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Ecofriendly Approach for the Control of a Common Insect Pest in the Food Industry, Combining Polymeric Nanoparticles and Post-application Temperatures

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ABSTRACT: One of the most common insect pests is *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), which affects different food commodities. A new effective approach for the management of insect pests is the development of new formulations based on essential oils (EO). However, few works informed about the relationship between insecticidal activity of EO or essential oils loaded polymeric nanoparticles (EOPN) and post-application temperature. In our work, palmarosa [*Cymbopogon martinii* (Roxb.) Watson], geranium (*Geranium maculatum* L.), and peppermint (*Mentha piperita* L.) oils were formulated in a polyethylene glycol 6000 matrix to obtain EOPN. Geranium and palmarosa EOPN had sizes of 259 and 191 nm, respectively; the encapsulation efficiency (EE) was close to 90%, and the samples were monodisperse. The sizes from peppermint EOPN were around 380 nm, with an EE of 72%, and were polidisperse. In a contact toxicity bioassay, the insecticidal effect of the oils was increased by all EOPN, with palmarosa oil being the most toxic. In addition, the oils and their nanoparticles showed a significantly negative temperature coefficient when applied by contact. In a fumigant bioassay, just palmarosa and peppermint EOPN enhanced the oil activity and palmarosa EO and EOPN showed the highest toxic effect. In this case, the EO and EOPN insecticidal activity was unaffected by environmental temperature variation.

KEYWORDS: *Plodia interpunctella, essential oils, polymeric nanoparticle, temperature coefficient*

INTRODUCTION

More than 70 species of moths infest different stored products, producing quantitative and qualitative losses in food commodities.[1] Every year important economic losses are caused by *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), Indian meal moth, which is a cosmopolitan insect pest that attacks cereal products, dried fruit, nuts, and legumes.[2]

In the last century, synthetic insecticides appeared as new tools for insect control. However, their indiscriminate application contributed to environmental contamination and pest resistance and affected non-target organisms.[3] Essential oils (EO) appear as new ecofriendly insecticides, which show good biological activity against insect pests.[4]

EO are mixtures of different volatile compounds, which are synthesized through complex metabolic pathways with the aim of protecting plants.[5] Plant EO and their constituents could be used in integrated pest management (IPM) programs as ecofriendly tools, because of their low toxicity to humans and rapid degradation in the environment.[6] The EO have shown toxic, fumigant, repellent, and antifeedant effects in *P. interpunctella*.[7−10]

In recent years, nanotechnology has been applied in the biopesticide area. EO nanoformulation is an innovative technique to contribute to the protection of the active compounds from environmental conditions and prevent the loss of EO over time.[11] In addition, nanoformulations have shown a reduction in the amounts of bioactive compounds that need to be used and a reduction of the harmful effects for humans and the environment.[12] Different types of proteins, synthetic emulsifiers, polysaccharides (e.g., starch and chitosan), and polyethers (e.g., polyethylene glycol and poly-ε-caprolactone) have been evaluated to nanoformulate the EO or their constituents.[13−16] Over the last few decades, polyethylene glycol 6000 (PEG 6000) has been extensively studied for medical application, food industry, and pest control. PEG 6000 has a wide range of solubility, an absence of antigenicity and inmunotoxicity, and a lack of toxicity and is easily excreted from living organisms.[17] PEG 6000 polymeric nanoparticles loaded with EO (EOPN) are considered as one of the most important emerging trends in insect pest control.[18−21]

As mentioned previously, the toxicity of EO toward stored product pests may also be enhanced or reduced by numerous factors, one being the environmental temperature.[22] The post-application environmental temperature is one such limiting factor that could have an important effect on EO efficacy.[23]

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Previous work studied the effect of the post-application temperature and synthetic insecticides.[24−27] Moreover, Bohinc et al.[28] and Pavela and Sedlák[23] analyzed this effect in EO. To the best of our knowledge, very little has been reported about the effect of EOPN and the post-application temperature. The aim of this work was to evaluate the insecticidal activity of EO and EOPN on *P. interpunctella* adults at different environmental temperatures. Understanding the interaction between the post-application temperature and EOPN insecticidal efficacy will allow for the implementation of future control programs for *P. interpunctella* based on these ecofriendly products.

MATERIALS AND METHODS

Insects. Adults of *P. interpunctella* were acquired from an established laboratory population (more than 25 generations, outcrossed once) at the Laboratorio de Zoología de Invertebrados II, Universidad Nacional del Sur, Argentina.

