Ecotoxicology and Environmental Safety 130 (2016) 11–18

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

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

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ARTICLE INFO

Article history:
Received 3 February 2016
Received in revised form
22 March 2016
Accepted 29 March 2016
Available online 7 April 2016

Keywords:
Blatella germanica
Polymer-based nanoparticles
Geranium and bergamot essential oils
Repellency
Nutritional physiology

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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http://dx.doi.org/10.1016/j.ecoenv.2016.03.045
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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 nontoxic 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

Table 2
Z-average size, polydispersion index (PDI) and loading efficiency of the PEG-based EO nanoparticles.

Product	Size (ηm)	PDI	Loading efficiency (%)
Geranium NP	234	0.268	82
Bergamot NP	184	0.198	78

nanoparticles (NP) are presented in Table 2.

2.4. Determination of sublethal concentration

To evaluate the contact toxicity of EOs and NPs against adult males of *B. germanica*, similar procedures previously described by Werdin González et al. (2015) were used. Briefly, plastic containers (7 cm diameter x 5 cm high) were treated with EOs hexanic solutions at 0.075, 0.15, 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 mg/cm^2. Additionally, other containers were treated with NPs (in the solid form) at 1.0, 2.0, 3.5, 6.5, 13.0, 16.0 and 20.0 mg/cm^2 (equal concentrations). Plastic container treated with hexane or PEG 6000 alone (processed as in 2.3.) were used as controls. For each concentration and controls, six independent replicates were performed. Then, six adult's males were introduced in each plastic container. The sex of the insects was determined under magnifying stereoscopic glass (10 × to 50 ×) (*B. gemanica* males are characterized by the presence of asymmetric genital plate on the left side). After 12 h exposure, insect mortalities were recorded; this time was selected taking into account previous mortality data (Werdin González et al., 2015). The mortality data were submitted to probit analysis using the statistical software SPSS 15.0; Lethal Concentration 50% (LC_{50}), LC_{25} and 95% confidence intervals were estimated. The LC values were considered significantly different if their 95% confidence intervals did not overlap.

2.5. Repellent activity

Filter paper discs (18 cm diameter) were divided on two halves: one was treated with EO hexane solution or with NP at LC_{25} concentration (Table 2); the other one was untreated. Then, both halves were fixed together and, to prevent escape of the insects, the experimental arena was delimited with a PVC ring. Two *B. germanica* males were released into the arena in the untreated zone; 2 min were given to acclimate to the experimental arena and then, their behaviour was recorded for 30 min with a video camera. After this period, the papers were stored for use in successive filming sessions that were held for 144 h. Controls were treated with hexane or PEG 6000 (processed as in 2.3.). For each product and controls, six independent replication were performed.

The videos were analyzed using software developed specially, which was able to track all insects inside the arena. The software featured the ability to detect, track, and keep record of the paths of each of the insects, even in conditions where collisions between them occurred, or occlusions caused by the PVC ring walls would not allow the cockroaches to be seen for a little time.

The software had four main parts: region-of-interest (*ROI*) detection, segmentation of the insects, tracking, and statistical analysis.

2.5.1. ROI detection

ROI detection was implemented as a four-step pipeline, which consisted on applying a Canny Edge filter (Canny, 1986) on a video frame, in which contours were detected by applying a border-following algorithm (Suzuki, 1985). Afterwards, nested contours are removed; leaving only the outer ones, and finally the right contour is selected by picking the biggest and roundest between the remaining ones (Fig. 1).

