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Essential oils nanoformulations for stored-product pest control – Characterization and biological properties

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HIGHLIGHTS

- PEG 6000 nanoparticles containing essential oils (EO) are <235 nm in size.
- Nanaoparticles show a loading efficiency >75%.
- Nanoparticles prevent the rapid evaporation and degradation of the EO.
- Nanoparticles promote a sustained controlled release of the EO.
- Nanoparticles enhance the biological effects of the EO against stored products pest.

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ABSTRACT

The lethal and sublethal activity of poly(ethylene glycol) (PEG) nanoparticles containing essential oils (EO), also the physicochemical characterization, were determined against *Tribolium castaneum* and *Rhizopertha dominica*. The 10% ratio EO-PEG nanoparticles showed an average diameter <235 nm (PDI < 0.280) and a loading efficacy >75%; after 6 month of storage their size did not change significantly and the amount of the EOs decreased 25%, approximately. Furthermore, during this period, no chemical derivates were observed. The EOs nanoparticles produced a notable increase of the residual contact toxicity apparently due to the slow and persistent release of the active terpenes. In addition, the nanoformulation enhanced the EO contact toxicity and altered the nutritional physiology of both stored product pest. The results indicated that these novel systems could be used in integrated pest management program for *T. castaneum* and *R. dominica* control.

1. Introduction

Stored foods are prone to postharvest loss in quality and quantity due to infestation by different groups of insects. Among the stored product beetles, *Tribolium castaneum* Herbst. (Coleoptera: Tenebrionidae) is a major secondary pest in storage of grain-based products and *Rhizopertha dominica* Fab. (Coleoptera: Bruchidae) is a major cosmopolitan primary pest of stored cereals, principally corn, rice and wheat (Saroukolai et al., 2010; Edde, 2012). Control of these insects relies heavily on the use of synthetic insecticides such as organophosphates, pyrethroids and fumigants (mainly phosphine and methyl bromide) (Kljajic and Peric, 2006; Islam et al., 2010). These chemicals are simple and cost-effective, but their massive use has caused problems such as resistant behavior and environmental pollution with negative side effects on human health and on arthropods, disrupting biological control (Desneux et al., 2007; Pimentel et al., 2009; Islam et al., 2010; Ali et al., 2012). In order to reduce the effects of conventional synthetic pesticides, biopesticides based on essential oils (EOs) appear to be a complementary or alternative method in crop production and integrated pest management (Tripathi et al., 2009; Werdin González et al., 2011, 2013). EOs showed toxic, repellent and antifeedent effects on stored product insects (Regnault-Roger, 1997; Isman, 2006; Regnault-Roger, 2013). Despite these promising properties, problems related with the EO volatility, poor water solubility, and aptitude for oxidation have to be resolved before they are used as an alternative pest control system (Moretti et al., 2002).

Nanoformulation of the EOs could solve these problems protecting them from degradation and losses by evaporation,

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achieving a controlled release of these products and facilitating their handling (Martín et al., 2010). Furthermore, this kind of formulation is expected to be more effective than the bulk substances (Anjali et al., 2010, 2012). On the other hand, it has been found that pesticide nanoformulation showed less toxicity towards non-target organisms compared with bulk or commercial formulations and therefore a higher specificity was observed (Frederiksen et al., 2003).

Nanoparticles (NPs) can be classified on the basis of the kind of material into metallic, semiconductor and polymeric nanoparticles (Liu, 2006); the last ones are the most promising for EOs nanoformulation. Their sizes vary from 10 to 1000 nm (Soppimath et al., 2001). In this work, poly(ethylene glycol) (PEG) was used as coating or carrier material for NPs formulation. It was selected because its wide range of solubility, lack of toxicity, absence of antigenicity and inmunotoxicty, non-interference with enzymatic activities and conformations of polypeptides and ease of excretion from living organisms (Danprasert et al., 2003). PEGs are water soluble synthetic polymers based on oxyethylene, with the general structure $H\text{-}[\text{-}0\text{-}CH_2\text{-}CH_2]_n\text{-}OH$. These materials are available in a wide range of molecular weights, ranging from liquids at room temperature (PEG 200-600), semisolids (PEG 1500), semicrystalline solids (PEG 3000–20 000 and above) and resinous solids for higher molecular weights (>100 000) (Craig, 1995). Recently, nanosystems based in PEG have noticed their potential in insect control (Yang et al., 2009; Loha et al., 2012). Other studies reported the insecticidal activity of various NPs on stored pest; Stadler et al. (2010) successfully applied nano-alumina against *R. dominica* and *Sitophilus oryzae* L. (rice weevil) (Copeloptera: Curculionidae). Silica, aluminum oxide, zinc oxide and titanium dioxide NPs and silver NPs synthesized using an aqueous leaves extracts were also found to be effective against *S. oryzae* (Goswami et al., 2010; Debnath et al., 2011; Abduz Zahir et al., 2012).

The aim of this study was to obtain and characterize polymeric nanoparticles containing essential oils (EOs-NPs) and to evaluate different biological activities against adults of *R. dominica* and *T. castaneum* compared with the EOs alone.

2. Materials and methods

2.1. Compounds

Commercial essential oils namely geranium and bergamot 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).

2.2. Insects

R. dominica and *T. castaneum* used in this study were obtained from Laboratorio de Zoología de Invertebrados II (Universidad Nacional del Sur) cultures maintained for the last 10 years. *R. dominca* was reared on wheat whole grains, maintained in the dark in incubators at 30 ± 2 °C and 70–80% RH (Dalvo, model MCI/2 V.c.a 220, Argentina). *T. castaneum* was reared on wheat flour mixed with yeast and (13:1, w/w) and maintained at 27 ± 2 °C, 60–70% RH and a 12: 12 h L: D photoperiod. Adults used in all experiments were 3–4 d old, of unknown sex and matting status.

2.3. Essential oils – nanoparticles (EOs-NPS) preparation

EOs-NPs were prepared according to the procedure previously described by Yang et al. (2009) with modifications. 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, 5.0, 7.5, 10.0, 12.5 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. Then, the mixture was cooled at −4 °C for 2 h in order to form the NPs spontaneously; 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.

2.4. EO-NPs characterization

2.4.1. EOs loading efficiency

Aliquots of PEG 6000 and geranium or bergamot EOs were diluted with absolute ethanol–H_2O (75:25) in the same relation above mentioned (see Section 2.3), and heated at 50 °C for 30 min. A serial dilution was made to obtain different concentrations for each mixture. The colorimetric assay was carried out for absorbance of the respective concentration using a UV–visible spectrophotometer [Shimadzu UV- 1203 photometer with the Kinetics-2-Program Pack P/N (206-62029-10; Shimadzu Corp., Kyoto, Japan)] at 290 nm. It was obtained the calibration plot of concentration versus absorbance of EOs-PEG.

After 2 d of storage, EOs-NP samples (0.1 g per part) were dissolved separately in 2 mL of absolute ethanol–H_2O (75:25). The mixture was heated at 50 °C for 30 min, to completely dissolve. The absorbance of the solution was determined at 290 nm and the obtained values were compared to that of the standard curve. The loading efficiency of EO was calculated comparing these observations with the original quantity of EO incorporated. Each test was repeated four times. For NP prepared with 10% ratio EO to PEG, the EO content was recorded during the storage process at 8, 16 and 24 weeks.