Colonies were reared in plastic containers (13 cm diameter × 30 cm height) covered by a fine mesh cloth for ventilation in a growth chamber at 27 ± 1 °C, 45−50% relative humidity, and 16:8 h light/dark. Each one contained a mixture of maize flour, honey, glycerin, flour, and powdered milk of analytical grade (2:1:1:1:1, w/w/w/w/w). For the bioassays, to determine the lethal concentration of 50% (LC_{50}) values, adults (less than 48 h old) from different cohorts were used.

Chemicals. EO, namely, palmarosa [*Cymbopogon martinii* (Roxb.) Watson], geranium (*Geranium maculatum* L.), and peppermint (*Mentha piperita* L.), were purchased from Swiss-Just (Switzerland). PEG 6000 was acquired from Merck, Germany. Analytical-grade hexane (Dorwill, Argentina) was used as the solvent.

Nanoparticle Preparation. The melt-dispersion method was used to obtain EOPN. The methodology was previously informed by Werdin-González et al.[17] On a hot plate stirrer, 20 g of PEG 6000 was heated at 65 °C. After that, 2 g of EO was incorporated into the melted PEG 6000. At the same time, PEG 6000 with EO was stirred using a D-500 hand-held homogenizer (DLAB Instrument, Limited) for 15 min at 15 000 rpm. Then, the EOPN were spontaneously formed, when the mixture was chilled at −4 °C for 45 min. Finally, the mixture was completely ground in a mortar box refrigerated at 0 °C, and the product was sieved using a stainless-steel sieve (230 mesh). The EOPN were stored in airtight polyethylene pouches at 27 ± 2 °C in desiccators containing calcium chloride, during 7 days, prior to experiments.

EOPN Size. A Malvern Nano ZS90 equipment was used to measure the EOPN size and polydispersity index (PDI) at 25.0 °C. The PDI was calculated by the square of the standard deviation divided by the square of the mean size, which is an indicator of the homo/heterogeneity of the size distribution of particles.[29] Samples (0.2 g) of each EOPN were suspended in 10 mL of distilled water for 30 min. Then, the dispersion was filtered using Whatman no. 1 filter paper and equilibrated for 2 h. Data were compared using one-way analysis of variance (ANOVA), followed by least significant difference (LSD) ($N = 4$).

Scanning Electron Microscopy (SEM). For sample preparation, EOPN were dispersed and sonicated in water. The samples were placed in a coverslip and, after water evaporation, coated with a layer of gold using an argon plasma metallizer. The images were visualized using a LEO EVO 40-XVP microscope, from Centro Científico Tecnológico (CCT), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Bahía Blanca, Argentina. The observation was made at a voltage of 10 kV and a magnification of 85000×.

EOPN Encapsulation Efficiency (EE). According to Werdin-González et al.,[17] the EE was evaluated spectrophotometrically; EOPN (0.1 g) were dissolved separately in 2 mL of absolute ethanol/H_2O (75:25). The mixture was centrifuged at 9000 rpm for 10 min. The supernatant was collected and analyzed by ultraviolet−visible (UV−vis) spectrophotometry (Shimadzu UV-1203 photometer with the Kinetics-2-Program Pack P/N 206-62029-10, Shimadzu Corp., Kyoto, Japan) at a wavelength of 290 nm. Four samples were analyzed, and the amount of EO was calculated by an appropriate calibration curve of free EO in ethanol. EE was determined from

$$\text{EE}\ (\%) = \frac{\text{weight of loaded EO}}{\text{weight of initial EO}} \times 100$$

One-way ANOVA and LSD were used to compare the data ($N = 4$).

Chemical Composition of EO Pre- and Post-formulation. To detect EO compounds in the remaining solution after encapsulation, a second extraction was performed, increasing solvent polarity. Therefore, *n*-butanol was selected. After that, the ether, *n*-butanol, and aqueous phases were analyzed by gas chromatography−mass spectrometry (GC−MS). As expected, EO components were only present in the ether phase.

The compounds were identified comparing their retention indices (Kováts indices) to those of known compounds and comparing their mass spectra to those stored in the MS database (NBS75K.L MS DATA). Also, when the appropriate standard was available, compounds were identified by comparison of their retention times to those of the reference compounds. 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 mm film thickness). The carrier gas was helium with a flow of 1 mL/min. The GC oven temperature was held at 50 °C for 2 min, programmed at 5 °C/min to 200 °C, and then held at this temperature for 15 min. Mass spectra were recorded at 70 eV. The mass range was from m/z 35 to 350 amu. The temperature of the injection block was 280 °C. Each EO was diluted with ethyl ether to a concentration of 0.001 mg mL^{-1} (0.1%, v/v), and 1 μL of that solution was injected in GC−MS for component analysis. A standard solution of *n*-alkanes was used in the same conditions to determine the Kováts indices of each peak.