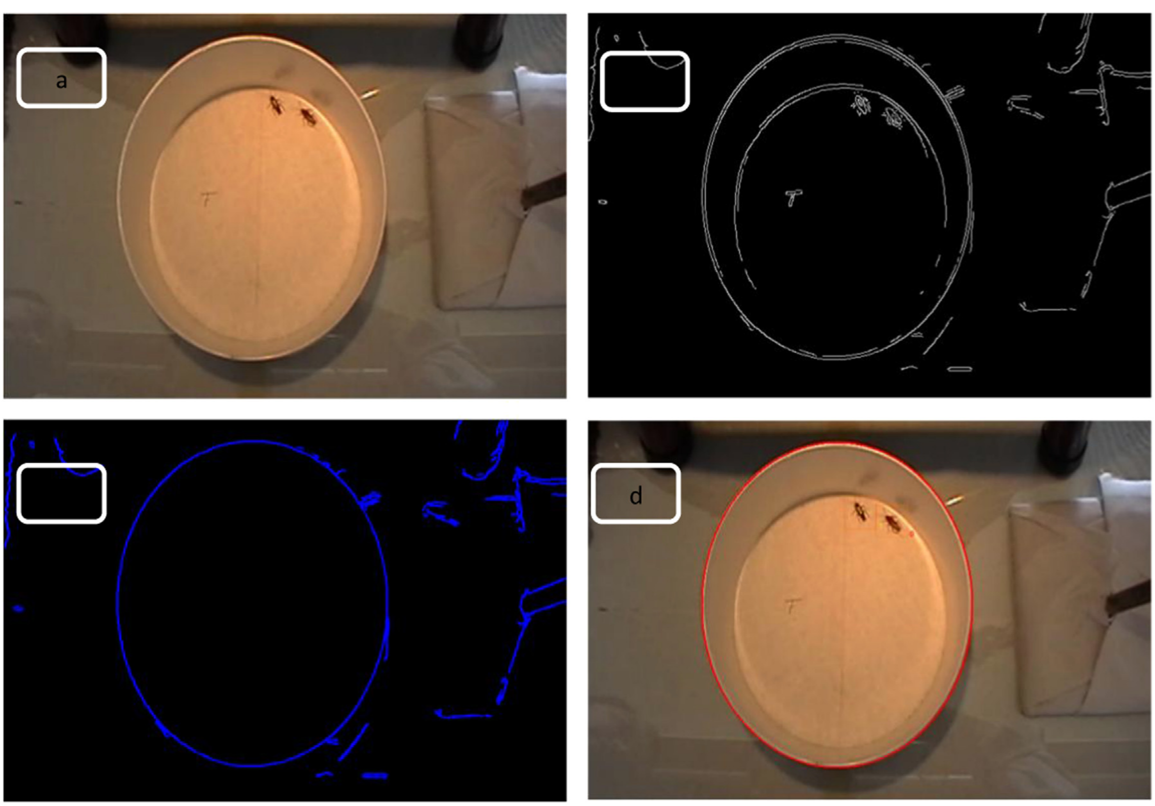

Fig. 1. The ROI detection pipeline. (a) Original video frame, (b) Canny-Edge filtered frame, (c) detected contours on the Canny-Edge image, and (d) selected contour drawn on top of the original video frame.

2.5.2. Segmentation of the insects

The system initially recognizes the cockroaches by applying a clustering algorithm (Mac Queen, 1967) on the pixels inside the ROI that test positively in a comparison against a colour-characteristic centroid. With each insect position now defined by one of the cluster centroids, we trap each one of them inside a bounding box. From now on, every insect will be identified by using their bounding box, movement vector, and a path history that will allow us to draw the trails of each insect and calculate its tortuosity.

2.5.3. Tracking

Every frame, each pixel on a bounding box is compared against the colour-characteristic centroid, capturing the movement of the characteristic pixels inside the boxes (noise is reduced using erosion/dilation techniques). Every bounding box is relocated on the new mean position of the positive pixels found (which is added to the trail history).

When two boxes overlap, the intersection area is ignored. By discarding the overlapping pixels, each bounding box keeps tracking only one insect. Naturally, it could occur that no positive pixels are detected due to a large overlapping area between boxes. In this case, the clustering algorithm is reapplied using every positive pixel inside the ROI. The algorithm returns a new set of centroids that correspond to the centre of each of the colliding roaches. Since several pixels might have been discarded in previous frames due to being in overlapped boxes, the new centroids will probably not match the registered k bounding boxes centres perfectly, and will need to be adjusted. To decide which bounding box corresponds to each insect, a probabilistic model is used: the movement vector of each colliding roach is obtained by analysing its recent movement history. A new hypothetical position is obtained by projecting the movement vector, and each of the bounding boxes are assigned to the free detected centroid closest to the new hypothetical position (Fig. 2).

Each bounding box position is checked to see whether or not an insect has trespassed to the treated area. The total number of trespassing's can be compared to the total number of frames to obtain the time percentage spent on each half of the arena. Because a history of the trail of each insect is stored, and a timestamp is inherently associated for a position on each frame, a tortuosity index can also be provided after the analysis for each half of the arena, and for each cockroach.

