

Research Article

Insecticidal efficacy of nanoemulsion containing *Mentha longifolia* essential oil against *Ephestia kuehniella* (Lepidoptera: Pyralidae)

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Abstract: In recent years, different formulations such as nanoemulsions have been widely used for the target delivery, and enhanced biological functions of pesticides combinations. In this study, contact toxicity of *Mentha longifolia* L. essential oil compared with its nanoemulsion on *Ephestia kuehniella* Zeller has been investigated. The experiments were conducted and executed at 27 ± 1 °C, and $75 \pm 5\%$ relative humidity under dark conditions. Chemical composition of this plant extract was also studied. The main constituents were pulegone (28.84%), 1,8-cineol (19.6%), p-menthan-3-one-cis (8.2%), β -pinene (6.46%) and p-menthan-3-one-trans (5.86%). Results indicated that the mortality rate of 5th instar larva of *E. kuehniella* increased with rising concentrations (10000-40000ppm) for oil and (8000-20000 ppm) for nanoemulsion, respectively. The essential oil LC₅₀ was 21352ppm, while this value for nanoemulsion was 14068ppm. According to the results, nanoemulsion was more effective than essential oil. *M. longifolia* oil had lower durability and the 50% persistent time (PT₅₀) was 2.39 day compared to the nanoemulsion (PT₅₀ = 17.13 day) in the highest concentration of essential oil. The nanoparticle size was 14-36 nanometers (nm) when the transmission electron microscope (TEM) was applied. The surface morphology of nanoemulsion was also studied by TEM. The average size of the particles was estimated 234nm by using laser light scattering apparatus. The overall results of this study show that by using nanoemulsion formulation, the effect of essential oil contact toxicity and its durability increases. Hence, the nanoemulsion slow-release formulation may represent a new category of biopesticides and this should be considered in the integrated pest management program.

Keywords: Contact toxicity, *Ephestia kuehniella*, Essential oil, *Mentha longifolia*, Nanoemulsion

Introduction

The main quantitative and qualitative losses of stored products can be related to storage pests (Rumbos *et al.*, 2016). One of the major pests in industrial flour mills in temperate climates is

the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) (Jemaa *et al.*, 2013). Moth larva can decrease the quality of stored products by feeding, webbing and fecal material (Hansen and Jensen, 2002). The excessive usage of fumigant chemical pesticides (e.g. phosphine and methyl bromide) in order to control stored product pests can raise emergence of resistant insects, residues in agriculture products and in the environment (Dent, 2000; Fields and White,

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2002). Methyl bromide has also been phased out in 2005 as an ozone depleter (Rajendran and Sriranjini, 2008). Therefore, it is necessary to develop safe alternatives that are inexpensive, environmentally friendly, and convenient for usage.

Various aromatic plant preparations like powders (Tesfu and Emanu 2013), solvent extracts (Moharramipour and Nazemi Rafih, 2008), and essential oils (Ziaee *et al.*, 2014a) have been investigated for their insecticidal activity. These compounds can be used as fumigants and contact biopesticides for control of stored foodstuff pests (Gusmao *et al.*, 2013). The mints, *Mentha longifolia* L. species is from the family of Labiatae (Lamiaceae), which are widely grown in Eurasia, Australia, and South and North Africa (Sharapov, 2012). This species also is one of the most common aromatic plants in Iran that has considerable toxicant effects on various storage pests (Shahmirzaei *et al.*, 2016).

Nowadays, there is a considerable interest towards the biological properties of essential oils (EOs). Different methods and procedures have been developed to conserve EOs, because they are unstable and susceptible to degradation when exposed to environmental stresses such as light, oxygen and temperature. One of the best techniques is encapsulation in different colloidal systems including nanoemulsions. Encapsulation is an efficient approach to improve the physical stability of EO, protect it from evaporation, and enhance EO bioactivity, because of controlled release of oil as the active ingredient (Majeed *et al.*, 2015; Moharramipour and Negahban, 2014). Nanocarrier systems are classified in two categories: polymer-based nanoparticles and lipid-based nanoparticles (Anandharamakrishnan, 2014). Monoglycerides are esters of fatty acids and glycerol. Thanks to their emulsifying property, monoglycerides have diverse applications as components of nano and liposomal formulations in drug delivery systems. One of the common industrial monoacylglycerols is Glyceryl monostearate (GMS). GMS is used as a preservative agent, emulsifying agent for oils, waxes and solvent,

and also solidifier and controlled release factor in formulations. The mentioned compound is used to produce various lipid-based nanoparticle delivery systems such as nanoemulsion (Kavadia *et al.*, 2017).