2.4.2. EO-NPs size

The average size and size distribution were determined using a dynamic light scattering particle size analyzer (LB-550, Horiba, Japan) at 25 °C. After 2 d of storage, 0.2 g samples of each EOs-NPs type were suspended in 10 mL distilled water for 30 min. Then, the dispersion was filtered using filter paper Wathman No. 1. Each test was repeated four times. For 10% EOs-NPs, the size was recorded during the storage process at 8, 16 and 24 weeks.

2.4.3. EO composition pre-/post-nanoformulation

The chemical composition of each oil pre-/post-nanoformulation was determined by Gas chromatography-mass spectrometry. For the extraction of the oil from the 10% EOs-NPs, 0.5 g of each formulation were dissolved in 5 mL distilled water and heated at 50 °C for 30 min.; then, was added 4 mL of absolute ether to recollect the EO extracted.

The compounds were identified comparing their retention indices (Kovats Indices) with those of known compounds and also comparing their mass spectra with those stored in the MS databases (NBS75K.L MS DATA). Relative percentage amounts were obtained directly from GC peak areas. GC–MS analyses were performed with a Hewlett-Packard 6890 chromatograph connected to a Hewlett-Packard 5972A mass spectrometer equipped with a capillary column (HP-5, 25 m × 0.25 mm, 0.25 μm film thickness). The carrier gas was helium with flow 1 mL min^{-1}. The GC oven temperature was held at 50 °C for 2 min, programmed at 5 °C min^{-1} to 200 °C, then held at this temperature for 15 min. Mass spectra were recorded at 70 eV. Mass range was from m/z 35 to 350 amu. The temperature of the injection block was 280 °C.

2.5. Biological activity of EO and EO and EOs-NPs against adults of T. castaneum and R. dominica

2.5.1. Residual contact toxicity

Samples of 20 g wheat were treated with geranium or bergamot EOs alone or 10% EOs-NPs. Against *T. castaneun*, the EOs concentrations ranged from 0.05% to 0.25% and the EOs-NPs from 0.6% to 3% (w/w) (equal concentrations taking account the EO loading efficiency). In the case of *R. dominica*, the EOs concentrations were from 0.003% to 0.05% and the EOs-NPs from 0.04% to 0.6% (w/w).

For the EOs, the wheat samples were treated with 3 mL of the hexane EOs solutions and were allowed to dry during 1 h, before being placed in 100 mL airtight glass container. EOs-NPs were mixed with the wheat and vigorously shaken to spread the compounds (this procedure was repeated weakly). Wheat samples treated with hexane or PEG 6000 alone (processed as in Section 2.3.) were used as controls.

The samples were stored at 27 ± 2 °C, 60–70% RH for 6 months. The bioassays were conducted periodically (1 d, 1 week, and 2–24 weeks). For each period, 20 insects were introduced in the glass container. Mortality, defined as the inability to move when disturbed, was recorded after 72 and 120 h. Four independent replicates were performed.

2.5.2. Fumigant toxicity

The fumigant toxicity of the EOs and 10% EOs-NP were evaluated in an enclosed chamber. 5 g wheat was impregnated with 1 mL of the hexanic EO solutions (0.25% for *T. castaneum* or 0.05% for *R. dominica*) and were allowed to dry during 1 h. The EOs-NPs were mixed with the wheat (3% for *T. castaneum* or 0.6% for *R. dominica*). Afterwards, the wheat was being placed on the bottom of a Petri dish (8.5 cm diameter × 2 cm high), covered with a lid with a fine wire sieve attached over the central hole, where 20 insects were released. Finally, each Petri dish was covered with another one, and all of them were fitted together with an adhesive film. Each concentration and control were replicated independently 4 times. Insect mortalities were determined after 24, 72 and 120 h.

2.5.3. Nutritional indices and antifeeding activity

Similar procedures to those described by Stefanazzi et al. (2011) were used to evaluate the antifeeding activity and the alteration in nutritional physiology promoted by the EO or the 10% EOs-NP. 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 hexanic solutions at 1 and 2 mg/disc concentrations. The disks were allowed to dry during 12 h. Control disks were treated with 200 μL of hexane only. To study the 10% EOs-NP effects, the discs were prepared as mentioned above but adding the NPs to the mixture of flour suspension to obtain an equal concentration as used to evaluate the EO alone. The control group consisted in PEG 6000 alone (processed as in Section 2.3.).

The discs were weighed and put in separated plastic containers. 10 adult of *T. castaneum* or 15 adults of *R. dominica* previously weighed on a scale (FX/FY series FX400, Frankfurt, Germany) were put into each container (the insects were starved for 24 h before starting the experiment).

After maintaining them for 72 h in controlled conditions for each insect as mentioned in Section 2.2., the weight of the discs, mortality and the weight of insects alive were registered. Four replicates were prepared. The nutritional indices were calculated:

- The relative growth rate (RGR): (A–B)/B × day, where A is the weight of alive insects on the third day divided by the number of insects alive on the third day, 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 on the third day.
- 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.
- 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.

2.6. Statistical analysis

Data from EO loading efficiency and size and from nutritional indices and antifeeding activity were analyzed by Anova and DMS (data mortality were transformed by $\sqrt{x}$). The mortality data from residual contact toxicity obtained at 72 h were submitted to probit analysis using the statistical software SPSS 15.0; Lethal Concentration 50% (LC50) and 95% confidence intervals were estimated. The LC50 values were considered significantly different if their 95% confidence intervals did not overlap.

3. Results and discussion

In this works we found that the incorporation of geranium or bergamot EO into a solid controlled-release nanoformulation using PEG 6000 as a coating material, prevent their rapid evaporation and degradation and enhance their stability and insecticidal activities by contact and ingestion, decreasing the amount of the active principle used due to increase its bioavailability. Considering that an important variable to determine seed's quality is the relative humidity, the solid EOs nanoformulations designed are desirables for control of stored grain insect pests.

3.1. EO-NPs characterization

The sizes, polidispersion index (PDI) and loading efficiency for the 8 EOs-NPs manufactured are resumed in Table 1. The PDI is a measure of the size distribution of nanoparticles; values lower than 0.3 indicate that the NPs have a rather narrow size distribution. The 10% ratio EO-PEG showed the best relationship between the 3 variables analyzed; i.e., had the smallest size, a low PDI (<0.3) and a high EO loading efficiency. Even though 12.5% EOs-NP showed the higher loading efficacy, these NP had the biggest size and the PDI was >0.3.

As a consequence of our results, the 10% EOs-NPs were selected to characterize the size and the EO content and chemical composition during 6 months of storage and to evaluate the biological activity.

After 6 month, the 10% EOs-NPs did not alter significantly their sizes ($P > 0.05$) (Fig. 1). The amount of EOs decreased during this period from 83% to 60% (geranium NP) and from 78% to 52% (bergamot NP).