2.5.4. Statistical analysis

Finally, the results are expressed as preference index (PI)=(AI-AII)/(AI+AII), where AI is the proportion of pictures that insects remain in the area untreated and CII is the proportion of pictures in the treated area. The significance of PI values (EO, EO-NP and control) were determined by ANOVA and LSD. Values higher than 0.1 indicate that the product generates repellency; values lower than −0.1, attractancy and values between −0.1 and 0.1 are included in the neutral zone (Benzi et al., 2009).

2.6. Nutritional indices and antifeeding activity

Similar procedures to those described by Werdin González et al. (2014) were used to evaluate the antifeeding activity and the alteration in nutritional physiology promoted by the EO or the nanoformulations. Wheat flour discs (1.6 cm diameter) were prepared by taking aliquots (200 µl) from a flour suspension in water (10 g+50 ml) and putting them on plastic dishes that were placed at 27 °C temperature and 60–70% RH during 12 h.

To analyze the EO activity, each flour disc was treated with 5 µl of EOs hexanic solutions to obtain equal concentration of LC_{25} and LC_{50} for the EO alone (Table 2). The disks were allowed to dry for 12 h. Control disks were treated with 200 µl of hexane only. To study the NP effects, the discs were prepared as mentioned above but adding the NPs to the mixture of flour suspension to obtain an equal concentration of LC_{25} and LC_{50} of the NPs (Table 2). The

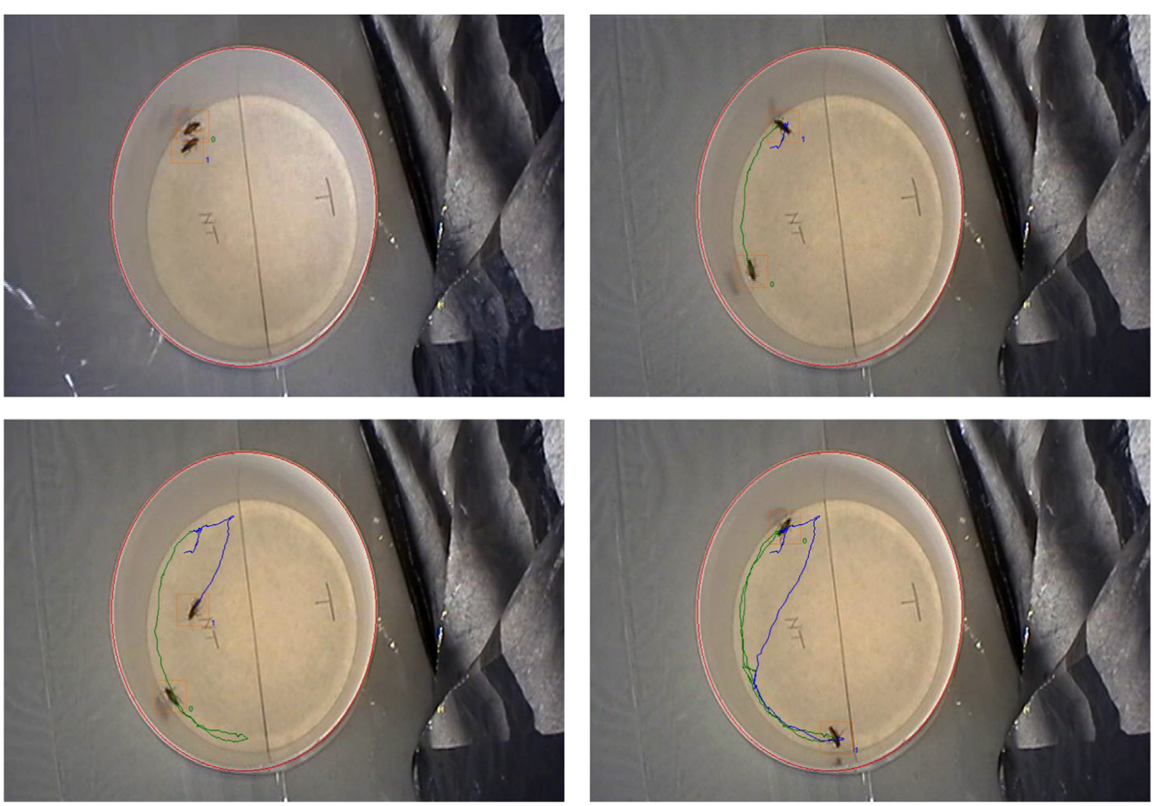

Fig. 2. The system tracking two insects for several minutes. The trail left by each insect can be seen in a distinctive colour.

control group consisted of PEG 6000 alone processed as in 2.3.

