

Design and development of aqueous nanoformulations for mosquito control

Antonela Rita Montefuscoli, Jorge Omar Werdin González, Santiago Daniel Palma, Adriana Alicia Ferrero & Beatriz Fernández Band

Parasitology Research
Founded as Zeitschrift für
Parasitenkunde

ISSN 0932-0113
Volume 113
Number 2

Parasitol Res (2014) 113:793-800
DOI 10.1007/s00436-013-3710-y



Your article is protected by copyright and all rights are held exclusively by Springer-Verlag Berlin Heidelberg. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".

Design and development of aqueous nanoformulations for mosquito control

Antonela Rita Montefuscoli · Jorge Omar Werdin González ·
Santiago Daniel Palma · Adriana Alicia Ferrero ·
Beatriz Fernández Band

Received: 13 November 2013 / Accepted: 17 November 2013 / Published online: 29 November 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract Microemulsions (ME) are thermodynamically stable isotropic mixtures of oil, water, and surfactant; they would also be attractive as potential insecticidal products due to the high bioavailability of the active ingredient, attributable to the small sizes of the oil drops. A laboratory study was conducted in order to compare the biological effect of oil in water (o/w) geranium essential oil (EO) and geraniol MEs and emulsions, against *Culex pipiens pipiens* mosquito larvae. The systems were based on three nonionic surfactants (Cremophor EL, Brij 35, Tween 80). The MEs showed dispersed phase diameters in the range of 8 to 14 nm and had low PDI values (<0.2). The MEs were analyzed by TEM, indicating that they had nearly spherical morphology. The microemulsified systems based on geranium EO and those of geraniol produced a notable increase of the larvicidal activity when compared with the respectably emulsions, concluding that the biological effect is related with the diameter of the dispersed phase. The smallest drops achieved the highest larvicidal activity, being the aqueous nanoformulations based on geraniol most effective than those of geranium EO. However, geranium microemulsions are preferred due to their residual toxicological profiles. The results indicate that these novel systems

could be used in integrated pest management program for the *C. pipiens pipiens*.

Introduction

Mosquitoes are insects of major public health concern because many species are vectors of diseases. *Culex pipiens pipiens* Say (Diptera: Culicidae) is one of the main vectors of lymphatic filariasis caused by *Wuchereria bancrofti* (Nematoda: Filarioidea) (Becker et al. 2010). The most efficient method for controlling these ectoparasites is to target larvae at their breeding sites, being chemical insecticides the primary management tool (Chung et al. 2009; Conti et al. 2010). In Argentina, the common insecticides used for adults and larvae are pyrethroids and organophosphates, respectively (Lucia et al. 2013). Moreover, insect growth regulators such as diflubenzuron and methoprene are generally used for the control of mosquito larvae (Jahan et al. 2011). The overuse of synthetic pesticides has created a number of ecological problems such as the development of resistant insect strains, ecological imbalance, and harm to mammals. Besides, their residues in soil, water resources, and crops affect public health (Cheng et al. 2009a, b, c). On the other hand, large amounts of organic solvents are usually employed to dissolve these materials in order to achieve effective and uniform application which potentiate the anterior problems enumerated (Stackelberg et al. 2001). Thus, actually the studies focus on the searching of new biodegradable and selective mosquitocidal products in order to generate eco-friendly formulations.

In this way, essential oils (EO) from plants are an excellent option to synthetic pesticides since they reduce negative impacts to human health and the environment being an alternative method for integrated pest management (Tripathi et al. 2009; Werdin González et al. 2011, 2013). EO are complex

A. R. Montefuscoli · J. O. Werdin González (✉) ·
B. Fernández Band
FIA Laboratory, Analytical Chemistry Section, INQUISUR
(UNS-CONICET), Universidad Nacional del Sur, Av. Alem 1253,
B8000CPB Bahía Blanca, Buenos Aires, Argentina
e-mail: jowerdin@gmail.com

J. O. Werdin González · A. A. Ferrero
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

S. D. Palma
Departamento de Farmacia, Facultad de Ciencias Químicas,
UNITEFA (UNC-CONICET), Ciudad Universitaria,
Córdoba 5000, Córdoba, Argentina

mixtures of volatile organic compounds produced as secondary metabolites in plants; they are constituted by hydrocarbons (terpenes and sesquiterpenes) and oxygenated compounds (alcohols, esters, ethers, aldehydes, ketones, lactones, phenols, and phenol ethers) (Nerio et al. 2010). Various EOs exhibit acute toxics and sublethal effects against different mosquitoes, including *Culex* species (Prajapati et al. 2005; Amer and Mehlhorn 2006a, b; Senthilkumar et al. 2008; Gillij et al. 2008; Pavela et al. 2009; Govindarajan 2011; Manimaran et al. 2012; Park and Park 2012; Senthilkumar and Venkatesalu 2012; Vatandoost et al. 2012; Maheswaran and Ignacimuthu 2013).