PEG has a helical conformation consisting of seven chemical units and two turns in a fiber identity period of 1.93 nm (Craig, 1995). In the molten state, PEG chains are randomly orientated. As the melt cools, nucleation can occur by either homogeneous or heterogeneous mechanisms (Chidavaenzi et al., 2001). The addition

Table 1
Nanoparticle (NPs) size, polidispersity index (PDI) and essential oil (EO) loading efficiency of EOs-NPs.

Essential oil	Percentage of EO (w/w%)	NP size (nm)	PDI	Loading efficiency (%)
Geranium	5	411 ± 37 **b**	0.569 ± 0.023	78 ± 5 **cd**
	7.5	481 ± 42 **cd**	0.411 ± 0.050	75 ± 5 **ab**
	10	234 ± 25 **a**	0.253 ± 0.027	83 ± 3 **cd**
	12.5	618 ± 139 **e**	0.323 ± 0.057	84 ± 3 **de**
Bergamot	5	363 ± 40 **bc**	0.601 ± 0.037	77 ± 4 **bc**
	7.5	236 ± 24 **a**	0.309 ± 0.043	71 ± 3 **a**
	10	184 ± 18 **a**	0.279 ± 0.037	78 ± 3 **cd**
	12.5	581 ± 210 **de**	0.619 ± 0.110	89 ± 6 **e**

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

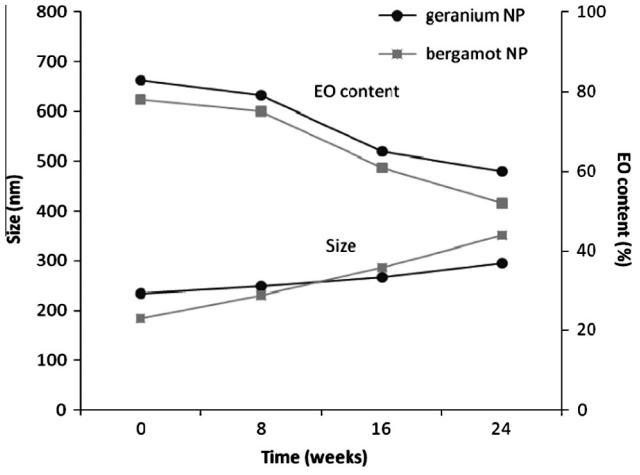

Fig. 1. EOs-NPs size and EOs release profile during 6 month of storage.

of a material (such as the EO) or the fast cooling of the melted PEG (as the method evaluated in this work) could act as an inhibitor of crystallization resulting in a higher percentage of amorphous and imperfectly crystalline material; the amorphous character is common with polymeric molecules used as carrier. This state could contribute to a higher EOs loading efficiency and the storage stability, as has been previously informed in other systems (Westesen et al., 1997; Chidavaenzi et al., 2001). In fact, the 10% ratio EO/PEG has also been the optimal proportion for other nanosystems (Yang et al., 2009).

It is also known that in amorphous materials, diffusive transport processes are usually significantly more rapid than in crystals (Hancock and Zografi, 1997), hence this could explain the release profile of the EO from the NPs observed in our experiments.

Otherwise, no problems were detected when the EOs-NPs were solubilized in distilled water. This could be a consequence of the nanoparticle size and the amorphous state of PEG achieved during nanoparticles formation process, from which is more soluble than its crystalline counter parts (Hancock and Zografi, 1997; Yu, 2001).

The qualitative analyses of both EO (pre/post-formulation) during the 6 month experience were performed by using GC–MS (Table 2). The results indicated that both commercial EOs are complex terpenes (mono- and sesquiterpenes and derivates) mixtures naturally found in the geranium and bergamot fruits and flowers.

It was found that the PEG 6000 could stabilize the EO in a polymeric matrix enable to significantly reduce the volatility of the terpene constituents. Moreover, we also found that the chemical composition of the EO in the nanoformulation was not modify during the storage time, thus were not found oxidized or hydrolytic derivates from the original compound, except the variation on the minor components. This indicate that no breakdown of active components had occur, enhancing the effectiveness of the EOs.

For geranium EO, the major compounds preformulation were citronellol and geraniol (26.14% and 23.19%, respectively). These monoterpenes were maintained as the principal EO components of NP during the 6 month of storage. Three other terpenes were secondarily important: linalool, citronellyl formate and menthone; the last one was not detected in NP of 24 weeks. At this time, the minor components were also not found.

For bergamot EO, the principal component found in the commercial product was linalyl acetate, representing the 58.27%. This compound was the major one found in the NP until the end of the experience. In this case, two other compounds were important: limonene and linalool; the first one decreased its amount after the formulation process and finally, at 24 weeks, it was not detected; on the other hand, linalool was maintained during all the storage time. At the end, the minor components were not found.

In many cases, the activity of essential oils mainly depends on the synergist effects of the major components and it has been demonstrated that terpenoids possess biological activity against several post-harvest Coleoptera (Tripathi et al., 2009; Kostyukovsky and Shaaya, 2011; Regnault-Roger et al., 2012). Previous reports showed that the main monoterpenes loaded in our nanoparticles produced lethal and sublethal effects in *T. castaneum* and *R. dominca* (Rozman et al., 2007; Abdelgaleil et al., 2009; Ukeh and Umoetok, 2011; Zhang et al., 2011).

3.2. Biological activity of EO and EOs-NPs against adults of T. castaneum and R. dominica

The residual effects of EOs and EOs-NPs in both stored-product beetles at the highest concentration are showed in Fig. 2 (data mortality obtained after 120 h exposure). A remarkable increase in the residual toxicity of the EO was achieved by its nanoformulation. Furthermore, the EO formulation using PEG 6000 as polymer matrix enhance the biological activity of these products.

In *T. castaneum*, the geranium and bergamot NP produced contact toxicity during 16 and 12 weeks while the EOs alone just for 4 and 2 weeks, respectively. In *R. dominica*, the bergamot NP caused mortality for 20 weeks while geranium NP during all the experience; moreover, at 24 weeks the last nanoformulation produced more than 50%. In counterpart, the EOs produced mortality during 4 (bergamot) and 6 weeks (geranium).

This could be indicating that an extended EO releases over time was achieved by the nanoformulation. Actually, the pesticide nanoformulation aims towards measured releases of necessary and sufficient amounts of these products for a period of time to obtain the fullest biological efficacy (Ghormade et al., 2011). Furthermore, Isman et al. (2011) pointed out that a principal disadvantage of EO using as pesticide is their lack of persistence which required two or more applications to exert a satisfactory management of pests. The EOs-NPs evaluated in this work will provide an alternative method for EOs application: on the one hand, the frequency may be reduced because its sustained controlled release

Table 2
Chemical composition EO pre/post-nanoformulation (after 1, 12 and 24 weeks) and percentage content of each components.

