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Research Article

Application of a nanoformulation based on essential oil against *Ephestia kuehniella* larvae: Characterization and bioactivity

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Abstract: This study aimed to produce and characterize solid lipid nanoparticles containing the essential oil (SLN-EO) of *Ziziphora clinopodioides* Lam. The preparation was carried out using the high shear homogenization and ultrasound method. The biological activities of the prepared nanoformulation were evaluated against Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) larvae under laboratory conditions. The particle size of SLN-EO was estimated to be under 150 nm (polydispersity index, PDI < 0.2) and zeta potential was negative. Morphology of nanoparticles was in globular form as demonstrated by transmission electron microscopy analysis. The loaded essential oil (EO) in SLN was calculated as 92% using the filtration-centrifugation method. The fumigant toxicity of EO as SLN formulation against *E. kuehniella* larvae was three times greater than that of pure EO. Similar results, but to a lesser extent, were obtained from comparing their contact toxicities. The fumigant durability of EO was enhanced by nanoformulation for up to two weeks. The nutritional indices of larvae, including relative growth rate (RGR), relative consumption rate (RCR), and feeding deterrence (FDI), were influenced considerably by SLN-EO compared to pure EO. The findings suggested the solid lipid nanoparticles as a suitable nanocarrier for EO in sustainable control management of Mediterranean flour moth.

Keywords: nanoformulation, *Ziziphora clinopodioides*, *Ephestia kuehniella*, durability, nutritional indices

Introduction

Ephestia kuehniella Zeller (Lepidoptera: Pyralidae) is a severe storage pest and feeds on different kinds of cereals (Karabörklü *et al*., 2011), especially in flour mills or bakeries (Pandir and Hatice, 2016). The quality of these products decreases by direct damage caused by their feeding in addition to the presence of larvae and their webbing (Johnson *et al*., 1997). Due to the environmental pollution and resistance of pests to chemical insecticides, considerable attention has been paid to the botanical materials (Ayvaz *et al*., 2010). Several studies have evaluated the insecticidal activity of some essential oils (EO) against different stored product pests (Ebadollahi and Taghinezhad, 2020; Ma *et al*., 2020; Mustapha *et al*., 2020; Najem *et al*., 2020; Pang *et al*., 2020; Pavela *et al*., 2020).

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Nanotechnology is the central zone of science, mainly because of its extensive application in various fields such as engineering, medicine, chemistry, and biology (Paula *et al*., 2010). Essential oils consist of degradable components when exposed to oxygen, light, and heat (Moretti *et al*., 2002). Nanoparticles can be employed to produce nanoinsecticides and pest repellants (Owolade *et al*., 2008). The aims of the preparation of nanoformulation are: to increase the solubility of active components in water, to preserve active ingredients against degradation, and to release them in a controlled release system (Ragaei and Sabry, 2014). In the nanoencapsulation process, the active component is enclosed by coating material such as chemical polymers, eco-friendly polymers, lipids, and phospholipids to preserve them from environmental factors (such as light, humidity, and temperature). (Kumari *et al*., 2010).

Recently, solid lipid nanoparticles (SLN) have attracted more attention because of tremendous benefits such as big scale, nontoxicity, controlled release properties, and active compound protection. Encapsulation using SLN can be a valuable weaknesses compensation method for applying essential oils (Müller *et al*., 2000; Singhal *et al*., 2011). Asnawi *et al*. (2008) demonstrated that geranium oil obtained by ultrasonic-solvent emulsification technique is efficient mosquito repellent. In another study, frankincense and myrrh oils, using Compritol 888 ATO (as a lipid phase), soybean lecithin, and Tween (as emulsifiers), were prepared for oral delivery (Shi *et al*., 2012). Nasseri *et al*. (2016) prepared loaded *Zataria multiflora* Boiss. in SLN to enhance the efficacy of EO to control some fungal pathogens. Al-Haj *et al*. (2010) encapsulated *Nigella sativa* L. pure oil using SLN and applied it for dermal and cosmetic purposes. *Zingiber officinale* Roscoe EO-loaded nanofiber was prepared and evaluated against *Phthorimaea operculella* (Zeller) (Mahdavi *et al*., 2018).

The present study aimed to prepare encapsulation of *Z. clinopodioides* EO using SLN. The prepared nanoparticle was characterized using particle size analysis and transmission electron microscopy (TEM). The biological activities of solid lipid nanoparticle essential oil (SLN-EO) and pure EO were evaluated and compared in terms of fumigant and contact toxicity and their effects on nutritional indices of the 3rd instar *E. Kuehniella* larvae.

Materials and Methods

Insect rearing

The larvae of *E. kuehniella* used in this investigation were provided by the Plant Protection Department, Ferdowsi University of Mashhad, Iran. The insects were reared in a growth chamber set at 27 ± 1 °C, 65 ± 5% RH, and dark conditions. The 3rd instar larvae were used in the experiments. The larvae were cultured on a natural composition containing wheat flour: wheat germ: yeast (10:2:1 w/w) (Ebadollahi et al., 2010).