In contrast, their chemical instability, volatility, poor water solubility, and aptitude for oxidation are inconvenient for the massive use of the EO (Moretti et al. 2002). In consequence, the incorporation of the EO in nanoformulations, such as microemulsions, could solve these problems offering several advantages, for example, a significant increase in water solubility, dissolution rate and dispersion uniformity, and major bioviability upon application (Lai et al. 2006).

Microemulsions (ME) are transparent isotropic thermodynamically stable dispersions of two immiscible liquids (water and oil) containing appropriate amounts of surfactant. The dispersed phase consists of small droplets with diameter in the range of 10–100 nm (Srinivasa Rao et al. 2009). Surfactants are amphiphilic molecules containing both polar and nonpolar group, so they tend to adsorb in the interface O-W; in this way, the dispersion of oil nanodroplets in an aqueous external phase is stabilized by an interfacial film of surfactant (Vandame et al. 2002). As they require a minimum energy for formation, they are ease of manufacturing and scale up. Due to their small droplet size, MEs may appear transparent, and Brownian motion prevents sedimentation or creaming, hence offering increased stability (Mehta et al. 2009).

In contrast, emulsions are thermodynamically unstable systems with droplet size larger than 100 nm (Sharma and Shah 1985). These systems tend to coalescence and creaming and are optically turbid or milky in color (Rao et al. 2009).

The aim of the present work was to formulate and characterized aqueous microemulsions containing three nonionic surfactants and geranium EO or geraniol (the EO main constituent) as dispersed phase. We also evaluated the acute and residual toxicity of emulsions and microemulsions against larvae from *C. pipiens pipiens*.

Materials and methods

Compound

Brij 35, Tween 80, Cremophor EL, and Geraniol were purchased from Sigma-Aldrich Commercial. Essential oil namely

geranium was from Swiss-Just (manufactured under supervision and control of Ulrich Justrich AG, Walzenhausen, Switzerland).

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 *C. pipiens pipiens*. The larvae were maintained under suitable temperature for acclimatization.

Construction of phase diagram

The MEs were formed using five components: geraniol or geranio EO as the oil phase, a mixture of surfactants (Cremophor EL, Brij 35, Tween 80; 1:1:2), and double distilled water as the aqueous phase.

Pseudoternary phase diagrams were constructed keeping the ratio of surfactants constant and varying the remaining two components. For convenience, the diagrams were graphed by drawing “water dilution lines” representing increasing water content and decreasing surfactants levels. The mixture of surfactants/oils titrated with water along dilution lines drawn from the surfactants apex (100 % mixture of surfactants) to the opposite oil side of the triangle (100 % oil). The line was arbitrarily denoted as the value of the line intersection with the oil scale (e.g., 20:80, 30:70).

If turbidity appeared followed by a phase separation, the samples were considered to be biphasic so they were not graphed in the phase diagram. In contrast, transparent (monophasic) and low viscosity samples were marked as points in order to determine the ME region of existence. Furthermore, the mixtures which correspond to each points in the diagram were examined by ocular inspection in a cross polarizer to determine birefringence. The MEs appeared completely dark when observed under a cross polarizer so these observations confirm that all of the formulations that constitute de ME region were optically isotropic colloidal dispersions.

Droplet size

The average droplet size and the polydispersity index (PDI) of the MEs were measured by dynamic light scattering (DLS) using a Zetasizer nano instrument ZEN 3690 model (Malvern, UK). Each measurement was carried out at room temperature in triplicate.

Transmission electron microscopy

The morphology of the MEs was determined by transmission electronic microscopy. A drop of the diluted samples was

transferred onto a carbon-coated copper grid, followed by negative staining with uranyl acetate solution for 1 min. After the replica was dried at room temperature, 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.

Acute larvicidal activity

Bioassays were performed using WHO (1996) with four instar larvae of *C. pipiens pipiens*. Microemulsions and emulsions (recorded in Table 1) and geraniol and geranium EO alone were added to plastic container with 80 ml of tap water. The concentration ranged from 10 to 130 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 surfactants alone (in the corresponding ratio). Mortality data were subjected to probit analysis in order to obtain LD50 and LD99 using SPSS 15.0 statistical software. The LD50 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 solutions of the emulsion (namely GOH 70 and Ger 70), microemulsion (GOH 20 and Ger 20), and geraniol and geranium EO were prepared using the LD99 values and maintained in darkness at 27 ± 2 °C and 60–70 % RH. During 28 days, the larvicidal effects against four instar larvae of *C. pipiens pipiens* was evaluated as above described. Percentage of mortality was recorded after 24 h.

Results and discussion

Characterization of microemulsions

The phase diagrams of the water/surfactant/oil (geranium EO or geraniol) at 25 °C are shown in Fig. 1a, b. Only microemulsion points were plotted, so the shaded area in the figure referred to the ME region while the outside area indicates multiphase turbid regions. The ME region for geranium EO was larger than the area of geraniol. This could be due to the presence of different solubilization enhancers present in the EO such as short-chain alcohols (Edris and Malone 2013), which may act as co-surfactants by further reducing the interfacial tension also by partitioning between the surfactants molecules in the interface and changing the curvature of the interfacial layer (Sottman and Stubenrauch 2008).