Contact Toxicity Assay. The contact toxicity bioassays of the EO and EOPN against *P. interpunctella* adults were performed using glass Petri dishes (9.4 cm diameter × 1.9 cm height); 0.7 mL of EO and hexane solution was put on a filter paper (69.39 cm^2), and the concentrations used in the EO assay were 25−400 $\mu g\ cm^{-2}$. After solvent evaporation, the filter papers were introduced in each vial. In the case of the EOPN bioassay, nanoparticles were dispersed on the filter papers at an equal concentration for the EO (on the basis of EE). A total of 10 unsexed adults were added on each Petri dish and then covered with a lid with a fine wire sieve. Hexane or PEG 6000 alone (processed as in the nanoparticle preparation) was used as the control. All experiments were performed in quadruplicate using different cohorts of insects. To evaluate the temperature effect, all treatments were put in growth rooms with s constant relative humidity of 45−50% and varied temperatures of 17, 24, and 31 ± 1 °C. After 24 h of exposure, the mortality was evaluated and the insects were considered dead when no body movement was produced. The mortality data were submitted to probit analysis using the statistical software SPSS 15.0 to obtain LC_{50}, and 95% confidence intervals were estimated. The LC_{50} values were considered significantly different if their 95% confidence intervals did not overlap.

Fumigant Toxicity Assay. The fumigant toxicity effects of the EO and EOPN against *P. interpunctella* adults were performed using glass Petri dishes (9.4 cm diameter × 1.9 cm height); 0.7 mL of EO and hexane solution was put on a filter paper (69.39 cm^2), and the concentrations used in the EO assay were 25−400 $\mu g\ cm^{-2}$. After solvent evaporation, the filter papers were introduced in a glass Petri dish (9.4 cm diameter × 1.9 height). Then, it was covered with a lid with a fine wire sieve. Batches of 10 unsexed adults were placed over the sieve to prevent the direct contact of insects with the test compounds. Each unit was then covered with a plastic container (350 mL), and all of them were fitted together with an adhesive film. In the case of the EOPN bioassay, nanoparticles were dispersed on the filter papers at concentrations equivalent to the EO. Hexane or PEG 6000 alone (processed as in the nanoparticle preparation) was used as the control. All experiments were performed in quadruplicate using different cohorts of insects. To evaluate the temperature effect, all treatments were put in growth rooms with a constant relative humidity of 45−50% and varied temperatures of 17, 24, and 31 ± 1 °C. Insect mortality was determined after 24 h of exposure, to calculate LC_{50} and 95%

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confidence intervals. The LC_{50} values were considered significantly different if their 95% confidence intervals did not overlap.

Effect of the Temperature. To determine the post-application temperature effect on toxicological bioassays, a temperature coefficient (TC) was calculated. TC is measured by the ratio between temperature-dependent activity changes and the temperature range in which the change occurred and is used to indicate the relationship between the environmental temperature and toxicity of insecticides.[30] TC was calculated for each temperature range from

$$TC = \frac{\text{larger } LC_{50} \text{ value}}{\text{smaller } LC_{50} \text{ value}}$$

TC was considered positive if the toxicity increases as the temperature increases, negative if the toxicity decreases as the temperature increases, and no effect if the TC was unaffected by the temperature increase. TC was considered significantly different when the LC_{50} values from which they were calculated were significantly different.[31,32]

RESULTS

Nanoparticle Characterization. The first step of the study was the characterization of the EOPN. The particle sizes of all treatments were kept below 390 nm (Table 1). The EOPN sizes from palmarosa were significantly lower than the others ($p < 0.05$), followed by geranium and peppermint nanoparticles ($p < 0.05$). Peppermint EOPN showed the highest sizes. The PDI was relatively low (below 0.25) for palmarosa and geranium EOPN, and these data indicated that the samples were monodisperse; conversely, peppermint EOPN samples were polydisperse (PDI > 0.4).