Two discs were weighed and put in separated plastic containers (previously weighed). Six adult males of *B. germanica* were put into each container (the insects were starved for 24 h before starting the experiment and weighed before the introduction). During the bioassay, the insect received water *ad libitum*.

After maintaining the insects for 12 h in controlled conditions (27 °C/60–70% RH), the weight of the discs and the container, mortality and the weight of insects alive were registered. Four replicates were prepared.

2.6.1. Nutritional indices and statistical analysis

The nutritional indices were calculated:

- the relative growth rate (RGR): (A–B)/B × day, where A is the final weight of alive insects divided by the number of insects alive after bioassays, and B is the original weight of the insects divided by the total number of insects;
- the relative consumption rate (RCR), which indicates the consumption of the insects related to their initial weight and the duration of the assay: RCR=D/B × day, where D is the biomass ingested (mg) divided by the number of insects alive after bioassay;
- the efficiency of conversion of ingested food (ECI) (%), which indicates the quantity of food used for weight gain in the insects: ECI=(RGR/RCR) × 100;
- the approximate digestibility (AD): (D-F)/F × 100, where, F is the weight of feces produced (estimated by the differences in plastic container weight before and after bioassay).
- a feeding deterrence index (FDI): FDI (%)=(C–T)/C × 100, where C is the consumption of control discs and T is the consumption of treated discs. Positive values expressed a feeding deterrent effect and negative values expressed a feeding stimulant effect.

Data from nutritional indices and antifeeding activity were analyzed by ANOVA and LSD.

3. Results

The geranium NP dispersed in distilled water possessed a Z-average size of 234 ηm with a PDI of 0.268; the NPs presented a loading efficiency of 82%. The bergamot NP active ingredient presented a Z-average size of 184 ηm with a PDI of 0.198 and a loading efficiency of 78% (Table 2).

The LC_{50} and LC_{25} values for EO alone and the NPs on adult males of *B. germanica* are summarized in Table 3. No control mortality was observed during the study. Based on LC_{50} values the toxicity order was geranium NP > geranium EO > bergamot NP > bergamot EO. The slope of the log-dose probit relationship (homogeneity of response) was also reported for each probit analysis (Table 2). A large slope (> 2) indicates a homogenous population and a small slope (< 2) indicates a heterogeneous population (Simon, 2015). The slopes in this study were higher than 2 and no significant differences were observed between them.

To evaluate the repellent effects of EO and NP, a software was specially improved to analyze the videos recording. The system currently detects and tracks the insects effectively in every normal condition presented on the videos, being able to generate percentage about the time spent by each cockroach on treated and non-treated regions of the arena.

Fig. 3 shows the PI values for the EO alone and the polymer-based EO nanoparticles. All those compounds produced significant repellent effects during the first 6 h when applied at the LC_{25} values (P < 0.05). Our results showed that the nanoparticles extended the EO repellent effects against adults males of *B. germanica*.

For the EO alone, geranium EO was more effective than bergamot, showing higher PI values during the first 6 h. Then, both EOs fell in the neutral zone.

The nanoformulation of the EOs enhances the repellent effects of the products; the NPs promote a higher PI and a longer repellency period than the EOs alone. Bergamot NP extended repellency during 72 h, while geranium NP until the end of the bioassay (144 h). During all the experiment, geranium NP was more effective than bergamot NP, showing the highest PI values (Fig. 3).

The statistical analysis of the nutritional indices and the antifeeding activity showed that at LC_{50} concentration, NPs significantly reduced the RGR, RCR, ECI and AD (P < 0.05), while the EOs alone did not modify these nutritional indices (P > 0.05) (Table 4). Moreover, at this concentration, significant differences were found in FDI between NPs and EOs alone (P < 0.05). At LC_{25} concentration, just RGR and RCR were affected by NPs; no differences were observed in the other parameters studied (Table 3).

Each point represents the mean of six independent replicates ± SE. Asterisks indicate a significant difference from the control (P < 0.05).

Table 3
Comparative contact toxicity effects between EO alone and NPs against adults of *B. germanica*. LC_{50} and LC_{25} values (mg/cm^2) obtained with data mortality after 12 h exposure.