Preparation of essential oil

The blue mint bush plants, *Ziziphora clinopodioides*, known as kakuti-e kuhi in the Persian language, were collected in the mountainous areas of Bojnord, North Khorasan Iran, during the spring season. Species identification was confirmed by the Department of Botany, Ferdowsi University of Mashhad, Mashhad, Iran. They were dried at room temperature. Aerial parts of plants were hydro-distilled in a Clevenger apparatus (600 ml water and 50 g dried plant) for 4 hours. The extracted oil was preserved at 4 °C in a refrigerator (Faraone *et al*., 2012).

Preparation of nanoencapsulation

Preparation of nanoformulations was performed according to Kumar *et al.* (2018). The following materials in this study were from specified sources. They were applied without more refining: Poloxamer 188 (Uniqema, Belgium), glyceryl behenate (Compritol® 888 ATO) (Gattefossé, France), glyceryl monostearate (Gattefossé, France), glyceryl

palmitostearate (Precirol® ATO 5) (Gattefossé, France), and Tween 80 (Croda-international PLC, UK). So,

To obtain appropriate nanoformulations, 10 ml of diluted water with emulsifiers (Tween 80 and Poloxamer 188, 2.5% w/v) was added to molten lipids (Compritol 888 ATO, Precirol® ATO 5, and glycerol monostearate, 5% w/v) containing the volume of EO (0.5, 1, 2 and 2.5% w/v) at 75 °C. Afterward, homogenization was performed at 24000 rpm for 5 min with ultraturax homogenizer. The sonication of the obtained emulsion was done by a prob sonicator (Bransonic, USA). This experiment was conducted in 3 cycles of 30-second sonication at 15-second intervals. Nanoemulsions were cooled to room temperature.

Characterization of solid lipid nanoparticles loaded with EO

Estimations of particle size, zeta potential, and polydispersity index of SLN-EO were conducted using Dynamic Light Scattering (DLS) (Zetasizer Nano-Zs; Malvern Instruments Ltd., United Kingdom). The Zeta potential of specimens specifies if nanoparticles tend to adhere together or not. Total sizes were achieved in triplicate (25 ± 2 °C). Stored nanoparticles kept in the refrigerator at 8°C were assessed for physical stability at 24 hours, 1 month, 2 months, 4 months, and 6 months time points.

The samples of SLN-EO were prepared according to Layegh *et al*. (2013) and analyzed by transmission electron microscopy (TEM) (CEM 902A; Zeiss, Germany) to specify the shape of SLN-EO and its distribution. Briefly, water was added to nanoformulation and located on a carbon-coated copper grid. Afterward, extra water was removed by filter paper (after 30 seconds). Subsequently, uranyl acetate 2% (20 µl) was added to SLN-EO and eliminated after 30 seconds. The prepared grid was left at room temperature to dry.

Filtration–centrifugation technique was applied for calculating entrapment efficiency (EE) (Nasseri *et al*., 2016). Pulegone is the major constituent of *Z. clinopodioides* oil, used as an index. Different solutions of pulegone (100, 500, 1000, 2000, 3000, and 4000 ppm) were prepared. The calibration curve of pulegone was drawn using the gas chromatography procedure. For purification of SLN-EO, 1 ml of prepared nanoformulations was poured in an amicon (Amicon Ultra-15, PLHK Ultracel-PL Membrane, 100 KDa, Millipore) and centrifuged at 14000 rpm for 30 minutes. A clear section gathered at the bottom of amicon was used for GC-MS analysis. The entrapment efficiency of SLN-EO (%) was determined by the equation (C - N) / C ×100, in which N and C refer to concentrations of pulegone before and after purification, respectively.

According to Kheirkhah *et al*. (2015) Gas chromatography-mass spectrometry analysis was conducted according to Kheirkhah *et al*. (2015. An equipped thermoQuest-Finnigan by DB-1 fused-silica capillary column (60m-0.25mm i.d., 0.25 mm film thicknesses) was used. The oven temperature was gradually increased from 60 to 250 °C at 5 °C/min. The detector and injector temperatures were set at 280 °C and 250 °C, respectively. A flame ionization detector (FID) and the carrier gas, helium, were utilized at a 1.1 ml /min constant flow. The ionization voltage was 70 eV.