Retention time (min)	Compound	Pre-formulation (%)	Post-formulation (%)		
			1 week	12 week	24 week
EO geranium					
13.06	Linalool	12.67	8.44	12.47	8.72
14.64	Menthone	11.14	4.16	3.75	–
16.74	Citronellol	26.14	36.10	31.27	35.27
17.48	Geraniol	23.19	43.70	47.37	54.99
17.98	Citronellyl formate	10.27	2.14	3.48	1.02
18.70	Geranyl formate	7.94	1.85	1.66	–
20.83	Geranyl acetate	1.51	0.74	–	
21.88	Caryophillene	2.00	0.45	–	
23.07	Neryl acetate	2.78	0.88	–	
24.36	Citronellyll butyrate	0.78	1.05	–	
25.13	Geranyl butyrate	1.58	0.49	–	
EO bergamot					
9.51	β-Pinene	2.38	–	–	–
11.04	Limonene	17.49	7.95	2.37	–
11.89	3-Carene	4.77	–	–	–
13.06	Linalool	9.46	10.85	14.40	13.05
17.57	Linalyl acetate	58.27	72.83	78.68	86.95
21.88	Caryophillene	7.63	8.37	4.55	–

Table 3
Nutritional and feeding deterrence indices of adults of *T. castaneum* and *R. dominica* exposed to flour discs treated with EO or EOs-NPs (data mortality obtained after 72 h exposure).

Insect	Concentration (mg/disc)	Treatment	Relative growth rate/day (mg mg^{-1} day)	Relative consumption rate/day (mg mg^{-1} day)	Efficiency of conversion of ingested food (%)	Mortality (%)	Feeding deterrence index (%)
T. castaneum	1	Control	0.0129 **a**	0.0778 **a**	3.388 **a**	0	
		EO geranium	0.0087 **a**	0.0726 **ab**	2.738 **a**	0	12
		EO bergamot	0.0106 **a**	0.0645 **ab**	3.309 **a**	0	14
		NP geranium	−0.0245 **b**	0.0553 **b**	−9.162 **c**	0	26
		NP bergamot	−0.0184 **b**	0.0627 **b**	−5.833 **b**	0	19
	2	Control	0.0216 **a**	0.1140 **a**	6.508 **a**	0	
		EO geranium	0.0283 **a**	0.1037 **a**	9.206 **a**	0	11 **a**
		EO bergamot	0.0364 **a**	0.1175 **a**	10397 **a**	0	3 **a**
		NP geranium	−0.0449 **b**	0.0478 **b**	−33889 **c**	0	60 **b**
		NP bergamot	−0.0386 **b**	0.0549 **b**	−23889 **b**	0	54 **b**
R. dominica	1	Control	0.0248 **a**	0.0584	13333 **a**	0 **a**	
		EO geranium	−0.0055 **b**	0.0321	0.388 **b**	0 **a**	41
		EO bergamot	−0.0145 **b**	0.0223	−13011 **b**	0 **a**	60
		NP geranium	−0.0760 **d**	0.0509	−51066 **c**	44 **b**	45
		NP bergamot	−0.0423 **c**	0.0436	−47704 **c**	28 **b**	42
	2	Control	0.0238 **a**	0.0433	17778 **a**	0 **a**	
		EO geranium	−0.0520 **b**	0.0276	−61481 **cd**	32 **b**	57 **ab**
		EO bergamot	−0.0383 **b**	0.0125	−114811 **e**	41 **b**	83 **b**
		NP geranium	−0.0849 **c**	0.0378	−91049 **de**	45 **b**	48 **a**
		NP bergamot	−0.0563 **c**	0.0352	−36148 **bc**	69 **c**	39 **a**

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

pattern; on the other hand, an aqueous application could be done, because, as above mentioned, the NPs are soluble in water; therefore, no auxiliary organic solvent will be required, which are commonly used in the chemical insecticide application and which potentiate the ecotoxicology effects of these harmful products.

The LC50 values obtained from contact toxicity data after 3 d exposure are informed in Fig. 3. In both insects, the toxicity was time depending, decreasing during the experiment. In *T. castaneum*, the LC50 values from geranium NP were lower than those from bergamot but no significant differences were found ($P < 0.05$), except at 8 weeks. Similar effects were observed in *R. dominica*, but LC50 values from geranium NP were significantly lower than bergamot NP from 1 to 12 weeks.

For all experiments, *R. dominica* was found to be more susceptible than *T. castaneum*. This differential toxicity pattern was previously informed for different stored products insect species. Some possible reasons could be the minor size and the slight development of detoxifying metabolic routes and excretion of *R. dominica* for phytochemical and or/the thickness of the cuticle or its composition (Korunic, 1998; Ogendo et al., 2008; Stefanazzi et al., 2011; Hashemi and Safavi, 2012).

The nanoparticles exhibit unique properties compared with their bulk counter parts, including a higher toxicity (Anjali et al., 2010). In this way, we compared the biological efficacy of the EO alone and the EO in the nanoparticles form (Fig. 4). The results indicated that the nanoformulation enhanced the EO contact activity (data mortality obtained after 72 h exposure). In *T. castaneum*, at 1 d both EOs-NPs produced significantly lower LC50 values than the EOs alone ($P < 0.05$), but at 7 d just bergamot NP was more efficient than the oil. In *R. dominica*, a different situation was observed; at 1 d, no significant differences were found between

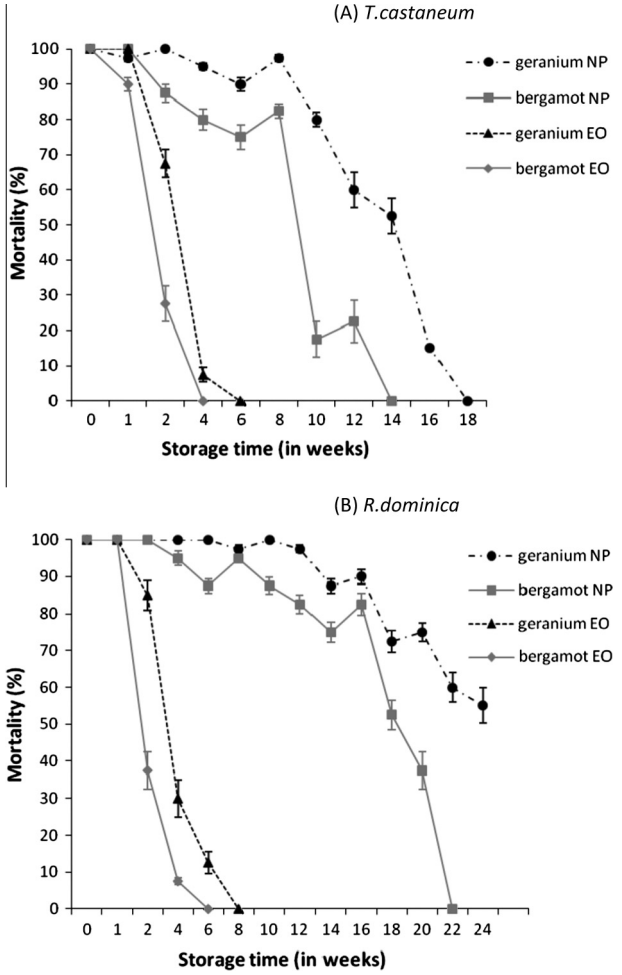

Fig. 2. Residual contact toxicity of EO alone and EOs-NPs at the highest concentration after 120 h exposure against adults of (A) *T. castaneum* at 3% EOs-NPs (equal to 0.25%) EO (B) *R. dominica* at 0.6% EOs-NPs (equal to 0.05% EO).