In order to analyze the biological activity of the geraniol and geranium EO formulations, 10 samples were prepared: five were microemulsions (three were geranium EO MEs and only two were based on geraniol). The other five mixtures corresponded to oil/water emulsions (Table 1).

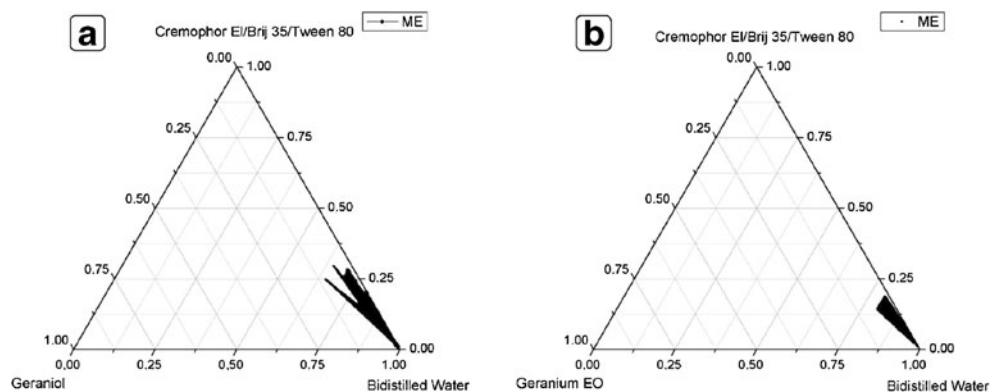
It is known that there is no direct relationship between the particle size of the emulsified droplets and the physicochemical characteristics of the used surfactants (Chaiyana et al. 2010); so the smallest droplet size that can be achieved is fundamentally a function of the system composition. The novel microemulsions obtained in this work included a mixture of three nonionic biocompatible surfactant: a polysorbate (Tween 80), a polyoxyethylene alkyl ether (Brij 35), and a polyoxyethylene castor oil (Cremophor EL).

Mean particle size and particle size distribution are the most important parameters in designing a novel formulation and are routinely the first to be measured. The saturation solubility and the rate of dissolution are strongly dependant on particle size and, accordingly, are the biological performance, the physical stability, and the shelf life of the product (these parameters would be improved with small sizes) (Sasson et al. 2007).

Table 1 General aspects of the designed formulations

Formulation	Oil/surfactant relation (g)	Diam. size (nm)	General properties
GOH 10 %	1:9	8.2	Transparent microemulsion
GOH 20 %	2:8	10.1	Transparent microemulsion
GOH 30 %	3:7	154.1	Turbid emulsion
GOH 35 %	3.5:6.5	356.7	Turbid emulsion
GOH 70 %	7:3	482.6	White emulsion
Ger 10 %	1:9	9.9	Transparent microemulsion
Ger 20 %	2:8	11.1	Transparent microemulsion
Ger 30 %	3:7	13.8	Transparent microemulsion
Ger 35 %	3.5:6.5	186.1	Turbid emulsion
Ger 70 %	7:3	525.3	White emulsion

Fig. 1 Pseudoternary phase diagram of surfactant (Brij 35, Tween 80, and Cremophor L), bidistilled water and **a** geraniol or **b** geranium EO. The shaded area represents the microemulsion region



A complete picture of the size population and distribution could be obtained by analysis of data generated by DLS, also refer as photon correlation spectroscopy, which allows to measure diameters in the range 3–3000 nm. DLS can also be used for the determination of the PDI which is the ratio of standard deviation to mean droplet size (Patel et al. 2013). A PDI value of 0.1–0.25 indicates a narrow size distribution whereas a PDI >0.5 indicates a very broad distribution (Sasson et al. 2007).

The droplet sizes and the macroscopic properties of the systems are shown in Table 1. The smallest sizes were obtained with the highest surfactant/oil ratio (9:1). When large amounts of surfactants are adsorbed to form the interface, a negative interfacial tension results and energy is available to increase the interfacial area, effectively reducing the droplets sizes (Sottman and Stubenrauch 2008).

The MEs showed dispersed phase diameters in the range of 8 to 14 nm and had low PDI values (<0.2). This indicates the uniformity of the drops within the formulations that would approached to monodisperse stable systems which probably would have an effective larvicidal activity owing to the largest surface area of the internal oil phase (active ingredient).

The macroscopic properties described in Table 1 depend, basically, on droplet size and their interactions with the visible light; it is known that when the dispersed phase diameter is lower than 20 nm it may be obtained transparent systems, so no interaction occurred; with higher diameter, the particles can interact with the light producing optical effects.

The TEM images of GOH 20 and Ger 20 microemulsion are shown in panels a and b of Fig. 2, respectively. They revealed that droplet sizes were in nanometric range (according with DLS results) and had nearly spherical morphology with regular distribution.