Table 1. Average Sizes [Mean Value ± Standard Error (SE)], PDI (Mean Value ± SE), and EE (Mean Value ± SE) of Different EOPN, after 7 Days Post-formulation[a]

	palmarosa	geranium	peppermint
size (nm)	191 ± 5 a	259 ± 12 b	381 ± 29 c
PDI	0.232 ± 0.015 a	0.228 ± 0.007 a	0.532 ± 0.013 b
EE (%)	89.75 ± 2.5 a	90.5 ± 2.32 a	72.25 ± 1.6 b

[a]Different letters within the same row indicate statistical differences (LSD; $p < 0.05$).

The morphology of EOPN was evaluated using SEM. Figures 1 and 2 showed geranium and palmarosa EOPN with an 85000× magnification. These nanoparticles had circular shapes with uniform sizes. In contrast, the peppermint EOPN showed an irregular shape and heterogeneity sizes (Figure 3). The dynamic light scattering (DLS) graphs in the figures showed that the average sizes were influenced by the EO added.

Table 1 shows that, for all EOPN, the EE was >70%. There was no significant difference between geranium and palmarosa EE, which showed values close to 90% ($p > 0.05$). However, the EE of peppermint was significantly lower ($p < 0.05$).

From the chemical characterization of EOPN, it was shown that, in palmarosa oil, the main compound was geraniol (Table 2). In the case of geranium EOPN, β-citronellol and geraniol were the major compounds (Table 2). Components, such as linalool, menthone, citronellyl formate, and geranyl formate, which, in the pre-formulation sample, were between 8 and 11%, had a significant reduction after formulation (<1.7%). On the other hand, the minor components of the original sample (<3%) were not detected after formulation. Finally, menthol was the major compound in peppermint oil and their nanoparticles (Table 2). After the formulation, a slight reduction was observed in isomenthone, *p*-menthen-3-one, and menthol acetate contents. The minor components of the original sample (<6%) were not detected after formulation.

Contact Toxicity Assay. At all temperatures, the EO toxicity order based on LC_{50} values was palmarosa > geranium > peppermint and significant differences were found between all of them ($p < 0.05$). For EOPN, at 17 and 24 °C, the toxicity order was palmarosa > geranium > peppermint ($p < 0.05$). However, at 31 °C, the toxicity order was palmarosa = geranium > peppermint; only peppermint EOPN showed a significantly higher LC_{50} value ($p < 0.05$).

Table 3 showed that palmarosa oil at 17 °C produced the highest toxicity effect (44.74 $\mu g\ cm^{-2}$; $p < 0.05$). At 24 and 31 °C, no differences were found in oil activity ($p > 0.05$). The palmarosa nanoparticles showed a similar trend, and the highest mortality was achieved at 17 °C (17.56 $\mu g\ cm^{-2}$). It must be noted that the polymeric nanoparticle highly improves the EO contact insecticidal activity between 1.77 and 2.53 times ($p < 0.05$).

Table 4 shows a comparison of the lethal concentration in geranium EO. At 17 °C, this oil showed the highest efficacy, with LC_{50} values of 73.15 $\mu g\ cm^{-2}$. At 24 and 31 °C, not significant different was observed ($p < 0.05$). Geranium EOPN showed a similar behavior, with LC_{50} estimated as 59.85 $\mu g\ cm^{-2}$ at 17 °C. The EOPN significantly enhanced the insecticidal activity of the bioactive compound between 1.54 and 1.90 times ($p < 0.05$).

At each temperature, peppermint EO and EOPN produced the lowest insecticidal activity (Table 5). At 17 °C, EO showed the highest toxicity effect (126.90 $\mu g\ cm^{-2}$), followed by 24 °C (202.05 $\mu g\ cm^{-2}$) and finally 31 °C (386.64 $\mu g\ cm^{-2}$; $p < 0.05$). Similar to the trend recorded with the oil, this insect was more sensitive to EOPN at 17 °C than at any other temperature. The EOPN significantly increased peppermint EO potency by 2.12- and 3.54-fold.

Fumigant Toxicity Assay. At 17 and 24 °C, the EO toxicity order was palmarosa > geranium > peppermint and significant