Some studies have been conducted on different oil formulations. For instance, Moretti *et al.* (2002) stated that microcapsulation is a suitable technique for conservation and controlled release of *Rosmarinus officinalis* L. oil. Then, Lai *et al.* (2006) conducted a research about the effect of solid lipid oil of *Artemisia arborescens* L. on *Bemisia tabaci* (Gennadius). They concluded that this constitute has a high physical stability in various temperatures (4-40 C) in 60 days. In other study, the high ability of *Artemisia sieberi* Besser nanocapsulated essential oil on repellent and nutrition properties of *Plutella xylostella* (Lep. Plutellidae) was confirmed (Negahban *et al.*, 2013 a & b). Various researchers used high energy emulsification approach for the generation of EO loaded nanoemulsion. For example, Laing *et al.* (2012) encapsulated peppermint oil in starch based nanoemulsions to increase its stability and bioavailability. The strong influence of nanogel from *Carum copitum* L. was also reported against *Sitophilus granarius* L. and *Tribolium confusum* (Herbst) (Ziaee *et al.*, 2014b). However, there is no study about nanoemulsion properties from *M. longifolia* oil until now.

The structure of nanoemulsion includes a lipid phase dispersed in an aqueous continuous phase, where each oil droplet is surrounded by a thin interfacial layer consisting of emulsifier molecules (Ranjan *et al.*, 2016). The mentioned compounds (due to their very small size) provide an appropriate method to enhance the physical stability of the encapsulated active ingredients and raise the distribution of insecticidal agents in the target products (Topuz *et al.*, 2016).

The objectives of this study are: a) evaluating major chemical composition (GC-MS) of *M. longifolia* oil, b) investigating about surface morphology (TEM) and particle size distribution of prepared nanoemulsion from the

mentioned oil, c) comparing the contact activities of *M. longifolia* essential oil before and after nano-formulation on the mortality of *E. kuehniella*, and d) studying about the effect of nanoencapsulation on the durability of the oil on *E. kuehniella* larva.

Materials and Methods

Experiments were carried out in the laboratory of Toxicology at Lorestan University, Khorramabad, Iran. All experiments were conducted in an incubator at 27 ± 1 °C and $75 \pm 5\%$ relative humidity in continuous darkness.

Plant material

In July 2016, aerial parts (Leaves, flowers and stems) of *M. longifolia* were collected at full flowering stage from Khorramabad ($47^{\circ}41'E32^{\circ}56'N$), Iran. The plant material was air-dried at room temperature ($20-25$ °C) with suitable ventilation, for seven days until crisp. The dried samples were maintained at -24 °C and then applied to extract its essential oil.

Isolation and analysis of essential oil

The air-dried samples were exposed to hydrodistillation (100g of each sample in 1200 ml of distilled water) using a Clevenger type apparatus for 4 hours. The oils were dried over anhydrous sodium sulphate and stored in a refrigerator at 4 °C.

The chemical composition of the essential oil was determined with the help of GC-MS technique, and by using a Shimadzu GC-9A with helium (a carrier gas) at a linear velocity of 0.3m/s on a DB-5 column (30m \times 0.25mm i.d, film thickness: 0.25 μ m). The oven was programmed to increase the temperature of 60 °C (3 min) isotherm to 210 °C at a rate of 3 °C/min. Temperatures of injector and detector were, respectively, 300 and 270 °C. The Gas chromatography-mass spectrometry (GC-MS) analysis was performed on a Varian 3400 equipped with a DB-5 column with the similar characteristics as the one used in GC. Here, the transfer line temperature and the ionization energy were, respectively, 260 °C and 70 eV.

By comparing the essential oil components GC retention times and their mass spectra with known combinations, the unknown essential oil components were clarified.

Insect cultures

The Mediterranean flour moth, *E. kuehniella*, was reared on artificial diets consisting of 65% wheat flour, 25% wheat bran and 10% baker's yeast in plastic dishes with 33 cm diameter and 13cm height. The thin fabrics were employed as dishes lid. The cultures were kept in a rearing room at 27 ± 1 °C and $75 \pm 5\%$ relative humidity under dark condition.