Insecticidal activity

One of the major drawbacks to the use of chemical insecticides for larval mosquitoes control is the potential risk of environmental contamination and indiscriminate effects of

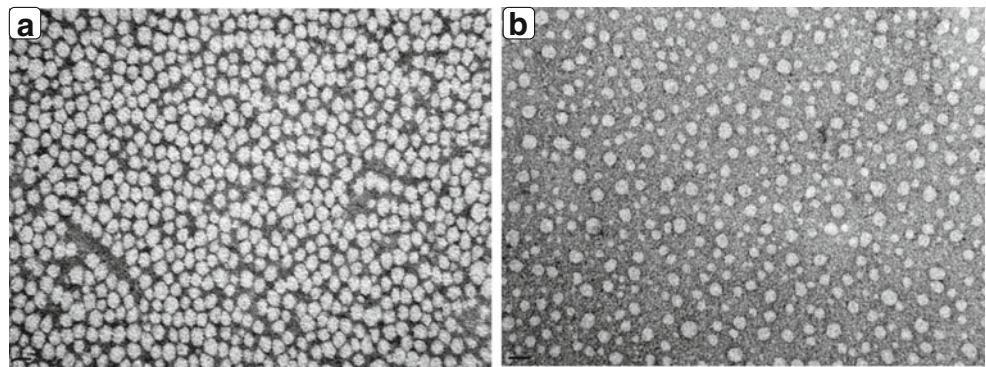
nontarget organisms. Many studies have been conducted to identify safer larvicides, being the EO good candidates for this purpose. They have the potential of being acute ovicidal, fumigant, insect growth regulator, and insecticidal against various insect species (Prajapati et al. 2005) and concurrently being developed as ecologically sensitive pesticides (Isman 2000). Their physicochemical characteristics facilitate them to interfere with basic metabolic, biochemical, physiological, and behavioral functions of insects (Nishimura 2001). Due to their lipophilic nature, larvicides based on EO have to be formulated to effectively apply in water.

Consequently, in this work, aqueous microemulsions were designed where the larvicidal principles (geraniol and geranium EO) were dispersed on a water phase, in order to have high diluted stability upon application. Moreover, the MEs obtained are solvent free and based on surfactants with not ecotoxicity effects due to their high levels of biodegradability (Chan and You 2010).

The LD50 values for fourth-instar larvae of *C. pipiens pipiens* treated with formulation based on geraniol and geranium EO (ME and emulsions) at the end of 24 h are shown in panels a and b of Fig. 3, respectively. For geraniol formulations, the LD50 values of MEs were close to 32 ppm and no significant differences were observed between GOH 10 and 20 ($P < 0.05$); the emulsions present significantly higher values than ME ($P < 0.05$) which were superior to 56 ppm. The LD50 value for geraniol not formulated was 95.94 ppm (CI 84.3–107.5). In the case of geranium EO MEs (Ger 10, 20, and 30), the LD50 values were close to 56 ppm and no significant differences were observed between them ($P < 0.05$), while for emulsions the values were significantly higher than 77 ppm ($P < 0.05$). The LD50 value for geranium EO not formulated was 89.77 ppm (CI 80.6–95.8).

Probably, the higher toxicity effects produced by ME, when compared with emulsions, would be due to their small droplets size which would provide a greater surface area that improves a better penetration into insect tissues and an effective distribution of the active ingredient, enhancing the insecticidal activity (Nel et al. 2006, 2009). This effect would be

Fig. 2 Transmission electron microscopic image of microemulsions. **a** Geraniol; **b** geranium EO