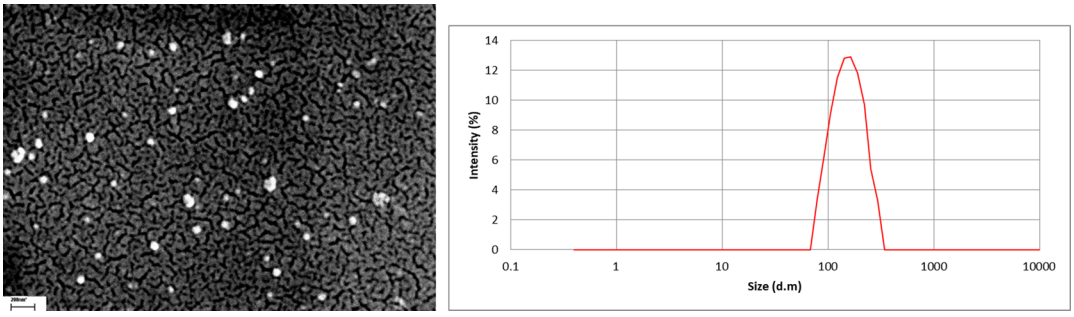

Figure 1. (A) SEM image (magnification of 85000×) and (B) DLS curves of palmarosa EOPN.

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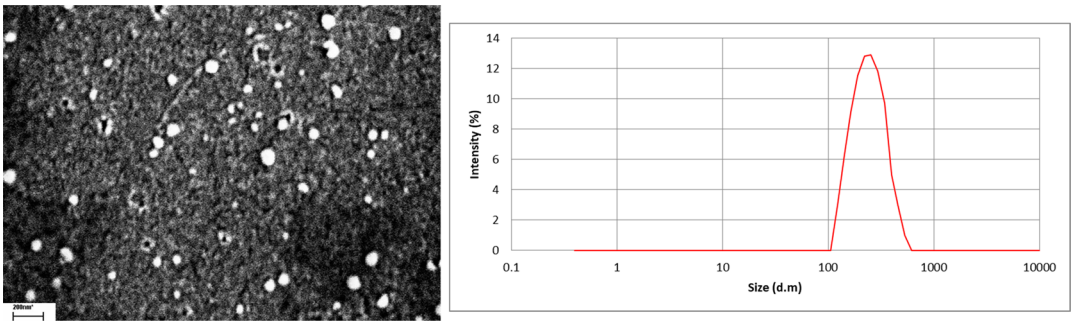

Figure 2. (A) SEM image (magnification of 85000×) and (B) DLS curves of geranium EOPN.

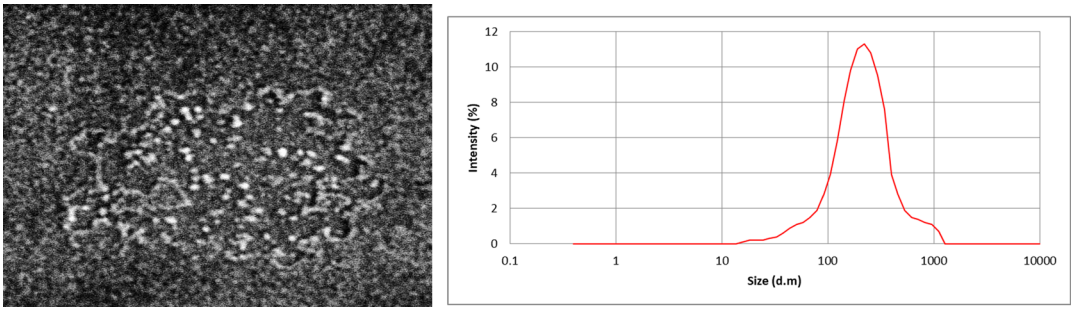

Figure 3. (A) SEM image (magnification of 85000×) and (B) DLS curves of peppermint EOPN.

Table 2. Chemical Analysis of Pre-/Post-formulation of the Oils from Palmarosa, Geranium, and Peppermint

		palmarosa EO		geranium EO		peppermint EO	
RT (min)	component	pre-formulation	post-formulation	pre-formulation	post-formulation	pre-formulation	post-formulation
7.16	α-pinene					1.92	
8.36	β-pinene					1.85	
9.87	limonene					3.36	
9.93	1,8-cineole					5.88	
13.06	linalool	2.55	2.29	12.67	9.95		
13.55	isomenthone					16.90	6.95
13.85	menthone			11.14	1.38		
14.10	menthol					52.51	81.37
14.35	*p*-menten-3-one					10.43	7.57
16.14	β-citronelol	9.94	10.12	26.14	38.12		
16.48	geraniol	77.07	76.38	23.19	47.89		
16.98	citronellyl formate			10.37	1.71		
17.70	geranyl formate			7.94	0.95		
18.04	menthol acetate					7.15	4.11
20.85	geranyl acetate	4.55	5.30	2.01			
20.86	caryophyllene	5.86	1.39	2.58			
23.70	neryl acetate			2.98			