Preparing of nanoemulsion

For preparation of the aqueous phase, 90ml of purified water and 3g of tween 80 (Tween®80, Ph Eur, JP, NF, 1130g/mol, Merck, GER) were mixed by using mechanical mixer (HEITORQUE, Heidolph, Germany) in a 300-ml beaker at $5-10$ °C. The rotating speed was 500-1000rpm that was regulated manually (about 50rpm per 3 minutes). Glyceryl monostearate (GMS) (2g) (99%, 358.56g/mol, Merck, GER) were heated at 80 °C by using magnetic stirrer (RH basic 2, IKA, GER) at rotating speed of 200 to 1000rpm (50rpm per second). After being melted, 2g of tween 80 was added. Then, temperature was decreased (1 °C in each 15 second) until reaching to 20 °C. In the next stage, 5g of pure essential oil of *M. longifolia* were mixed with GMS and tween 80 drop by drop under agitation to form the nanoemulsion oil phase. Then this compound was added to the aqueous phase drop by drop and mixed by using homogenizer apparatus (T 18 basic ULTRA-TURRAX package, IKA, GER) with rotating speed of 10000 to 20000rpm for 30 minutes (15 minute at 10000 rpm and 15 minute at 20000rpm) until the reaction was ended.

Survey of nanoemulsion surface morphology and nanoparticle size

The transmission electron microscope (TEM) (Zeiss-EM10C-100KV, GER) was used for observation the surface and morphology of nanoemulsion. Particle size distribution of

nanoemulsion was estimated by dynamic light scattering (DLS) (Nano ZS, Malvern, UK) apparatus.

Contact toxicity

To evaluate the contact toxicity of *M. longifolia* essential oil and its nanoemulsion on *E. kuehniella* 5th instar larva, the method of spraying on surface of insect body and dishes was selected (Morishita, 2001). Preliminary tests were scheduled to select the proper concentration ranges of *M. longifolia* oil and its nanoemulsion. Oil was diluted in 10% ethanol whereas water was used as solvent for nanoemulsion. Oil was used at concentrations of 10000, 14125.3, 19952.6, 28183.8 and 40000ppm, and nanoemulsion at concentrations of 8000, 10000, 12589.2, 15848.9 and 20000ppm. Ten 5th instar larva of *E. kuehniella* were placed at the bottom of each petri dish (9 cm) with a perforated lid, covered with thin fabric to allow gas exchange. One ml each of the estimated concentrations from oil and nanoemulsion was diffused on each petri dish. In order to uniformly spray the larvae in petri dishes, the potter tower apparatus (Potter tower, Burcard, UK) was used.

Pressure of the device at spraying time was adjusted to 8 bars. Experiments were done in 5 replications for each concentration. After 24 hours from the experiment assembly, mortality percentage was evaluated. Ethanol 10% was used for essential oil control group. There were two control groups for nanoemulsion (water and nanoemulsion without essential oil).

Durability of contact toxicity

The experiments were performed at 40000ppm (lethal concentration for 80% mortality) of *M. longifolia* oil, as achieved from contact toxicity bioassay. The used concentration was left for one day in glass vials exposed to air. Ten *E. kuehniella* 5th instar larva were placed at the bottom of each petri dish, 9cm diameter. Then, predetermined concentration of each

treatment was sprayed by potter tower apparatus on larva body and dishes. The spraying rate was 1ml for every replication. After 24 hours, the number of dead larva was counted and the mortality percentage was calculated. Counting were made at intervals of 2 days until no mortality was observed. It is noteworthy that there is 2 days off among experiments. Experiments were repeated 5 times at estimated concentration of each combination. After spraying, the petri dishes were closed by thin fabrics.

Data analysis

By using Abbott's (1925) formula the mortality data was corrected to control mortality rate. The analysis of variance (ANOVA) was used for each experiment. Probit analysis (Finney, 1971) was also employed to estimate 50% lethal concentration (LC₅₀) and 50% persistent time (PT₅₀) values by SAS 9.4 (SAS Institute, 1996). The Tukey's multiple range test was applied at the value of P = 0.05 to clarify differences among multiple means using SAS 9.4 (SAS Institute, 1996).