Bioassay of nanoformulation and pure oil fumigant bioassay

To assess the fumigant toxicity of EO and SLN-EO, their LC_{50} values were determined -for *E. kuehniella* larvae using a 28-ml glass jar. The 2-cm-diameter pieces of filter paper (Whatman No. 1) were impregnated with six concentrations with equally logarithmic intervals based on equivalent pure essential oil in the air (11.11, 18.52, 33.33, 59.26, 103.7 and 185.18 µl $EO.l^{-1}$ air for EO, and 8.14, 10.37, 13.33, 17.41, 22.22 and 29.63 µl $EO.l^{-1}$ air for SLN-EO). The lowest and highest concentrations were chosen based on several preliminary trials, causing 10 and 90% mortality. The filter paper was attached to the undersurface of the screw cap of the glass jar. The cap was screwed tightly onto the jar

containing 20 3rd-instar larvae. Non-impregnated filter papers and those impregnated with non-loaded SLN were served as controls in the bioassay of EO and EO-SLN, respectivel. The jars were placed randomly into the growth chamber, and mortality was recorded after 24-hour exposure. Each bioassay was replicated 5 times.

Contact bioassay

Nine cm diameter filter papers (Whatman No. 1) placed in the bottom of glass Petri dishes were impregnated with six concentrations with equal logarithmic intervals based on equivalent pure EO per m^2 area. The Concentrations consisted of 0.79, 0.83, 0.89, 0.97, 1.04 and 1.12 $ml.m^{-2}$ for EO and 0.47, 0.53, 0.6, 0.68, 0.75 and 0.85 $ml.m^{-2}$ for SLN-EO, dissolved in 1 ml acetone and distilled water, respectively. The solvents were allowed to evaporate for ten minutes before introducing *E. kuehniella* larvae. Control dishes were treated with either distilled water or acetone only. Each experimental unit consisted of 20 third-instar larvae, maintained into the growth chamber and replicated 5 times. Mortalities were recorded after 24 hours.

Antifeedant bioassay

Wheat flour discs were made to assess the antifeedant effects of EO and SLN-EO on nutritional indices of *E. kuehniella* larvae. 200 µl flour suspension (10 g wheat flour: 50 ml distilled water) was poured on plastic film. The discs were dried at room temperature for 4 hours and maintained overnight in a growth chamber. The mean weight of the flour discs was 36.9 ± 0.32 mg. The experiment was set as a completely randomized design. Based on a feeding toxicity test of EO on flour disc in 4 days, the concentrations of LC_{10}, LC_{15}, and LC_{25} were used for both EO and SLN-EO treatments, which were equivalent to 0.051, 0.058, and 0.067 µl $EO.disc^{-1}$, respectively. The concentrations were applied on the surface of flour discs using 10 µl of acetone and distilled water for EO and SLN-EO treatments, respectively. After evaporating the solvents for 15 minutes, two flour discs from each treatment and ten live larvae of the 3rd instar were weighed and placed at the bottom of a vial, considered the experimental unit. After four days, the weights of live larvae and discs were recorded. Four replications were carried out. The following formulae were used according to Huang *et al*. (2002) to calculate Relative growth rate (RGR), relative consumption rate (RCR), the efficiency of conversion of ingested food (ECI), and feeding deterrence index (FDI).

$RGR = (A - B) / (B \times P)$

$RCR = T / (B \times P)$

$ECI = (RGR / RCR) \times 100$

$FDI = (C - T) / C \times 100$

Where, A = final mean weight of live larvae (mg), B = initial mean weight of larvae (mg), P = duration of feeding period (day), T = weight of consumed food in treatment (mg), C = weight of consumed food in Control (mg).

Insecticidal durability

The biological durability of EO and SLN-EO were determined as described by Ziaee *et al*. (2014). LC_{80} value of *Z. clinopodioides* obtained from fumigant bioassay of EO (129.472 $\mu l.L^{-1}$ air) was applied in this investigation. From the onset of the experiment, 20 larvae were introduced to each vial of either EO or SLN-EO treatments at two-day intervals. The process of the persistence experiment was similar to one defined for fumigant bioassay. Mortalities were recorded from 24-hour post-exposure up to when the potential toxicities of SLN-EO or EO were diminished to their lowest level (Zero percent mortality).

Data analysis

Concentration-mortality data were subjected to probit analysis (Finey, 1971) using a Maximum Likelihood program (POLO-PC, LeOra Sotware, Berkeley, California) to determine the lethal concentration values, confidence limits, and slope of probit mortality regressions. There was no mortality in the control groups; therefore, no data correction was needed (Abbott, 1925). Data on antifeedant bioassays of EO and SLN-EO on nutritional indices were subjected to analysis of variance (ANOVA) using the "Univariate"

option of GLM within SPSS 19.0 statistical software (SPSS, 1998) followed by Tukey's test ($P < 0.05$) for post-hoc testing. Prior to analysis, data were examined and confirmed for normality by the Kolmogorov-Smirnov test.