Table 3. LC_{50} (μg cm^{-2}) Values of the Insecticidal Activity of Palmarosa EO and Its EOPN at 17, 24, and 31 °C in Adults of *P. interpunctella*

	contact					fumigant				
	EO		EOPN			EO		EOPN		
temperature (°C)	LC_{50}	CI[a]	LC_{50}	CI[a]	LC_{50} EO/LC_{50} EOPN	LC_{50}	CI[a]	LC_{50}	CI[a]	LC_{50} EO/LC_{50} EOPN
17	44.74	40.3−49.1	17.56	14.3−20.5	2.53[b]	42.33	37.2−49.1	23.81	15.0−29.5	1.77[b]
24	102.92	91.4−113.9	54.49	50.5−59.0	1.89[b]	40.66	35.7−45.2	23.30	20.0−27.3	1.74[b]
31	84.10	74.0−93.1	47.44	40.5−55.8	1.77[b]	38.87	31.8−46.8	30.73	26.6−35.8	1.26[b]

[a]The 95% lower and upper confidence intervals. [b]Significant difference between EO LC_{50} and EOPN LC_{50} (Cl overlap; $p < 0.05$).

differences were found between all of them ($p < 0.05$). However, at 31 °C, the toxicity order was palmarosa > geranium = peppermint. At all temperatures, the EOPN toxicity was palmarosa = peppermint > geranium.

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Table 4. LC_{50} ($\mu g\ cm^{-2}$) Values of the Insecticidal Activity of Geranium EO and Its EOPN at 17, 24, and 31 °C in Adults of *P. interpunctella*

	contact					fumigant				
	EO		EOPN			EO		EOPN		
temperature (°C)	LC_{50}	CI[a]	LC_{50}	CI[a]	LC_{50} EO/LC_{50} EOPN	LC_{50}	CI[a]	LC_{50}	CI[a]	LC_{50} EO/LC_{50} EOPN
17	73.15	67.1−79.7	47.44	40.9−51.3	1.54[b]	55.27	50.1−60.5	54.65	46.1−63.3	1.01
24	128.83	117.4−141.3	67.51	61.4−73.4	1.90[b]	55.78	51.6−63.3	54.61	49.9−60.2	1.02
31	111.46	97.1−124.1	64.40	52.2−76.2	1.73[b]	66.95	55.4−80.1	60.67	54.0−68.8	1.10

[a]The 95% lower and upper confidence intervals. [b]Significant difference between EO LC_{50} and EOPN LC_{50} (Cl overlap; $p < 0.05$).

Table 5. LC_{50} ($\mu g\ cm^{-2}$) Values of the Insecticidal Activity of Peppermint EO and Its EOPN at 17, 24, and 31 °C in Adults of *P. interpunctella*

	contact					fumigant				
	EO		EOPN			EO		EOPN		
temperature (°C)	LC_{50}	CI[a]	LC_{50}	CI[a]	LC_{50} EO/LC_{50} EOPN	LC_{50}	CI[a]	LC_{50}	CI[a]	LC_{50} EO/LC_{50} EOPN
17	126.90	115.1−138.6	59.85	52.2−68.9	2.12[b]	90.12	83.3−98.0	26.73	23.8−30.8	3.37[b]
24	202.05	191.1−212.7	86.84	76.7−98.2	2.32[b]	100.59	88.5−111.9	22.59	19.1−26.8	4.45[b]
31	386.64	348.5−387.5	109.18	93.4−128.0	3.54[b]	81.37	69.4−92.0	30.56	26.5−35.5	2.66[b]

[a]The 95% lower and upper confidence intervals. [b]Significant difference between EO LC_{50} and EOPN LC_{50} (Cl overlap; $p < 0.05$).

Table 6. Temperature Coefficients of EO and Its Polymeric Nanoparticles at 17, 24, and 31 °C in Adults of *P. interpunctella*

		contact			fumigant		
product		17−24 °C	24−31 °C	17−31 °C	17−24 °C	24−31 °C	17−31 °C
palmarosa	EO	−2.30[a]	1.22	−1.88[a]	1.05	1.05	1.09
	EOPN	−3.10[a]	1.14	−2.70[a]	1.02	−1.31	−1.29
geranium	EO	−1.76[a]	1.15	−1.52[a]	1.00	−1.20	−1.21
	EOPN	−1.42[a]	1.05	−1.36[a]	1.00	−1.11	−1.11
peppermint	EO	−1.59[a]	−1.91[a]	−3.05[a]	−1.12	1.24	1.12
	EOPN	−1.45[a]	−1.26	−1.82[a]	1.18	−1.35	−1.14

[a]Significant difference ($p < 0.05$) between the LC_{50} for each compound at two different temperatures (Cl overlap; $p < 0.05$). Each value was calculated from the data obtained in at least four replicates.