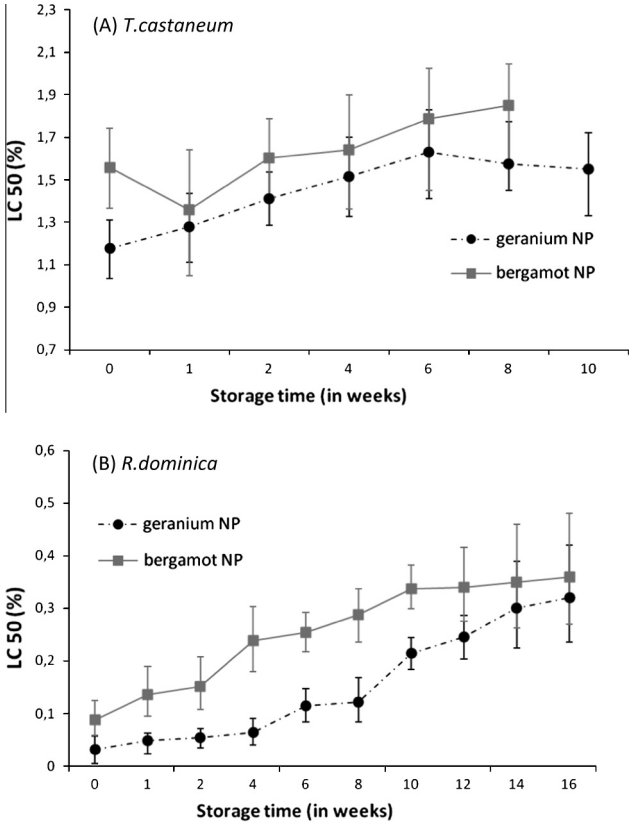

Fig. 3. LC50 values from EOs-NPs against adults of (A) *T. casteneum* and (B) *R. dominica* (data mortality obtained after 72 h exposure).

the EO and its nanoformulations; at 1 week, both formulation produced significant lower LC50 values: the LC50 for the geranium NP was 7.8 times lower than the EO while bergamot NP was 3.6 times. It is known that nanoparticles have a much higher chemical activity than the bulk material. Nanoparticles are also much more mobile, enabling better penetration into insect tissues and enhancing insecticidal activity. This can be achieved either by faster penetration by direct contact through the insect's cuticle, or by ingestion and penetration through the digestive tract (Margulis-Goshen and Magdassi, 2012).

When the fumigant activity was evaluated, both EO produced 100% mortality after 24 h exposure whiles the geranium and bergamot NPs, did not produce any effects after 120 h exposure. This could be indicating that the nanoformulation reduces terpenes volatility from the EO. In this way, Lai et al. (2006) produced solid lipid nanoparticles of *Artemisia arborescens* EO (200–294 nm) which showed controlled release of the products and decreased its rapid evaporation.

It is known that EO can modify nutritional indices and provoke feeding deterrence in insect stored pest; in consequence, the effects of EO-NPs against adults of *T. castaneum* and *R. dominica* were evaluated (Table 3). The physiological process involved in post-ingestive toxicity and feeding deterrence not necessary have to be related; Koul (2005) indicates that the behavioral rejection is not an adaptation to post-ingested effects but more an outcome of deterrent receptors with wide chemical sensitivity.

In *T. castaneum*, statistical analysis showed that at 1 and 2 mg/disc, EOs-NPs significantly affected the RGR, RCR and ECI ($P < 0.05$), while the EOs alone did not modify the nutritional indices ($P > 0.05$). Moreover, geranium NP produced a significantly reduction of ECI compared with bergamot NP ($P < 0.05$). On the other hand, just at 2 mg/disc significant differences were found in FDI between EOs-NPs and EOs alone ($P < 0.05$). Even though, no mortality was found after 3 d exposure. The differences observed between the EO and the EOs-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.

For *R. dominica*, both EO and EO-NP significantly modified the RGR and ECI ($P < 0.05$) but none affected the RCR ($P > 0.05$). At 2 mg/disc, the EOs alone produced the higher FDI, being the oil from bergamot the only one that differenced from EO-NP ($P < 0.05$). At 1 mg/disc, just the EOs-NPs produced mortality; and at 2 mg/disc, the EOs and the NPs produced mortality, but the bergamot NP determined the higher mortality (69%, $P < 0.05$), hence possible post-ingestive toxicity was observed.

Even the penetration mechanism, the level of transfer and the bioavailability of nanoparticles remain poorly understood, especially in insects, some general hypothesis could be done about the toxicological process involved in the biological activity found in this work. Some parameters to take into account to characterize the toxicological profile of the NPs are the penetration pattern, the bioavailability of the NPs and the detoxification mechanism involved, which could explain the enhancement of bioactivity of EO-NPs against this two stored product species.

The unusual physicochemical (and toxicological) properties of NP are attributable to their small size, chemical composition, solubility, aggregation, in others; but the nanosize (and a related high surface area) creates the opportunity for increased uptake and interaction with biological tissues (Nel et al., 2006, 2009). In the