Results

Characterization of SLN-EOs

The combinations of different preprations of SLN-EOs, assigned from F1 to F10 are accessible in table 1. Three lipids, including Precirol (P), glyceryl monostearate (GMS), and Compritol (C) were used to prepare different formulations of solid lipid nanoparticle loading by *Z. clinopodioides* EO. Nanoformulations containing Tween as surfactant became semisolid after a short time. Although F4, F5, F6, F8, and F9 had small size (less than 200 nm) and negative zeta potential after production, the nanoformulation F10 was chosen due to its good physical stability for further study on characterization and toxicity against *Ephestia* larvae.

Particle sizes and Polydispersity indices of F10 during 6 months are presented in Table 2. The particle diameter of the nanoformulation SLN-EO, F10 after 6 months of storage was 140.4 ± 2.49 nm, which was not significantly different from its diameter on the first day of storage (133.56 ± 1.76). ($p < 0.05$). Moreover, phase separation did not occur during storage. Spherical shape and homogenous dispersion of the nanoparticles were achieved as demonstrated by TEM analysis (Fig. 1). The calculated encapsulation efficiency of SLN-EO was 92% indicating proper entrapment of most ingredients of EO by lipid carriers. The results of GC-MS analysis indicated that the major components of essential oil were not modified before and after the encapsulation process (Fig. 2).

Table 1 The compositions of prepared nanoformulations.

SLN-EO	Lipid	Rate (%w/v)	Surfactant	Rate (%w/v)	EO (%w/v)	Outcome	Size (nm)
F1	GMS	5	Tween	2.5	0.5	Semisolid	-
F2	GMS	5	Tween	2.5	1	Semisolid	-
F3	GMS	5	Tween	3.5	0.5	Semisolid	-
F4	GMS + C	2.5 + 2.5	Poloxamer	2.5	1	Nanoemulsion	134.2
F5	GMS + C	2.5 + 2.5	Poloxamer	2.5	2	Nanoemulsion	121.8
F6	GMS + C	2.5 + 2.5	Poloxamer	2.5	2.5	Nanoemulsion	169.0
F7	GMS	5	Poloxamer+Tween	1.25 + 1.25	0.5	Semisolid	-
F8	GMS + P	2.5 + 2.5	Poloxamer	2.5	1	Nanoemulsion	114.3
F9	GMS + P	2.5 + 2.5	Poloxamer	2.5	2	Nanoemulsion	114.1
F10	GMS + P	2.5 + 2.5	Poloxamer	2.5	2.5	Nanoemulsion	136.7

F: Formulation; GMS: Glyceryl monostearate; C: Compritol; P: Precirol; *Ziziphora clinopodioides* EO: Essential oil.

Table 2 Particle size and polydispersity index (PDI) of formulation 10 (GMS+P 2.5%) during a 6-month storage period.

Parameter	Storage period				
	1 day	1 month	2 months	3 months	6 months
Size (nm) ± SE SE	133.56 ± 1.760	134.43 ± 2.750	137.73 ± 4.010	138.23 ± 1.440	140.4 ± 2.490
PDI ± SE	0.155 ± 0.006	0.159 ± 0.012	0.178 ± 0.005	0.177 ± 0.006	0.181 ± 0.007

GMS: Glyceryl monostearate; P: Precirol.
SE: Standard error.

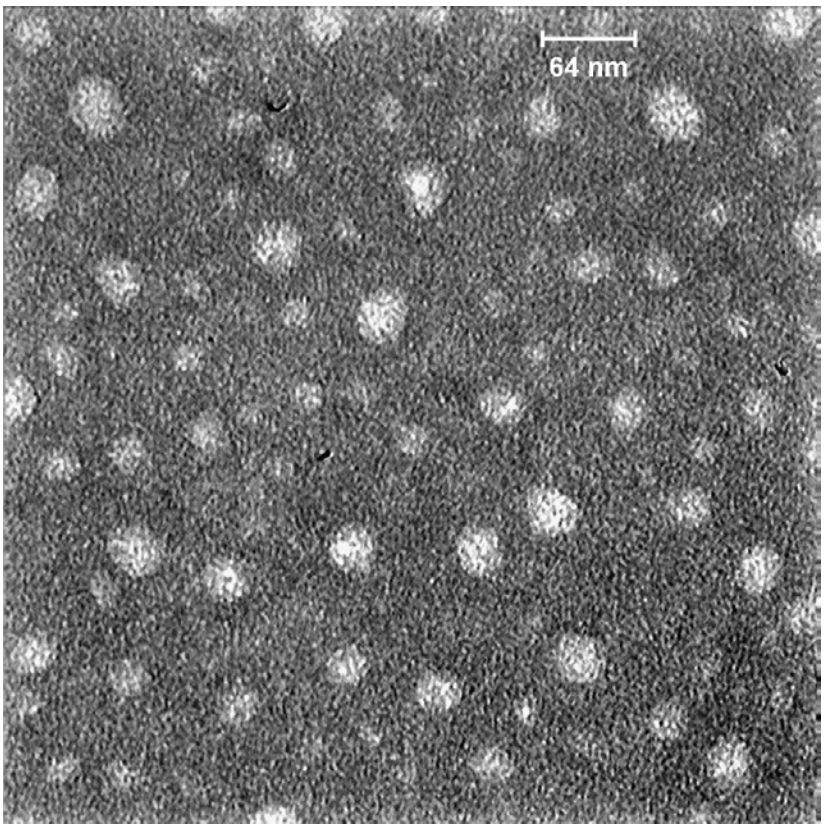

Figure 1 Transmission electron microscopy of SLN-EO.

