res 110:713–719

Rattan RS (2010) Mechanism of action of insecticidal secondary metabolites of plant origin. Crop Protect 29:913–920

Regnault-Roger C, Vincent C, Arnason JT (2012) Essential oils in insect control: low-risk products in a high-stakes world. Annu Rev Entomol 57:405–424

Ribeiro WLC, Macedo ITF, dos Santos JML, de Oliveira EF, Camurça-Vasconcelos ALF, de Paula HCB, Bevilaqua CML (2013) Activity of chitosan-encapsulated *Eucalyptus staigeriana* essential oil on *Haemonchus contortus*. Exp Parasitol 135:24–29

Roy A, Singh SK, Bajpai J, Bajpai AK (2014) Controlled pesticide release from biodegradable polymers. Cent Eur J Chem 12:453–469

Sakurada T, Kuwahata H, Katsuyama S, Komatsu T, Morrone LA, Corasaniti MT (2009) Intraplantar injection of bergamot essential oil into the mouse hindpaw: effects on capsaicin-induced nociceptive behaviors. Int Rev Neurobiol 85:237–248

Shah MA, Wani SH, Khan AA (2016) Nanotechnology and insecticidal formulations. J Food Bioengin Nanopro 1:285–310

Sherry M, Charcosset C, Fessi H, Greige-Gerges H (2013) Essential oils encapsulated in liposomes: a review. J Liposome Res 23:268–275

Sotelo-Boyás ME, Correa-Pacheco ZN, Bautista-Baños S, Corona-Rangel ML (2017) Physicochemical characterization of chitosan nanoparticles and nanocapsules incorporated with lime essential oil and their antibacterial activity against food-borne pathogens. LWT-Food Sci Technol 77:15–20

Tabari MA, Youssefi MR, Esfandiari A, Benelli G (2017) Toxicity of β-citronellol, geraniol and linalool from Pelargonium roseum essential oil against the West Nile and filariasis vector *Culex pipiens* (Diptera: Culicidae). Res Vet Sci 114:36–40

Tundis R, Loizzo MR, Bonesi M, Menichini F, Mastellone V, Colica C, Menichini F (2012) Comparative study on the antioxidant capacity and cholinesterase inhibitory activity of *Citrus aurantifolia* Swingle, *C. aurantium L.*, and *C. bergamia* Risso and Poit. peel essential oils. J Food Sci 77:H40–H46

Turek C, Stintzing FC (2013) Stability of essential oils: a review. Comp Rev Food Sci Food Saf 12:40–53

Varona S, Kareth S, Martín Á, Cocero MJ (2010) Formulation of lavandin essential oil with biopolymers by PGSS for application as biocide in ecological agriculture. J Supercrit Fluids 54:369–377

Varona S, Martín Á, Cocero MJ, Duarte CM (2013) Encapsulation of lavandin essential oil in poly-(ϵ-caprolactones) by PGSS Process. Chem Engine Technol 36:1187–1192

Vishwakarma GS, Gautam N, Babu JN, Mittal S, Jaitak V (2016) Polymeric encapsulates of essential oils and their constituents: a review of preparation techniques, characterization and sustainable release mechanisms. Polym Rev 56:668–701

Werdin González JO, Gutiérrez MM, Ferrero AA, Fernández Band BS (2014) Essential oils nanoformulations for stored-product pest control—characterization and biological properties. Chemosphere 100: 130–138

Werdin González JO, Stefanazzi N, Murray AP, Ferrero AA, Fernández Band BS (2015) Novel nanopesticides based on essential oils to control the German cockroach. J Pest Sci 88:393–404

Werdin González JO, Yeguerman C, Marcovecchio D, Delrieux C, Ferrero AA, Fernández Band BS (2016) Evaluation of sublethal effects of polymer-based essential oils nanoformulation on the German cockroach, *Blatella germanica*. Ecotoxicol Environ Safe 130:11–18

Westesen K, Bunjes H, Koch HJ (1997) Physicochemical characterization of lipid nanoparticles and evaluation of their drug loading capacity and sustained release potential. J Control Release 48:189–197

WHO (1996) Report of the WHO informal consultation on the "Evaluation and Testing of Insecticides". WHO, Genova

Woranuch S, Yoksan R (2013) Eugenol-loaded chitosan nanoparticles: I. Thermal stability improvement of eugenol through encapsulation. Carbohydr Polym 96:578–585

Yang FL, Li XG, Zhu F, Lei CL (2009) Structural characterization of nanoparticles loaded with garlic essential oil and their insecticidal activity against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). J Agric Food Chem 57:10156–10162

Zhaveh S, Mohsenifar A, Beiki M, Khalili ST, Abdollahi A, Rahmani-Cherati T, Tabatabaei M (2015) Encapsulation of *Cuminum cyminum* essential oils in chitosan-caffeic acid nanogel with enhanced antimicrobial activity against Aspergillus flavus. Ind Crop Prod 69:251–256

Zuidam NJ, Shimoni E (2010) Overview of microencapsulates for use in food products or processes and methods to make them. In: Zuidam NJ, Nedović VA (eds) Encapsulation technologies for active food ingredients and food processing. Springer, New York, pp 3–29