Product	LC_{50}[a,b]	LC_{25}[a,b]	Slope ± SE
Geranium EO	0.2983 (0.244–0.352) **b**	0.1808 (0.100–0.231) **a,b**	3.4 ± 0.3
Geranium NP	0.1391 (0.118–0.192) **a**	0.0703 (0.064–0.101) **a**	3.7 ± 0.3
Bergamot EO	0.9474 (0.893–1.181) **d**	0.3162 (0.223–0.418) **b**	3.9 ± 0.4
Bergamot NP	0.5918 (0.495–0.659) **c**	0.1985 (0.153–0.229) **b**	4.0 ± 0.5

[a] The 95% lower and upper confidence intervals are shown in parentheses.
[b] LC values followed by the same letters within the same column are not significantly different.

4. Discussion

The overuse of pesticides in bulk form has led to the contamination of groundwaters, soil, sediments, plants, and animals, besides damaging many non-target organisms (Anjali et al., 2010; Köhler and Triebskorn, 2013). In this context, the restriction on the use of same conventional insecticides along with resistance of arthropod pests are leaving an opportunity in the market place for new, safe, and effective insecticides formulations to control important pests in both agriculture and public health. The application of nanotechnology in insecticide delivery is relatively new and in the early stages of development (Nuruzzaman et al., 2016). In recent works, different polymer-based nanoformulations have been proposed for encapsulation of conventional insecticides and biopesticides (Perlatti et al., 2013). The evaluation of its toxicological profiles on different insect's pest, including lethal and sublethal effects, are a key point to further development and practical application of polymeric nanoinsecticides with huge potential.

The data presented here show that the LC values for the PEG-based EO nanoparticles were lower than those of EO alone; similar responses were observed in previous work (Werdin González et al., 2015, 2014); the high efficacy of the NPs compared with the EO alone could be due to their nanometric size which increased surface area, enabling better penetration into the cuticle of cockroach (Balaji et al., 2015). However, the magnitude of this enhancement was less pronounced that those previously studied. In

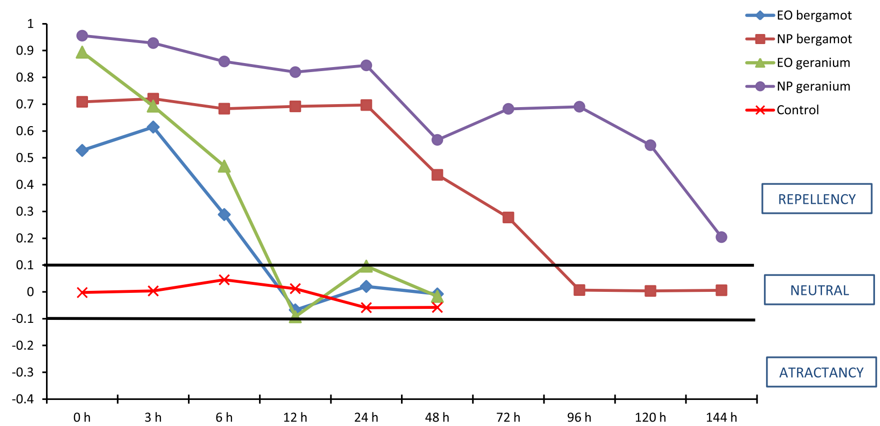

Fig. 3. Repellent effects of EO alone and NPs at LC_{25} on adults of *B. germanica*.

Werdin González et al. (2015) the LC_{50} values for *B. germanica* adults were obtained after 72 h of exposure with a LC_{50}-EO/LC_{50}-NP ratio of 10.57 for geranium EO and 16.11 for bergamot EO. In the present work, the ratio for geranium was 2.14 and for bergamot 1.60 obtained after 12 h of exposure; probably, the lower enhancement of the toxicological activity of the EO induced by nanoformulation observed in this work depend on the exposure time. 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/targated manner and/or C) a protection of the insecticide from premature degradation/ volatilization (de Oliveira et al., 2014; Werdin González et al., 2015, 2014).

Our results clearly showed PEG-based EO nanoparticles exerted sublethal effects against the mechanical vector, *B. germanica,* since NPs enhance repellency and negatively affected nutritional indices and FDI.