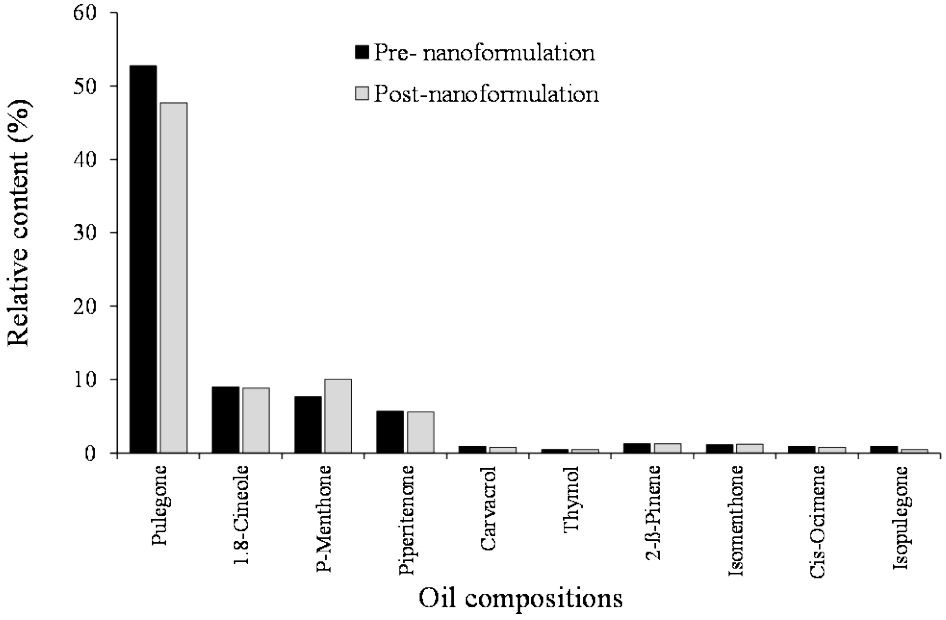

Figure 2 Chemical constituents of *Ziziphora clinopodioides* essential oil before and after nanoformulation.

Biological activity of nanoformulation and pure oil

The results of fumigant bioassay of EO and SLN-EO after 24 hours exposure showed that EO LC_{50} value on the 3rd instar larvae was 47.946 µl.L^{-1} air. In contrast, the corresponding value for the nanoformulation SLN-EO reduced to 16.176 µl.l^{-1} air (Table 3). The comparison of LC_{50} values revealed that the vapor toxicity of SLN-EO was 2.964 times higher than that of EO (95% confidence limit of the LC_{50} ratio: 2.552-3.443, $p < 0.05$).

Table 4 shows the influence of contact activity of EO and nanoformulation against *E. kuehniella* larvae. Based on the LC_{50} ratio of EO and SLN-EO, the contact toxicity of SLN-EO was 1.457 times more potent than that of EO (95% confidence limit of the LC_{50} ratio: 1.409-1.507, $p < 0.05$).

The antifeedant activities of EO and SLN-EO are presented in Tables 5 to 8. EO and SLN-EO were effective on nutritional indices of the 3rd instar *Ephestia* larvae. Both relative growth rate (RGR) and relative consumption rate (RCR) diminished with concentration increase of EO or SLN-EO. Nevertheless, the effects of nanoformulations in reducing the RGR and RCR of larvae were significantly greater than the effects of pure EO ($p < 0.05$). (Tables 5 and 6). The results indicated that the efficiency of conversion of the ingested food (ECI) of larvae was not significantly affected by EO and SLN-EO or their concentrations. (Table 7). The feeding deterrence indices (FDI) of *Ephestia* larvae were enhanced with increasing oil concentration. Means comparison indicated that the nanoformulation SLN-EO significantly increased the FDI of larvae more than that of EO counterpart ($p < 0.05$). (Table 8). The results of bioassays on the persistence of fumigant activity against *E. kuehniella* larvae showed that the SLN-EO formulation caused a uniform 90% mortality during the first week after exposure, after which its mortality was gradually reduced so that on the 14th day it was reduced to zero. While the EO form caused 85% larval mortality at the beginning of the test, it declined rapidly, reaching 10% at the end of the first week and was zero on the 8th day (Fig. 3).

Table 3 Fumigant lethal concentrations of *Ziziphora clinopodioides* essential oil and its nanoformulation against *Ephestia kuehniella* larvae.