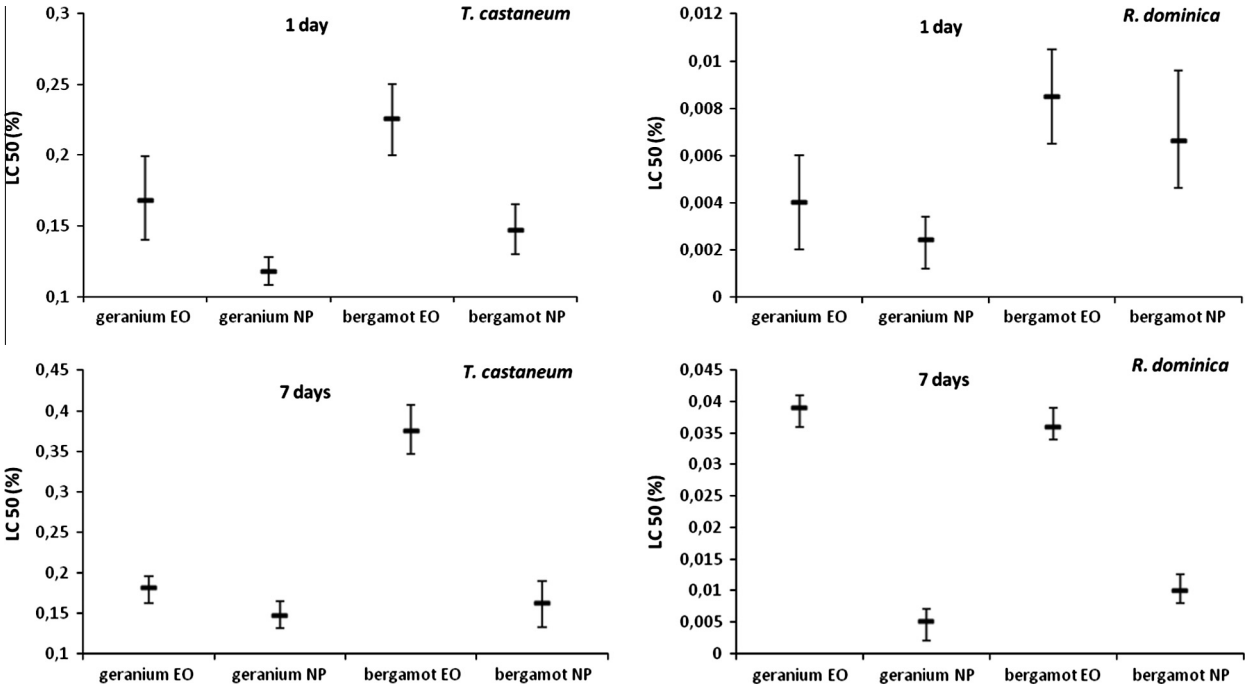

Fig. 4. Comparative contact toxicity effects between EO alone and EOs in the NP form against adults of *T. castaneum* and *R. dominica* at 1 and 7 d of storage (data mortality obtained after 72 h exposure).

case of the EO-NPs designed in this study, and taking into consideration the previous result, the major uptake routes involved in both insects could be the direct contact with the cuticle as the insects moved through the grain. Besides, as no fumigant toxicity was detected, probably low EO-NPs respiratory uptake occurred. Other route involved could be the digestive tract, especially in the case of *R. dominica* because of the post-ingestive toxicity observed.

Insects cuticles is secreted by a single layer of epidermal cells that covers the entire surface of the insect, extending into the tracheal system, fore- and hind-gut, and part of the genital system. It is composed by several layers, from the outside: cement and wax, then epicuticle, then exo- and endocuticle (Vincent and Wegst, 2004). Exo- and endocuticular layers consists of arrangements of highly crystalline chitin nanofibers embedded in a matrix of protein, polyphenols and water, with small amounts of lipid.

For the EOs alone (which are a complex mixture of nonpolar or minimally polar substances) it was proposed that constituents diffuse in the cuticle horizontally and/or vertically. By diffusing horizontally, they reach the tracheae system where they continue moving to the rest of the tissues in the organism and therefore reach their site or sites of action. By diffusing vertically, the substances cross from the tegument to the epidermis and enter to the organism (Sfara et al., 2009; Tarelli et al., 2009).

Terpenes found in the EO studied in this work have an apolar nature thus the presence of the external wax layer could facilitate their horizontal diffusion to the detriment of the vertical diffusion across the solid hydrophilic endocuticle. In contrast, the penetration of EO-NPs could be by horizontal and vertical diffusion since the NPs had a matrix of PEG 6000, which is an amphiphilic polymer with a particular solubility pattern, soluble in water and in some apolar organic solvents. This differential uptake pattern between EO and NP could be associated with the enhancement of the toxicological activity observed in this work. Moreover, as nanoparticles exhibit large specific surface, they can potentially cause higher adhesiveness of EO-NPs to insect's body, increasing the exposure time to the biological active molecules.

In relation with the bioavailability, some studies in mammals demonstrated that PEG 6000 increase this pharmacokinetic parameter for several drugs using PEGylated nanoparticles (Khoo et al., 2000; Zerrouk et al., 2001; Otsuka et al., 2003). It is known that PEGylation is an effective method to improve the delivery and stability of therapeutic molecules in the human internal environment. This technology involves the binding of PEG to active macromolecules, like peptides, proteins, oligonucleotides, and small organic compounds, to improve their water solubility and resistance to hydrolysis, and to reduce renal excretion, immunogenicity and antigenicity (Jevševar et al., 2010).

Jeffers and Roe (2008) proposed that PEG polymers can be used as a new method for increasing the activity of insecticidal proteins. For example, when the decapeptide trypsin modulatingoostatic factor (TMOF) was PEGlyated, it caused an increase of TMOF toxicity to the mosquito larvae *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) and to the lepidopterans neonates of *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae); besides, in *H. virescens* larvae the peptide-PEG conjugated was accumulated in the insect hemolymph (Jeffers et al., 2012). Nachman et al. (2012) demonstrated that pyrokinins analogs (multifunctional neuropeptides) conjugated with two PEG polymers promote their biostability when the pea aphid *Acyrthosiphon pisum* (Homoptera: Aphididae) was feed on a basal diet with the peptide analogs increasing their antifeedant activity.

It is important to note that naturally occurred EOs has a defensive role for the plants that produce them when are attacked by several organisms (Mithofer and Boland, 2012). In order to avoid the toxic effects, metabolism of these substances by insects can therefore represent a system of detoxification, making it possible to tolerate these toxins. Many terpenes found in the geranium and bergamot EOs have been proved to be detoxified by different intracellular biochemical pathway producing substrates more hydrophilic and thus readily excretable by the insect (Hendry, 1996; Miyazawa et al., 1998; Davoudi et al., 2011; Rossi and Palacios, 2013; Passreiter et al., 2004). Moreover, it has been

demonstrated the participation of the P450 oxidizing system in the detoxifying process (Rossi et al., 2012).

It is possible to suggest that when the insects were exposed to the EO-NPs, a decreased detoxifying ratio (compared with terpenes alone) occurred because the NPs kept in the extracellular media not being available to the detoxifying systems. Thus, more bioactive products could reach to the site(s) of action (Isman, 2000; Regnault-Roger et al., 2012) enhancing the toxic effects of the EOs. Summarizing, probably an increase penetration and bioavailability and a decrease detoxification could be promote by the NPs.

Further work need to be conducted to determine the mode of action of EO-NPs and to test the efficacy of EOs components in the nanoparticles form, because of their high potential and environment friendly relationship.

4. Conclusion

Nanotechnology has a huge potential to develop alternative pest control strategy and lower risk insecticidal molecules. The benefits of the EOs-NPs evaluated in this works are the enhancement of efficacy due to higher surface area, sustained controlled release, induction of systemic activity due to smaller particle size and higher mobility, and possibly lower ecotoxicity due to higher solubility in water which promote the elimination of organic solvents used for the application of conventionally pesticides. Moreover, taking into account that EO demonstrated biological efficacy and are eco-friendship products, this new designs could be use to promote the massive use of this biopesticides in grain stored systems.

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References

Abdelgaleil, S.A.M., Mohamed, M.E., Badawy, M.E.I., El-arami, A.A., 2009. Fumigant and contact toxicities of monoterpenes to *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) and their inhibitory effects on acetylcholinesterase activity. J. Chem. Ecol. 35, 518–525.

Abduz Zahir, A., Bagavan, A., Kamaraj, C., Elangi, G., Abdul Rahuman, A., 2012. Efficacy of plant-mediated silver nanoparticles against *Sitophilus oryzae*. J. Biopest. 52 (2012), 95–102.

Ali, A., Ahmad, F., Biondi, A., Wang, Y., Desneux, N., 2012. Potential for using Datura alba leaf extracts against two major stored grain pests, the khapra beetle *Trogoderma granarium* and the rice weevil *Sitophillus oryzae*. J. Pest. Sci. 85, 359–366.