New researches aiming to found alternative insect repellents from natural sources are in constant increase (Maia and Moore, 2011). This is due to EOs are believed to be safer than their synthetics counterparts, so they present consumer approval. Moreover, they are considered to be environment friendly (Regnault-Roger et al., 2012). However, the high volatility of the EOs makes them less efficient products than the synthetic repellents. Probably, the polymer-based nanoformulations prevent the fast evaporations of essential oils, so the repellency was enhanced. Even after 144 h, NP geranium was readily capable to produce repellent effects on *B. germanica*. Taking into account that behaviour cockroaches depends on olfactory cues (Bell et al., 2007), these long lasting repellents nanoformulations could be useful to eliminate harborages areas and/or to deter the insects from clean surfaces or food preparations. Moreover, the addition of nanoformulation to the materials used in food packaging could prove to be an effective way of keeping insects away from products (Licciardello et al., 2013). Since some EO (for example mint) are used in the United States as flushing-out agents and for perimeter treatments for controlling cockroaches, ants and termites (Alzogaray et al., 2013), the NP developed in this work could be useful in the context of these strategies aimed to prevent the establishment of pest population.

Growth, development, and reproduction of insects strongly depend on the quality and quantity of food consumed (Parra et al., 2012). The effects of phytochemical products on insect metabolism and the interactions between insects and their food could be elucidated by using feeding indices (Parra et al., 2012; Teimouri et al., 2015). It is known that EO can modify nutritional indices and provoke feeding deterrence in insect; in consequence, the effects of EOs alone and NPs against adults of *B. germanica* were evaluated. The physiological process involved in post-ingestive toxicity and feeding deterrence not necessary have to be related; Koul. (2005) indicates that the behavioural rejection is not an adaptation to post-ingested effects but more an outcome of deterrent receptors with wide chemical sensitivity.

The most useful nutritional indices were found to be RCR, ECI and AD (Parra et al., 2012); RCR is used for measurement exploitation of food by insect, this index shows the rate of feed connected weight in insects at certain time (Teimouri et al., 2015); ECI is an overall measure of an insect's ability to utilize the food ingested for growth and development; and finally, AD measures the assimilation of food ingested (Koul et al., 2004). The reduction

Table 4
Nutritional and feeding deterrence indices of adults of *B. gemanica* exposed to flour discs treated with EO alone and NPs.

Concentration	Treatment	Relative growth rate (RGR)		Relative consumption rate (RCR)		Efficiency of conversion of ingested food (ECI %)		Approximate digestibility (AD %)		Feeding deterrence index (FDI)	
LC_{25}	Control	0.0629	ab	0.0832	a	80.75	a	64.47	a		
	EO geranium	0.1031	a	0.0982	a	101.90	a	48.54	a	−2.50	a
	EO bergamot	0.0867	a	0.1112	a	79.73	a	88.03	a	4.54	a
	NP geranium	0.0303	b	0.0387	b	56.17	a	88.80	a	13.40	a
	NP bergamot	0.0333	b	0.0547	b	49.73	a	41.42	a	34.21	a
LC_{50}	Control	0.0250	a	0.0749	a	35.0	a	83.03	a		
	EO geranium	0.0807	a	0.0854	a	82.37	a	79.41	a	9.19	a
	EO bergamot	0.0744	a	0.0925	a	69.70	a	89.64	a	1.62	a
	NP geranium	−0.0186	b	0.0057	b	−450.00	b	−208.33	b	92.13	b
	NP bergamot	−0.0019	b	0.0112	b	−79.17	b	−937.50	b	85.39	b

Means with the same letters are not significantly different using LSD test at P=0.05.

of RGR, RCR, ECI, ECD promote by NPs have a direct relationship to fecundity and longevity of the adult insect, so it make them susceptible to diseases and natural enemies. Similar effects were informed by Werdin González et al. (2014) when they evaluated the effects of these products against stored products insect's pest. The differences observed between the EO and the NPs could be explained taking account that the products were allowed to dry during 12 h, so many volatiles could be lost from the EO but not from the nanoformulation. Moreover, the amorphous nature of NPs also attributes to its efficacy by enhancing the dissolution rate (Anjali et al., 2010) allowing a better distribution of the product within the insect.

Analysing the overall results from our previous work (Werdin González et al., 2015) and those presented herein, we completed the toxicological profile of the PEG-based EO nanoparticles on *Blatella germanica*. In consequence we demonstrated that these products produced direct contact toxicity increasing the residual activity for 1 year, enhanced the EO repellency and modified negatively the feeding indices on the German cockroaches. Along with other control agents, this novel nanoformulations must be consider for the integrated pest management of this medical insect pest.

Acknowledgements

The authors gratefully acknowledge Universidad Nacional del Sur (PGI, 24/ B187) and Consejo Nacional de Investigaciones Científicas y Técnicas (PIP-CONICET, 112 201201 00625 CO) for the financial support.

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