The results indicated there was no significance difference in the LC_{50} value from palmarosa EO at different temperatures (Table 3). The LC_{50} value ranged from 38.87 to 42.33 $\mu g\ cm^{-2}$ at 31 and 17 °C, respectively. For palmarosa EOPN, a similar behavior was observed and the LC_{50} ranged from 23.30 to 30.73 $\mu g\ cm^{-2}$ at 24 and 31 °C. The EOPN significantly enhanced the insecticidal activity between 1.26 and 1.77 times.

Table 4 shows the geranium EO fumigant toxicity effect. No significant differences were found in the LC_{50} value from geranium EO and EOPN at different temperatures. Moreover, the EOPN did not improve the EO fumigant insecticidal activity. In the contact bioassay, peppermint EO showed, at each temperature, the highest LC_{50} values (Table 5). No significant differences were found in LC_{50} values from peppermint EO at different temperatures. For peppermint EOPN, a similar trend was observed. The EOPN significantly increased peppermint EO potency by 2.26−4.45 times.

Effect of the Temperature. Table 6 shows the temperature coefficients in the ranges of 17−24, 24−31, and 17−31 °C on *P. interpunctella* exposed to EO and their nanoparticles. In the contact toxicity assay, all EO and EOPN had a significantly negative TC in the ranges of 17−24 and 17−31 °C ($p < 0.05$). Only peppermint EO had a significantly negative TC in the ranges of 24−31 °C. However, in the fumigant toxicity bioassay, all EO and EOPN showed that TC was unaffected by temperature variation.

DISCUSSION

The chemical analysis showed that all EO were diverse mixtures containing oxygenated and non-oxygenated monoterpenes and sesquiterpenes. In the present work, the EO from geranium, palmarosa, and peppermint were encapsulated in a PEG matrix by the melt-dispersion method to obtain EOPN. On one hand, geranium and palmarosa EOPN presented similar sizes (259 and 191 nm, respectively) and EE (close to 90%), and both EOPN were monodisperses (PDI < 0.25). On the other hand, peppermint EOPN had sizes of 381 nm and EE of about 72% and was polidisperse (PDI > 0.4). For all of the nanoparticles, the SEM images confirmed the previous data. Moreover, the post-formulation chemical analyses showed that all nanoparticles entrapped most of the main EO constituents. Recently, the melt-dispersion method was used to obtain EOPN loaded with lemon, mandarin, and orange oils.[18] In that work, the nanoparticles showed sizes from 212 to 240 nm, EE > 88%, and PDI between 0.23 and 0.34. Kumar et al.[33] used PEG 6000 and *Mentha piperita* oil to obtain EOPN of 331 nm, with EE close to 85% and PDI = 0.547. Yang et al.[21] demonstrated that the melt-dispersion technique can be produce garlic EOPN with sizes of 233 nm and EE close to 80% and the samples were monodisperse. Werdin-González et al.[16,17] informed that *Citrus bergamia* (Risso & Poit.) EOPN had sizes between 184 and 236 nm and EE and PDI close to 75% and 0.25, respectively. With these works taken into account, it is possible to conclude that the

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melt dispersion is a simple, convenient, and low-cost technique, which allows for EOPN to be obtained with sizes of >100 nm, a variable PDI, and high loading efficiency. Moreover, this technique could be applied at the industrial scale, minimizing the production cost, to obtain ecofriendly polymeric nanoparticles against insect pests.

In the contact toxicity bioassay, all of the EOPN significantly increase the insecticidal activity of the bioactive compounds from 1.54 to 3.54 times. In the case of fumigant assays, just palmarosa and peppermint potentiated the biological activity from 1.26 to 4.45 times. This enhanced activity can be used to treat and protect stored the product against *P. interpunctella*. A number of studies have demonstrated that EOPN increase the EO insecticidal activity. The nanoparticles loaded with citrus EO enhanced the toxicity by contact and ingestion on *Tuta absoluta* (Meyrick).[18] Werdin-González et al.[34] showed that nanoparticles loaded with geranium and bergamot EO increased the contact toxicity between 8 and 10 times on adults of *Blattela germanica* (L.). In addition, the authors also observed that this EOPN enhanced the biological activity on *Rhyzopertha dominica* (F.) between 7.8 and 3.6 times.[17] Moreover, Yang et al.[21] demonstrated that garlic nanoparticles could be a good alternative in the management of *Tribolium castaneum* (Herbst). In the present work, it was observed that EOPN from geranium did not potentiate the fumigant activity of the oil. Probably, the terpene volatility of geranium oil was reduced by the nanoformulation. Similar results were registered using these nanoparticles on *R. dominica* and *T. castaneum*.[17]