Anjali, C.H., Khan, S.S., Margulis-Goshen, K., Magdassi, S., Mukherjee, A., Chandrasekaran, N., 2010. Formulation of water-dispersible nanopermethrin for larvicida applications. Ecotox. Environ. Safe. 73, 1932–1936.

Anjali, C.H., Sharma, Y., Mukherjee, A., Chandrasekaran, N., 2012. Neem oil (Azadirachta indica) nanoemulsion – a potent larvicidal agent against Culex quinquefasciatus Pest. Manage. Sci. 68, 158–163.

Chidavaenzi, O.C., 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.

Craig, D.Q.M., 1995. A review of thermal methods used for the analysis of the crystal form, solution thermodynamics and glass transition behaviour of polyethylene glycols. Thermochimi. Acta 248, 189–203.

Danprasert, K., Kumar, R., Cheng, M., Gupta, P., Shakil, N.A., Prasad, A.K., Parmar, V.S., Kumar, J., Samuelson, L.A., Watterson, A.C., 2003. Synthesis of novel poly(ethylene glycol) based amphiphilic polymers. Eur. Polym. J. 39, 1983–1990.

Davoudi, A., Shayesteh, N., Shirdel, D., Hosseinzadeh, A., 2011. Effect of diethyl maleate on toxicity of linalool against two stored product insects in laboratory condition. Afr. J. Biotech. 10, 9918–9921.

Debnath, N., Das, S., Seth, S., Chandra, R., Bhattacharya, S., Goswami, A., 2011. Entomotoxic effect of silica nanoparticles against *Sitophilus oryzae* (L.) J. Pest. Sci. 84, 99–105.

Desneux, N., Decourtye, A., Delpuech, J.M., 2007. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 52, 81–106.

Edde, P.A., 2012. A review of the biology and control of *Rhyzopertha dominica* (F.) the lesser grain borer. J. Stored Prod. Res. 48, 1–18.

Frederiksen, H.K., Kristensen, H.G., Pedersen, M., 2003. Solid lipid microparticle formulations of the pyrethroid gamma-cyhalothrin—incompatibility of the lipid and the pyrethroid and biological properties of the formulations. J. Control. Release. 86, 243–252.

Ghormade, V., Desphande, M.V., Paknikar, K.M., 2011. Perspectives for nano-biotechnology enabled protection and nutrition of plants. Biotech. Adv. 29, 792–803.

Goswami, A., Roy, I., Sengupta, S., Debnath, N., 2010. Novel applications of solid and liquid formulations of nanoparticles against insect pests and pathogens. Thin Solid Films 519, 1252–1257.

Hancock, B.C., Zografi, G., 1997. Characteristics and significance of the amorphous state in pharmaceutical systems. J. Pharm. Scie. 86, 1–12.

Hashemi, S.M., Safavi, S.A., 2012. Chemical constituents and toxicity of essential oils of oriental arborvitae, *Platycladus orientalis* (L), against three stored-product beetles. Chil. J. Agr. Res. 72, 188–194.

Hendry, G., 1996. Why do plants have cytochrome P-450? Detoxification versus defence. New Phytol. 102, 239–247.

Islam, M.S., Hasan, M.M., Lei, C., Mucha-Pelzer, T., Mewis, I., Ulrichs, C., 2010. Direct and admixture toxicity of diatomaceous earth and monoterpenoids against the storage pests *Callosobruchus maculates* (F.) and *Sitophilus oryzae* (L.). J. Pest. Sci. 83 (2010), 105–112.

Isman, M.B., 2000. Plant essential oils for pest and disease management. Crop Prot. 19, 603–608.

Isman, M.B., 2006. Botanical insecticides, deterrents, and repellents inmodern agriculture and an increasingly regulated world. Annu. Rev. Entomol. 51, 45–66.

Isman, M.B., Miresmailli, S., Machial, C., 2011. Commercial opportunities for pesticides based on plant essential oils in agriculture, industry and consumer products. Phytochem. Rev. 10, 197–204.

Jeffers, L.A., Roe, R.M., 2008. The movement of proteins across the insect and tick digestive system. J. Insect Physiol. 54, 319–332.

Jeffers, L.A., Shen, H., Khalil, S., Bissinger, B.W., Brandt, A., Gunnoe, T.B., Roe, R.M., 2012. Enhanced activity of an insecticidal protein, trypsin modulating oostatic factor (TMOF), through conjugation with aliphatic polyethylene glycol. Pest. Manage. Sci. 68, 49–59.

Jevševar, S., Kunstelj, M., Porekar, V.G., 2010. PEGylation of therapeutic proteins. Biotechnol. J. 5, 113–128.

Khoo, S.M., Porter, C.J.H., Charman, W.N., 2000. The formulation of Halofantrine as either non-solubilising PEG 6000 or solubilising lipid based solid dispersions: physical stability and absolute bioavailability assessment. Int. J. Pharm. 205, 65–78.

Kljajic, P., Peric, I., 2006. Susceptibility to contact insecticides of granary weevil *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) originating from different locations in the former Yugoslavia. J. Stored Prod. Res. 42, 149–161.

Korunic, Z., 1998. Diatomaceous earths, a group of natural insecticides. J. Stored Prod. Res. 34, 87–97.

Kostyukovsky, M., Shaaya, E., 2011. Phytochemicals as natural fumigants and contact insecticides against stored-product insects. In: Dubey, N.K. (Ed.), Natural Products in Plant Pest Management. CAB International, London, pp. 175–190.

Koul, O., 2005. Insects Antifeedants. CRC Press, Florida.

Lai, F., Wissing, S.A., Müller, R.H., Fadda, A.M., 2006. *Artemisia arborescens* L. essential oil–loaded solid lipid nanoparticles for potential agricultural application: preparation and characterization. AAPS Pharm. Sci. Tech. 7, 1–9.

Liu, W.T., 2006. Nanoparticles and their biological and environmental applications. J. Biosci. Bioeng. 102, 1–7.

Loha, K.M., Shakil, N.A., Kumar, J., Singh, M.K., Srivastava, C., 2012. Bio-efficacy evaluation of nanoformulation of B-cyfluthrin against *Callosobruchus maculates* (Coleoptera: Bruchidae). J. Environ. Sci. Health B 47 (2012), 687–691.

Margulis-Goshen, K., Magdassi, S., 2012. Nanotechnology: an advanced approach to the development of potent insecticides. In: Ishaaya, I., Reddy, P.S., Rami, H.A. (Eds.), Advanced Technologies for Managing Insect Pests. Springer Science and Business Media, New York, pp. 295–314.

Martín, A., Varona, S., Navarrete, A., Cocero, M.J., 2010. Encapsulation and co-precipitation processes with supercrtitical fluids: applications with essential oil. Open Chem. Eng. J. 4, 31–41.

Mithofer, A., Boland, W., 2012. Plant defense against herbivores: chemical aspects. Annu. Rev. Entomol. 63, 431–450.