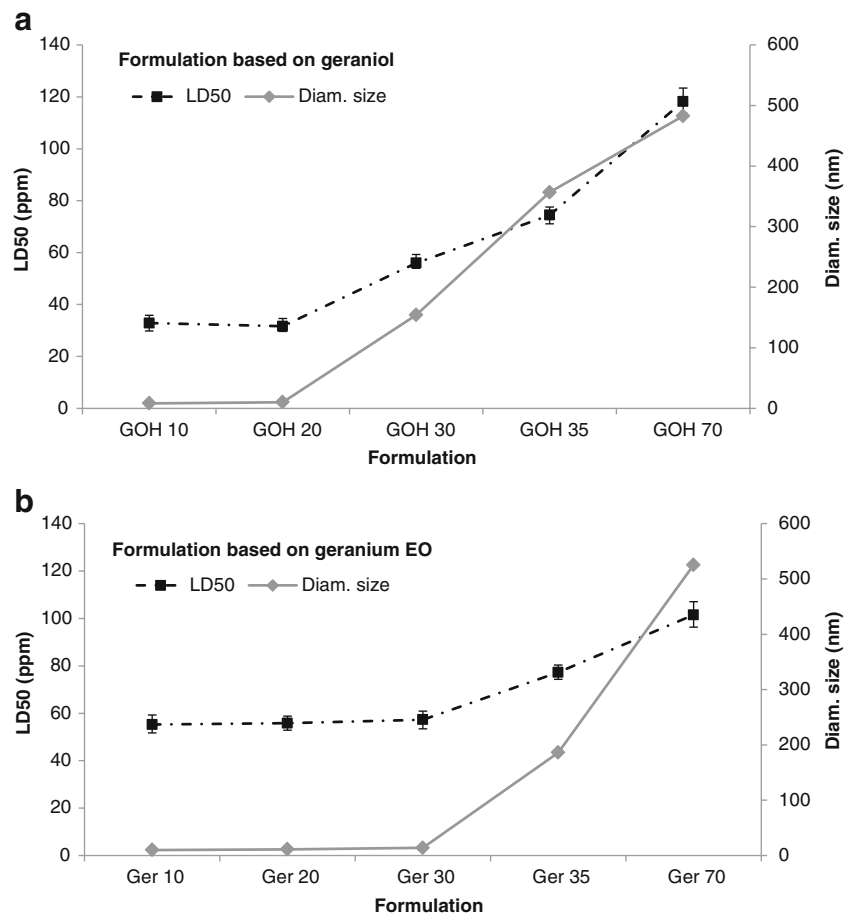


achieved either by faster penetration by direct contact through the insect's cuticle or by ingestion and penetration through the digestive tract (Margulis-Goshen and Magdassi 2012). Furthermore, Okonagi and Chaiyane (2012) informed that the anti-cholinesterase activity of ME based on *Zingiber cassumunar* EO was much higher than that of native oil, exhibiting 20 times higher inhibitory activity.

In addition, these systems have a very high level of thermodynamic stability. They will be formed spontaneously once the right balance between surfactants and

active ingredients is achieved. They would require a minimal mixing during manufacture. The stability of the formulation, apart from reducing the costs of production, removes all of those issues related to phase separation experienced with conventional products which could be seen in the form of creaming in the upper layer or sedimentation in the lower layer. Furthermore, these systems would provide high dilution stability and would leave no deposits in spray tanks so they will not cause spray blockage while being applied.

Fig. 3 Relationship between droplet size and larvial activity. **a** Formulation based on geraniol. **b** Formulation based on geranium EO



We also observed that geraniol formulations were more toxic than geranium EO. It is known that geraniol is a monoterpene with a high insecticide and repellent activity (Chen and Viljoen 2010). The toxicological mechanisms of geraniol (tested in cockroaches) involve neurophysiological effects (Prince and Berry 2006). Furthermore, geraniol proved to be effective in repelling mosquitoes (Omolo et al. 2004; Müller et al. 2009; Qualls and Xue 2009).

In the present work, the residual effects of the ME (Ger 20 and GOH 20), emulsion (Ger 70 and GOH 70) and not formulated active ingredients were also studied, showing that the toxicity was time dependent.

The larvicidal effects of geraniol and its formulation are shown in Fig. 4a. The emulsion was the most effective reaching total efficiency (100 % mortality) until the 7th day of storage, and then, it produced more than 50 % mortality until 25 days after the preparation. In contrast, the geraniol not formulated produced 81.5 % mortality after the first day of storage, and then more than 50 % until the 10th day. Finally, ME only produced 100 % mortality after the first day and then

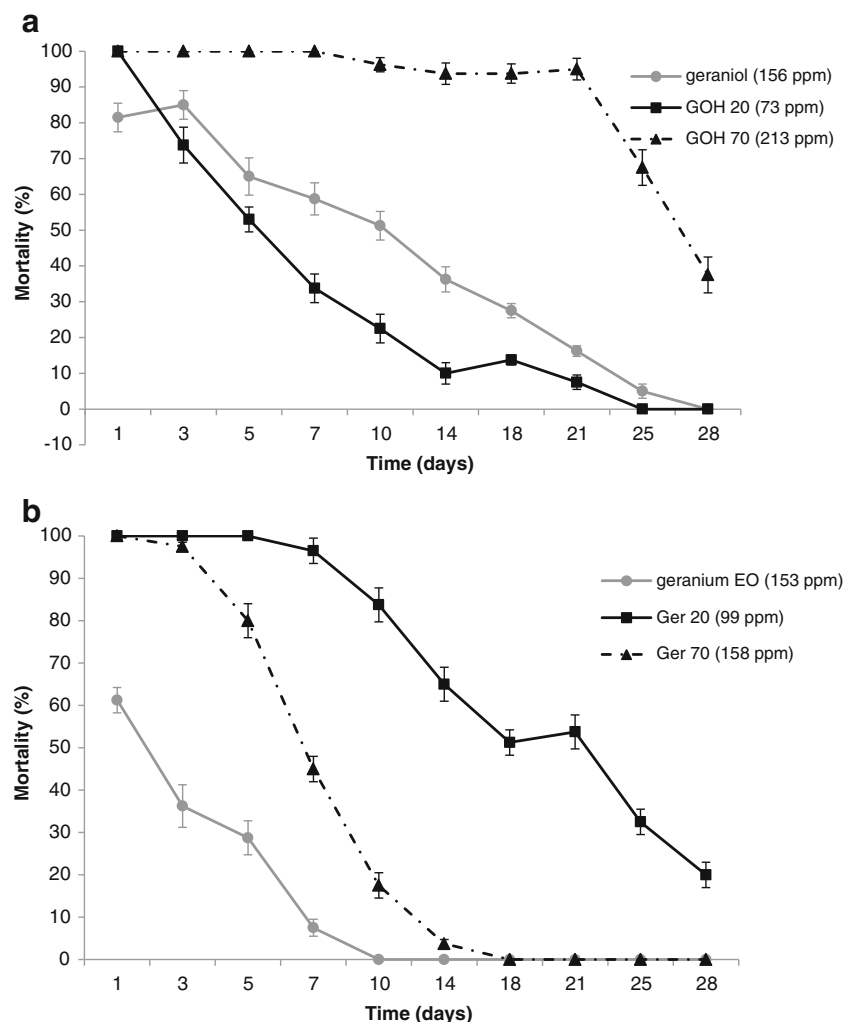
produced more than 50 % until the 5th day of storage. It is possible to summarize that 3 days after the suspension was prepared, the effectiveness for geraniol formulations was emulsion>geraniol not formulated>ME.