In contrast with bulk materials, nanomaterials modify their chemical and physical properties; furthermore, these materials have contributed to the development of botanical insecticides based on EO.[35] In this sense, polymeric nanoparticles could potentiate insecticidal activity through an enhanced cuticular penetration.[36] The insect cuticle can be considered a two-phased structure, with lipophilic (epi- and exocuticles) and hydrophilic (endocuticle) layers.[37] Generally, it is accepted that EO, which had a nonpolar nature, tend to migrate by horizontal diffusion through epi- and exocuticles as a result of the endocuticle acting as a hydrophilic barrier. On the other hand, the amphiphilic nature of PEG 6000 could promote EOPN to have horizontal and vertical diffusion across the insect cuticle. In this regard, Hashem et al.[38] demonstrated that EO nanoformulation increases the penetration throughout the cuticle and the products can enter into the insect more easily. Moreover, the exposure time of the bioactive compounds to the insect pests could be increased by the nanosize of EOPN, which can cover large areas of the insect cuticle. Even more, nanoparticles could also change the pattern delivery of EO active ingredients, increasing their efficacy.[39]

In this work, we also studied the environmental temperature on the bioactivity of EO and EOPN. To the best of our knowledge, this is the first work to evaluate the action of the environmental temperature on the toxicity activity of EOPN.

At all of the studied temperatures, palmarosa EO and EOPN showed the highest insecticidal activity by contact and fumigation. In a previous work, Jesser et al.[9] observed that palmarosa EO at 25 °C was the most effective oil against *P. interpunctella* in the contact toxicity assay. It is important to note that EO contained complex mixtures of compounds with different mechanisms of action, especially at the level of the nervous system.[40] For example, some compounds, such as geraniol, which is the main component of palmarosa oil, produce a reversible competitive inhibition in acetylcholinesterase.[41]

TC shows the difference in the toxicity of insecticides with temperature variations.[42] In this work, it was found that the insecticide efficacy of the EO and EOPN decreased with the temperature when these products were applied by contact against *P. interpunctella* adults. As revealed by TC analysis, palmarosa, geranium, and peppermint EO and EOPN exhibited a negative gradient of toxicity, similar to the case of other insecticidal substances with different modes of action. For example, pyrethroids and dichlorodiphenyltrichloroethane (DDT) show negative TC for some insects.[24,25] Pavela and Sedlák[23] also informed that EO from *Thymus vulgaris* (L.) has a negative TC in *Spodoptera littoralis* (Boisduval) topically applied but the EO in *Culex quinquefasciatus* (Say) showed a positive TC when applied by contact. Otherwise, Bohinc et al.[28] showed a positive TC on *Acanthoscelides obtectus* (Say) exposed to lavender powders applied by contact. The negative TC could help in understanding some basic physiological mechanisms involved in the toxicological effects. Possible mechanisms could be a drastic increment in intrinsic neurotoxicity, a reduction in the metabolism rate, and differences in penetration patterns.[31,32] Zubari and Cutkomp[27] also suggest that the cuticle sorbs more insecticide at a lower temperature and, thus, permits a great availability of the product. Besides these effects, a lower temperature leads to decreased insect activity, limiting the exposure of the insect to the products over time.[26]

To conclude, it has been found that palmarosa EOPN presented the highest insecticidal activity and the optimal post-application temperature was 17 °C. It was also observed that the temperature had a significant effect on the insecticidal activity of the EO and EOPN when applied by contact. Further studies are needed to determinate the mechanism responsible for the temperature−toxicity relationship in polymeric nanoparticles. This is a central topic for successful nanotechnology application for the management of *P. interpunctella* and other stored product insect pests.

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E. Jesser, A. P. Murray, and J. O. Werdin-González conceived and designed research. E. Jesser, C. Yeguerman, N. Stefanazzi, and R. Gomez elaborated the EOPN and conducted the experiment. E. Jesser and J. O. Werdin-González analyzed data and wrote the manuscript. All author approved the manuscript.

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Notes

The authors declare no competing financial interest.

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