Miyazawa, M., Wada, T., Kameoka, H., 1998. Biotransformation of (+)- and (−)-limonene by the larvae of common cutworm (*Spodoptera litura*). J. Agric. Food Chem. 46, 300–303.

Moretti, M.D.L., 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.

Nachman, R.J., Hamshou, M., Kaczmarek, K., Zabrocki, J., Smagghe, G., 2012. Biostable and PEG polymer-conjugated insect pyrokinin analogs demonstrate antifeedant activity and induce high mortality in the pea aphid *Acyrthosiphon pisum* (Hemiptera: Aphidae). Peptides 34, 266–273.

Nel, A.E., Madler, L., Velegol, D., Xia, T., Hoek, E.M., Somasundaran, P., Klaessig, F., Castranova, V., Thompson, M., 2009. Understanding biophysicochemical interactions at the nano-bio interface. Nat. Mater. 8, 543–557.

Nel, A.E., Xia, T., Madler, L., Li, N., 2006. Toxic potential of materials at the nanolevel. Science 311, 622–627.

Ogendo, J.O., Kostyukovsky, M., Ravid, U., Matasyoh, J.C., Deng, A.L., Omolo, E.O., 2008. Bioactivity of *Ocimum gratissimum* L. oil and two of its constituents against five insect pests attacking stored food products. J. Stored Prod. Res. 44, 328–334.

Otsuka, H., Nagasaki, Y., Kataoka, K., 2003. PEGylated nanoparticles for biological and pharmaceutical applications. Adv. Drug Deliv. Rev. 55, 403–419.
Passreiter, C.M., Wilson, J., Andersen, R., Isman, M.B., 2004. Metabolism of thymol and trans-anethole in larvae of *Spodoptera litura* and *Trichoplusia* ni (Lepidoptera: Noctuidae). J. Agric. Food Chem. 2004 (52), 2549–2551.
Pimentel, M.A.G., Faroni, L.R.D., Guedes, R.N.C., Sousa, A.H., Totola, M.R., 2009. Phosphine resistance in Brazilian populations of *Sitophilus zeamais* motschulsky (Coleoptera: Curculionidae). J. Stored Prod. Res. 45, 71–74.
Regnault-Roger, C., 1997. The potential of botanical essential oils for insect pest control. Integrated. Pest. Manage. Rev. 2, 25–34.
Regnault-Roger, C., 2013. Essential oils in insects control. In: Ramawat, K.G., MÃ©rillon, J.M. (Eds.), Handbook of Natural Products. Springer-Verlag, Berlin, pp. 4087–4102. doi 10.1007/978-3-642-22144-6-181.
Regnault-Roger, C., Vincent, C., Arnason, J.T., 2012. Essential oils in insect control: low risk products in a high-stakes world. Annu. Rev. Entomol. 57, 405–424.
Rossi, Y.E., Canavoso, L., Palacios, S.M., 2012. Molecular response of *Musca domestica* L. to *Mintostachys verticillata* essential oil, (4R)(+)-pulegone and menthone. Fitoterapia 83, 336–342.
Rossi, Y.E., Palacios, S.M., 2013. Fumigant toxicity of *Citrus sinensis* essential oil on *Musca domestica* L. adults in the absence and presence of a P450 inhibitor. Acta Trop. 172, 33–37.
Rozman, V., Kalinovic, I., Korunic, Z., 2007. Toxicity of naturally occurring compounds of Lamiaceae and Lauraceae to three stored-product insects. J. Stored Prod. Res. 43, 349–355.
Saroukolai, A.T., Moharramipour, S., Meshkatalsadat, M.H., 2010. Insecticidal properties of *Thymus persicus* essential oil against *Tribolium castaneum* and *Sitophilus* oryzae. J. Pest. Sci. 83, 3–8.
Sfara, V., Zerba, E.N., Alzogaray, A., 2009. Fumigant insecticidal activity and repellent effect of five essential oils and seven monoterpenes on first-instar nymphs of *Rhodnius prolixus*. J. Med. Entomol. 46, 511–515.
Soppimath, K.S., Aminabhavi, T.M., Kulkarni, A.R., Rudzinski, W.E., 2001. Biodegradable polymeric nanoparticles as drug delivery devices. J. Control. Release. 70, 1–20.
Stadler, T., Buteler, M., Weaver, D.K., 2010. Novel use of nanostructured alumina as an insecticide. Pest. Manage. Sci. 66, 577–579.
Stefanazzi, N., Stadler, T., Ferrero, A., 2011. Composition and toxic, repellent and feeding deterrent activity of essential oils against the stored-grain pests *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae). Pest. Manage. Sci. 67, 639–640.
Tarelli, G., Zerba, E.N., Alzogaray, A., 2009. Toxicity to vapor exposure and topical application of essential oils and monoterpenes on *Musca domestica* (Diptera: Muscidae). J. Econ. Entomol. 102, 1383–1388.
Tripathi, A.K., Upadhyay, S., Bhuiyan, M., Bhattacharya, P.R., 2009. A review of essential oils as biopesticide in insect-pest management. J. Pharmacog. Phytother. 1, 52–63.
Ukeh, D.A., Umoetok, S.B.A., 2011. Repellent effects of five monoterpenoid odours against *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (F.) in Calabar. Nigeria. Crop Prot. 30, 1351–1355.
Vincent, J.F.V., Wegst, U.G.K., 2004. Design and mechanical properties of insect cuticle. Arth. Struct. Dev. 33, 187–199.
Werdin González, J.O., Gutiérrez, M.M., Murray, A.P., Ferrero, A.A., 2011. Composition and biological activity of essential oils from Labiatae against *Nezara viridula* (Hemiptera: Pentatomidae) soybean pest. Pest. Manage. Sci. 67, 948–955.
Werdin González, J.O., Laumann, R.A., da Silveira, S., Moraes, M.C.B., Borges, M., Ferrero, A.A., 2013. Lethal and Sublethal effects of four essential oils on the egg parasitoids *Trissolcus basalis*. Chemosphere 92, 608–615.
Westesen, K., Bunjes, H., Koch, H.J., 1997. Physicochemical characterization of lipid nanoparticles and evaluation of their drug loading capacity and sustained release potential. J. Control. Release. 48, 189–197.
Yang, F.L., Li, X.G., Lei, C.L., 2009. Structural characterization of nanoparticles loaded with garlic essential oils and their insecticidal activity against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). J. Agric. Food Chem. 57, 10156–10162.
Yu, L., 2001. Amorphous pharmaceutical solids: preparation, characterization and stabilization. Adv. Drug Deliv. Rev. 48, 27–42.
Zerrouk, N., Chemtob, C., Arnaud, P., Toscani, S., Dugue, J., 2001. In vitro and in vivo evaluation of carbamazepine-PEG 6000 solid dispersions. Int. J. Pharm. 225, 49–62.
Zhang, J.S., Zhao, N.N., Liu, Q.Z., Liu, Z.L., Du, S.S., Zhou, L., Deng, Z.W., 2011. Repellent constituents of essential oil of *Cymbopogon distans* aerial parts against two stored-product insects. J. Agric. Food Chem. 59, 9910–9915.