Treatment	No. of insects	Lethal concentration (µl.l^{-1} air)		Slope ± SE	χ^2 (df)
		LC_{50} 95% CL (lower-Upper)	LC_{90} 95% CL (lower-Upper)		
EO	700	47.946 (41.962-54.921)	217.614 (171.153-297.643)	1.951 ± 0.15	1.483(4)
SLN-EO	700	16.176 (15.202-17.245)	32.912 (29.311-38.335)	4.154 ± 0.327	1.733(4)

CL: 95% confidence limits; SE: Standard error; EO: Essential oil.

Table 4 Contact lethal concentrations of *Ziziphora clinopodioides* essential oil and its nanoformulation against *Ephestia kuehniella* larvae.

Treatment	No. of insects	Lethal concentration (ml.m^{-2})		Slope ± SE	χ^2 (df)
		LC_{50} 95% CL (lower-Upper)	LC_{90} 95% CL (lower-Upper)		
EO	700	0.937 (0.922-0.952)	1.117 (1.086-1.16)	28.69 ± 2.277	0.837(4)
SLN-EO	700	0.643 (0.624-0.662)	0.895 (0.848-0.964)	15.26 ± 1.29	2.476(4)

CL: 95% Confidence limits; SE: Standard error; EO: Essential oil.

Table 5 The impacts of *Ziziphora clinopodioides* essential oil and its nanoformulation on relative growth rate (RGR) of 3rd instar *Ephestia kuehniella* larvae.

Concentration ($\mu l.disc^{-1}$)	RGR ($mg.mg^{-1}.day^{-1}$)		Mean ± SE
	EO	SLN-EO	
Control	0.1076 ± 0.0053a	0.1057 ± 0.0031 a	-
0.051	0.0954 ± 0.0026 ab	0.0744 ± 0.0058 b	0.0849 ± 0.01 a
0.058	0.0819 ± 0.0021 b	0.0651 ± 0.0044 b	0.0735 ± 0.0084 a
0.067	0.0736 ± 0.004 c	0.0623 ± 0.0052 b	0.0679 ± 0.0056 b
Mean ± SE	0.0836 ± 0.0031 A	0.0672 ± 0.003 B	

Means followed by similar small letter within a column and similar capital letter in the last row (Comparison between EO and SLN-EO) are not significantly different (Turkey's test at $p < 0.05$).

Table 6 The impacts of *Ziziphora clinopodioides* essential oil and its nanoformulation on relative consumption rate (RCR) of 3rd instar *Ephestia kuehniella* larvae.

Concentration ($\mu l.disc^{-1}$)	RCR ($mg.mg^{-1}.day^{-1}$)		Mean ± SE
	EO	SLN-EO	
Control	0.3269 ± 0.0198 a	0.3104 ± 0.0154 a	-
0.051	0.3181 ± 0.02 ab	0. 2728 ± 0.0218 ab	0.2998 ± 0.027 a
0.058	0.2512 ± 0.0161 b	0.2244 ± 0.0062 bc	0.2378 ± 0.0133 a
0.067	0.2008 ± 0.0174 b	0.1922 ± 0.0245 c	0.1965 ± 0.0043 b
Mean ± SE	0.2595 ± 0.0183 A	0.2298 ± 0.0142 B	

Means followed by similar small letter within a column, and similar capital letter in the last row (Comparison between EO and SLN-EO) are not significantly different (Turkey's test at $p < 0.05$).

Table 7 The impacts of *Ziziphora clinopodioides* essential oil and its nanoformulation on efficiency of conversion of ingested food (ECI) of 3rd instar *Ephestia kuehniella* larvae**.**

Concentration ($\mu l.disc^{-1}$)	ECI (%)		Mean ± SE
	EO	SLN-EO	
Control	33.9623 ± 1.1697 a	34.2795 ± 1.737 a	-
0.051	29.4674 ± 1.5639 a	27.3507 ± 1.1749 a	28.4091 ± 1.0583 a
0.058	33.1027 ± 2.6425 a	29.1592 ± 2.6281 a	31.1310 ± 1.9716 a
0.067	37.5813 ± 4.2932 a	33.9216 ± 4.7976 a	35.7514 ± 1.8298 a
Mean ± SE	33.3838 ± 1.8799 A	30.5725 ± 1.824 A	

Means followed by similar small letter within a column, and similar capital letter in the last row (Comparison between EO and SLN-EO) are not significantly different (Turkey's test at $p < 0.05$).