In the bioassays containing geranium EO and its formulations, a different situation was observed (Fig. 4b). The most effective formulation was the ME producing 100 % mortality until the 5th day of storage, and then, it produced more than 50 % mortality until 21 days after the preparation. On the other hand, the geranium EO emulsion only produced 100 % mortality after the first day of storage and then produced more than 50 % until the 5th day of storage. Finally, the geranium EO not formulated produced 61.55 % mortality after the first day of storage. For the emulsion and geranium EO not formulated, no more larval mortality was observed 18 days after the dilutions preparation of the samples. In conclusion, the effectiveness for geranium EO was ME>emulsion>geranium EO not formulated.

Considering the previous results, different behaviors of the systems occurred showing distinct residual toxicological

Fig. 4 Residual activity against *C. pipiens pipiens* at LD99.

a Effects of geraniol not formulated, microemulsion (GOH 20) and emulsion (GOH 70) **b** Effects of geranium EO not formulated, microemulsion (Ger 20) and emulsion (Ger70)



profiles: for geraniol, the emulsion was the most effective, and for geranium EO, the microemulsion was systems with more residual activity. This could be explained taking into account the composition of the dispersed phase. Geranium EO contains more than 10 monoterpenes while only one component constitutes the disperse phase of geraniol formulations. In the first case, probably, the diversity of internal forces produced between the monoterpenes and the hydrocarbonated chains of surfactants may minimize disruption in interfacial region when the ME samples were diluted. Consequently, this kind of ME would resist dilution more than those of geraniol. Possibly, geraniol ME collapse when a great dilution occurred reducing the toxicological effects expected to the nanoformulation. In consequence, the mortality could be related to the total amount of geraniol: GOH 70 (213 ppm)>Geraniol not formulated (156 ppm)>GOH (73 ppm).

To conclude, even in the acute toxicological bioassays, the geraniol ME were more toxic than those of geranium EO, the last ones are preferred to be applied as larvicidal formulations due to their stability after the application.

According with our results and taking into consideration that the development time for *C. pipiens pipiens* from egg to adults is 10–14 days (depending on temperature, humidity, and photoperiod) (Becker et al. 2010), once the geranium EO ME would be field applied, the residual toxicity could probably affect the new generation's first stage, controlling them until a new application.

Conclusion

Some properties of the designed microemulsions, such as the low toxicity surfactant systems, the excellent thermodynamic stability, and the extremely small droplet size, make these systems attractive owing to their high levels of biological efficacy, low environmental and operator impact, and relatively low cost of manufacture. The geranium EO microemulsions turn out to be the best option for the integrated pest management of *C. pipiens pipiens*, representing a good alternative to other pesticides for the control of vector-borne diseases. Consequently, the possibility for employing these aqueous nanoformulations may warrant further investigation.