Table 8 The impacts of *Ziziphora clinopodioides* essential oil and its nanoformulation on feeding deterrence of 3rd instar *Ephestia kuehniella* larvae**.**

Concentration ($\mu l.disc^{-1}$)	FDI (%)		Mean ± SE
	EO	SLN-EO	
0.051	7.0423 ± 5.1104 a	19.2488 ± 4.9685 a	13.1455 ± 6.1037 a
0.058	22.2744 ± 4.8499 ab	27.4178 ± 1.4737 a	24.8461 ± 2.5717 ab
0.067	30.5164 ± 4.8488 b	38.9671 ± 7.3735 a	34.7418 ± 4.2253 b
Mean ± SE	19.9443 ± 3.905 A	28.5446 ± 3.6514 B	

Means followed by similar small letter within a column, and similar capital letter in the last row (Comparison between EO and SLN-EO) are not significantly different (Turkey's test at $p < 0.05$).

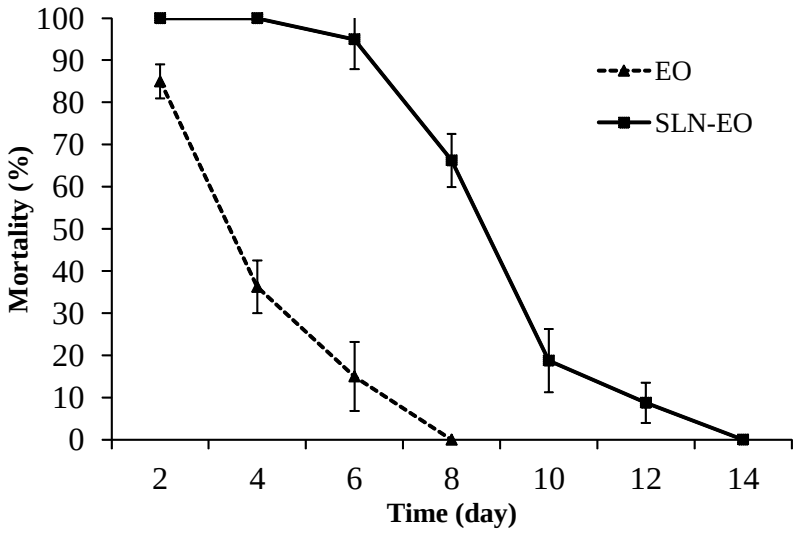

Figure 3 Mortality (mean ± SE) of 3rd instar *Ephestia kuehniella* larvae caused by pure EO and SLN-EO during fumigant durability test.

Discussion

A sufficient characterization of the SLN is a prerequisite to control the quality of the prepared formulation. Some factors, including physical stability, biofate of the nanoparticles, and release rate of the active loading component, are affected by size alteration (Abdelbary and Fahmy, 2009). In this research, SLN-EO methods confirmed the high shear homogenization and ultrasound are efficient by their nanometric size. Furthermore, the polydispersity index value showed narrow size distribution in SLN-EO. The high zeta potential resulting in this investigation provided good stability during storage time and prevented aggregation of nanoparticles. It is noteworthy that encapsulation efficacy is affected by factors such as temperature and pressure during preparation technique (Varona et al., 2010) and the constitution of EO (Kumar *et al*., 2014).

As mentioned in GC-MS analysis, pulegone was the principal constituent of *Z. clinopodioides* oil, and it was applied as an index for calculating entrapment efficiency. Because of the high lipophilicity of EO, high EE was obtained. The encapsulation efficacy of SLN-EO was 92%. A similar result was obtained by Fazly Bazzaz *et al*. (2018), who gained high encapsulation efficacy for prepared EO nanoformulation. In another survey, it has been concluded that the presence of GMS as lipid enhanced encapsulation efficacy of active components (Vivek *et al*., 2007). Our prepared SLN-EO exhibited exceptional stability during experiment time. Some factors such as nanometric size, negative zeta potential, the gradual motion of lipid into solid lipid nanoparticles, and attendance of GMS may cause physical stability of SLN-EO (Hou *et al*., 2003; Liu *et al*., 2007). The nanoformulation could be dispersed easily in distilled water. This finding is a result of the nanosize of SLN-EO. The TEM image confirmed the spherical shape and uniform size of SLN-EO. Similarly, other researchers have reported the round shape of solid lipid nanoparticles (Chen *et al*., 2006; Liu *et al*., 2007). GC-MS analysis showed that the major components before and after the preparation process did not alter considerably, suggesting that SLN is a suitable carrier for EOs and preserving monoterpene constituents.

Solid lipid nanoparticles improved essential oils' insecticidal activity (contact and fumigant toxicity) against *E. kuehniella* larvae. According to our results, the SLN-EO formulation causes a significant reduction in required EO concentrations to control *Ephestia* larvae,

solving the limitations of EO in pest management programs. Moreover, the longer persistence of the SLN-EO suggested that the prepared nanocapsules have controlled release properties. Similarly, Campolo *et al*. (2017) found that nanoemulsion of EO had substantial contact toxicity against eggs and larvae of *Tuta absoluta*. In another research, Louni *et al*. (2018) reported the high insecticidal activity of *Mentha longifolia* L nanoemulsion against *E. kuehniella*. They indicated that in the highest value of EO, the 50% persistent time (PT) of Mentha nanoemulsion was 17.13 days, while pure oil was less effective (PT50 = 2.39 days). Kumar *et al*. (2014), who produced nanoparticles of PEG-Mentha oil, reported similar results. They concluded that after the first week of application against housefly larvae, the mortality of nanoparticles was 93% and reached 57% in the 6th week. In other surveys that EO of garlic and geranium were incorporated in polyethylene glycol, a remarkable increase in contact toxicity persistence against two stored product pests was achieved because of controlled release of terpenes of EOs (Gonzalez *et al*., 2014; Yang *et al*., 2009).

The mobility of nanoparticles is more than their bulk, and they can penetrate the tissue of insects (Nel *et al*., 2009). On the other hand, when nanoparticles were held in the extracellular area, the percentage of detoxification decreased. Therefore, they are not available for detoxifying systems; thus, further bioactive compounds reach the action - site improving the toxic influences of pure oil (Regnault-Roger *et al*., 2012).

According to our finding, SLN-EO and EO significantly affected the feeding activity of the 3rd instar *E. kuehniella* larvae. Based on statistical analysis, the SLN-EO showed more influence on reducing relative growth and consumption rates than the EO. In other studies, the effects of EO on nutritional parameters in various stored product insects were investigated (Huang *et al*., 2002; Stefanazzi *et al*., 2011). Our findings agree with Gonzales *et al*. (2014), who assessed the capability of geranium and bergamot EOs nanocapsules on *T. castaneum* nutritional indices. They reported a notable reduction in RGR and RCR values. Furthermore, Bahrami *et al*. (2016) reported that the EOs of asafetida, geranium, and walnut changed the nutritional parameters of *Rhyzopertha dominica.* Heidarzade *et al*. (2019) demonstrated that *T. castaneum* feeding indices were influenced by both EOs and nanoformulated EO.

Conclusion

The findings of the current study confirmed the high insecticidal activity of SLN-EO nanoformulation against *E. Kuehniella* larvae. Besides, a solid lipid nanoparticle could improve the residual function of EO. It could be concluded that the nanoencapsulation using the high shear homogenization and ultrasound method may solve the constraints of the EO application. Further experiments are suggested to investigate solid lipid nanoparticles loaded by EO for long-time conservation of stored products and their influence on various insect species.

Conflict of Interest Statement

All authors state that they do not have any conflict of interest.

Authors' Contributions

All writers contributed similarly to the current study.

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کاربرد نانوفرمولاسیون حاوی اسانس گیاهی علیه لارو شب‌پره مدیترانه‌ای آرد *Ephestia kuehniella*: تعیین خصوصیات فرمولاسیون و فعالیت زیستی آن

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چکیده: هدف تحقیق حاضر، تولید و تعیین خصوصیات نانوذرات لیپیدی جامد حاوی اسانس کاکوتی بود. بدین‌منظور روش هموژناسیون با فشار کششی بالا و امواج فراصوت استفاده شد. فعالیت بیولوژیکی نانوفرمولاسیون تهیه شده علیه لارو شب‌پره مدیترانه‌ای آرد *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) در شرایط آزمایشگاهی مورد ارزیابی قرار گرفت. اندازه نانوذرات زیر ۱۵۰ نانومتر (شاخص پراکندگی کم‌تر از ۰/۲) و پتانسیل زتای منفی بود. شکل گرد نانوذرات با مطالعه توسط میکروسکوپ الکترونی عبوری، گزارش گردید. بازده بارگیری اسانس در نانوذرات لیپیدی جامد توسط روش فیلتراسیون- سانتریفیوژ، ۹۲ درصد محاسبه شد. طبق نتایج به‌دست آمده، نانوذرات لیپیدی جامد می‌تواند سمّیت‌تنفسی اسانس را افزایش دهد (۳ برابر). هم‌چنین نانوفرمولاسیون اسانس توانست دوام اسانس خالص را علیه لارو تا دو هفته افزایش دهد. شاخص‌های تغذیه‌ای لارو شامل شاخص نرخ رشد نسبی، شاخص نرخ مصرف نسبی و شاخص بازدارندگی تغذیه‌ای توسط نانوذرات لیپیدی جامد حاوی اسانس کاکوتی در مقایسه با اسانس به‌طور قابل‌توجهی تغییر کرد. براساس یافته‌های این پژوهش، نانوذرات لیپیدی جامد، به‌عنوان حامل مناسب اسانس جهت کنترل پایدار لارو شب‌پره مدیترانه‌ای آرد پیشنهاد می‌گردد.

واژگان کلیدی: نانوفرمولاسیون، *Ziziphora clinopodioides* Lam.، *Ephestia kuehniella*، دوام، شاخص‌های تغذیه‌ای