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

References

- Amer A, Mehlhorn H (2006a) Larvicidal effects of various essential oils against *Aedes*, *Anopheles*, and *Culex* larvae (Diptera, Culicidae). *Parasitol Res* 99:466–472
- Amer A, Mehlhorn H (2006b) Persistency of larvicidal effects of plant oil extracts under different storage conditions. *Parasitol Res* 99:473–477
- Becker N, Petric D, Zgomba M, Boaseâ C, Dahl C, Madonâ M, Kaiser A (2010) Mosquitoes and their control, 2nd edn. Springer, Berlin
- Chaiyana W, Saeio K, Hennink WE, Okonogi S (2010) Characterization of potent anticholinesterase plant oil based microemulsion. *Int J Pharm* 401:32–40
- Chan WC, You HY (2010) The influence of nonionic surfactant Brij 30 on biodegradation of toluene in a biofilter. *Afr J Biotechnol* 9:5914–5921
- Chen W, Viljoen AM (2010) Geraniol—a review of commercially important fragrance material. *S Afr J Bot* 76:643–651
- Cheng SS, Chua MT, Chang EH, Huang CG, Chen WJ, Chang ST (2009a) Variations in insecticidal activity and chemical compositions of leaf essential oils from *Cryptomeria japonica* at different ages. *Bioresource Technol* 100:465–470
- Cheng SS, Huang CG, Chen YJ, Yu JJ, Chen WJ, Chang ST (2009b) Chemical compositions and larvicidal activities of leaf essential oils from two eucalyptus species. *Bioresource Technol* 100:452–456
- Cheng SS, Liu JY, Huang CG, Hsui YR, Chen WJ, Chang ST (2009c) Insecticidal activities of leaf essential oils from *Cinnamomum osmophloeum* against three mosquito species. *Bioresource Technol* 100:457–464
- Chung IM, Seo SH, Kang EY, Park SD, Park WH, Moon HI (2009) Chemical composition and larvicidal effects of essential oil of *Dendropanax moribifera* against *Aedes aegypti* L. *Biochem Syst Ecol* 37:470–473
- Conti B, Canale A, Bertoli A, Gozzini F, Pistelli L (2010) Essential oil composition and larvicidal activity of six Mediterranean aromatic plants against the mosquito *Aedes albopictus* (Diptera: Culicidae). *Parasitol Res* 107:1455–1461
- Edris AE, Malone CFR (2013) Preferential solubilization behaviours and stability of some phenolic-bearing essential oils formulated in different microemulsion systems. *Int J Cosmetic Scie*. doi:10.1111/j.1468-2494.2012.00737.x
- Gillij YG, Gleiser RM, Zygadlo JA (2008) Mosquito repellent activity of essential oils of aromatic plants growing in Argentina. *Bioresource Technol* 99:2507–2515
- Govindarajan M (2011) Larvicidal and repellent properties of some essential oils against *Culex tritaeniorhynchus* Giles and *Anopheles subpictus* Grassi (Diptera: Culicidae). *Asian Pac J Trop Med* 4:106–111
- Isman MB (2000) Plant essential oils for pest and disease management. *Crop Prot* 19:603–608
- Jahan N, Razaq J, Jan A (2011) Laboratory evaluation of chitin synthesis inhibitors (Diflubenzuron and Buprofezin) against *Aedes aegypti* larvae from Lahore, Pakistan. *Pakistan J Zool* 43:1079–1084
- Lai F, Wissing SA, Muller RH, Fadda AM (2006) *Artemisia arborescens* L essential oil-loaded solid lipid nanoparticles for potential agricultural application: preparation and characterization. *AAPS PharmSciTech* 7:1–9
- Lucia A, Zerba E, Masuh H (2013) Knockdown and larvicidal activity of six monoterpenes against *Aedes aegypti* (Diptera: Culicidae) and their structure–activity relationships. *Parasitol Res*. doi:10.1007/s00436-013-3618-6
- Maheswaran R, Ignacimuthu S (2013) Bioefficacy of essential oil from *Polygonum hydropiper* L. against mosquitoes, *Anopheles stephensi* and *Culex quinquefasciatus*. *Ecotoxicol Environ Saf* 97:26–31
- Manimaran A, Cruz MMJ, Muthu C, Vincent S, Ignacimuthu S (2012) Larvicidal and knockdown effects of some essential oils against *Culex quinquefasciatus* Say, *Aedes aegypti* (L.) and *Anopheles stephensi* (Liston). *Adv Biosci Biotechnol* 3:855–862
- Margulis-Goshen K, Magdassi S (2012) Nanotechnology: an advanced approach to the development of potent insecticides. In: Ishaaya I, Reddy PS, Rami HA (eds) Advanced technologies for managing insect pests. Springer, New York, pp 295–314

- Mehta SK, Kaur K, Kaur G, Bhasin KK (2009) Percolating phenomenon in microemulsions: effect of external entity. In: Fanun M (ed) Microemulsion: properties and applications. CRC, Boca Raton, pp 59–76
- Moretti MDL, Sanna-Passino G, Demontis S, Bazzoni F (2002) Essential oil formulation useful as a new tool for insect pest control. APS Pharma Sci Tech 3:1–11
- Müller GC, Junnila A, Butler J, Kravchenko VD, Revay EE, Weiss RW, Schlein Y (2009) Efficacy of the botanical repellents geraniol, linalool, and citronella against mosquitoes. J Vector Ecol 6:2–8
- Nel AE, Xia T, Madler L, Li N (2006) Toxic potential of materials at the nanolevel. Science 311:622–627
- Nel AE, Madler L, Velegol D, Xia T, Hoek EM, Somasundaran P, Klaessig F, Castranova V, Thompson M (2009) Understanding biophysicochemical interactions at the nano-bio interface. Nature Mater 8:543–557
- Nerio L, Olivero-Verbel J, Stashenko E (2010) Repellent activity of essential oils: a review. Bioresource Technol 101:372–378
- Nishimura H (2001) Aroma constituents in plants and their repellent activities against mosquitoes. Aroma Res 2:257–267
- Okonagi S, Chaiyana W (2012) Enhancement of anti-cholinesterase activity of *Zingiber cassumunar* essential oil using a microemulsion technique. Drug Discov Ther 6:249–255
- Omolo MO, Okinyo D, Ndiege IO, Lwande W, Hassanali A (2004) Repellency of essential oils of some Kenyan plants against *Anopheles gambiae*. Phytochemistry 65:2797–2802
- Park HM, Park IK (2012) Larvicidal activity of *Amyris balsamifera*, *Daucus carota* and *Pogostemon cablin* essential oils and their components against *Culex pipiens pallens*. J Asia-Pacific Entomol 15:631–634
- Patel RB, Patel MR, Bhatt KK, Patel BG (2013) Formulation and evaluation of microemulsion-based drug delivery system for intranasal administration of olanzapine. Int J Biomed Pharma Sci 7:20–27
- Pavela R, Vrchotová N, Triska J (2009) Mosquitocidal activities of thyme oils (*Thymus vulgaris* L.) against *Culex quinquefasciatus* (Diptera: Culicidae). Parasitol Res 105:1365–1370
- Prajapati V, Tripathi AK, Aggarwal KK, Khanuja SPS (2005) Insecticidal, repellent and oviposition-deterrent activity of selected essential oils against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. Bioresource Technol 96:1749–1757
- Prince D, Berry M (2006) Comparison of effects of octopamine and insecticidal essential oils on activity in the nerve cord, foregut, and dorsal unpaired median neurons of cockroaches. J Insect Physiol 52: 309–319
- 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
- Qualls WA, Xue RD (2009) Field evaluation of three botanical repellents against *Psorophora ferox*, *Aedes atlanticus*, and *Aedes mitchellae*. J Am Mosq Control Assoc 25:379–381
- Rao YS, Deepthi KS, Chowdary KP (2009) Microemulsions: a novel drug carrier system. Int J Drug Deliv Technol 1:39–41
- Sasson Y, Levy-Ruso G, Toledano O, Ishaaya I (2007) Nanosuspensions: emerging novel agrochemical formulations. In: Ishaaya I, Nauen R, Horowitz AR (eds) Insecticides design using advanced technologies. Springer, Dordrecht, pp 1–32
- Senthilkumar A, Venkatesalu V (2012) Larvicidal potential of *Acorus calamus* L. essential oil against filarial vector mosquito *Culex quinquefasciatus* (Diptera: Culicidae). Asian Pac J Trop Dis 2: 324–326
- Senthilkumar A, Kannathasan K, Venkatesalu V (2008) Chemical constituents and larvicidal property of the essential oil of *Blumea mollis* (D. Don) Merr. against *Culex quinquefasciatus*. Parasitol Res 103: 959–962
- Sharma MK, Shah DO (1985) Introduction to macro- and microemulsion. In: Shah DO (ed) Macro- and microemulsions: theory and applications. ACS Symposium Series 272. American Chemical Society, Washington, pp 1–18
- Sottman T, Stubenrauch C (2008) Phase behaviour, interfacial tension and microstructure of microemulsions. In: Stubenrauch C (ed) Microemulsions. Background, new concepts, applications, perspectives. Wiley, Oxford, pp 1–47
- Srinivasa Rao Y, Sree Deepthi K, Chowdary KPR (2009) Microemulsions: a novel drug carrier system. Int J Drug Deliv Technol 1:39–41
- Stackelberg PE, Kauffmann LJ, Ayers MA, Baehr AL (2001) Frequently co-occurring pesticides and volatile organic compounds in public supply and monitoring wells, southern New Jersey, USA. Environ Toxicol Chem 20:853–865
- Tripathi AK, Upadhyay S, Bhuiyan M, Bhattacharya PR (2009) A review of essential oils as biopesticide in insect-pest management. J Pharmacog Phytother 1:52–63
- Vandame TF (2002) Microemulsions as ocular drug delivery systems: recent developments and future challenges. Prog Retin Eye Res 21: 15–34
- Vatandoost H, Dehkordi AS, Sadeghi SMT, Davari B, Karimian F, Abai MR, Sedaghat MM (2012) Identification of chemical constituents and larvicidal activity of *Kelussia odoratissima* Mozaffarian essential oil against two mosquito vectors *Anopheles stephensi* and *Culex pipiens* (Diptera: Culicidae). Exp Parasitol 132:470–474
- Werdin González JO, Gutiérrez MM, Murray AP, Ferrero AA (2011) Composition and biological activity of essential oils from Labiatae against *Nezara viridula* (Hemiptera: Pentatomidae) soybean pest. Pest Manag Sci 67:948–955
- Werdin González JO, Laumann RA, da Silveira S, Moraes MCB, Borges M, Ferrero AA (2013) Lethal and sublethal effects of four essential oils on the egg parasitoids *Trissolcus basalidis*. Chemosphere 92:608–615
- WHO (1996) Report of the WHO informal consultation on the “Evaluation and Testing of Insecticides”. WHO, Genova