Results

Chemical composition of *Mentha longifolia* essential oil

GC-MS analysis of *M. longifolia* oil is reported in Table 1. Major components were pulegone (28.84%), 1, 8-cineol (19.6%), p-menthan-3-one-cis (8.2%), β-pinene (6.46%) and p-menthan-3-one-trans (5.86%).

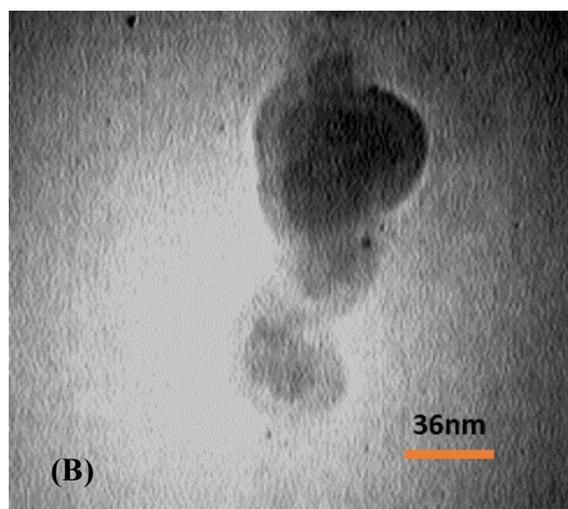
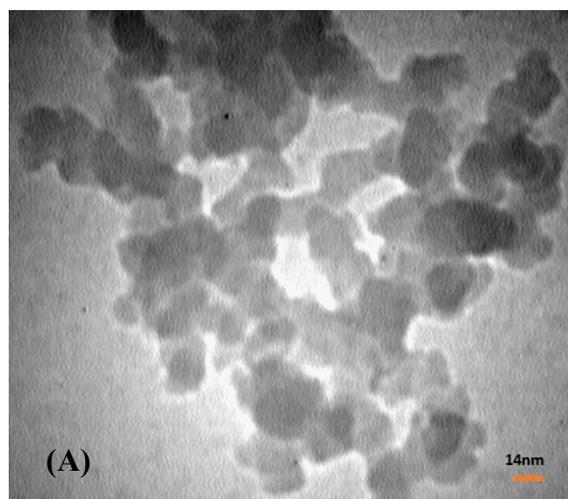
Nanoemulsion surface morphology and particle size distribution

TEM images (as can be seen from Fig. 1) show nucleus and wall structure of nanoemulsion. Nanoparticle sizes by using TEM were around 14-36nm. The mean size of particles, according to Fig. 2, was estimated about 234nm using laser light scattering apparatus (Fig. 2).

Table 1 Chemical composition of the essential oil from *Mentha longifolia*.

Compounds	RT	Content (%)
α -phellhandrene	5.21	0.2
α -pinene	5.3	3.3
Camphene	5.6	0.97
β -pinene	6.08	6.46
β -myrcene	6.34	1.47
α -terpinene	6.76	1.02
Limonene	6.92	1.92
1,8-cineol	7.18	19.6
δ -terpinene	7.4	1.87
Terpinolene	7.86	0.59
Cis- β -terpineol	8.32	1.51
Octyl-acetate	8.72	0.12
Linalool	8.8	0.09
Sabinenehydrate	8.93	0.17
Trans- β -terpineol	9.15	0.24
α -campholene aldehyde	9.29	0.1
Menthofuran	9.39	0.17
p-menthan-3-one-cis	9.69	8.2
Menthone	9.75	0.95
p-menthan-3-one-trans	9.9	5.86
α -Terpineol	10.03	0.6
Borneol	10.09	1.43
Cis-iso-pulegone	10.16	1.08
β -Fenchol	10.3	3.79
2-isopropyl-2,5-dimethyl-cyclohexanone	10.83	2.75
Pulegone	11.09	28.84
Caryophyllene	12.71	0.79
Piperitenone	12.88	1.65
Spathulenol	15.36	0.58
α -Caryophyllene epoxide	15.43	3.37
β -Caryophyllene epoxide	15.73	0.32

RT: retention time.

**Figure 1** A, B: Transmission microscope images of nanoemulsion core structure from *Mentha longifolia* oil.**Contact toxicity**

The contact toxicity of *M. longifolia* oil and its nanoemulsion was tested on 5th instar larva of *E. kuehniella*. The results showed that in both treatments (for oil: $F = 36.16$; $df = 5, 20$; $p > 0.0001$, and for nanoemulsion: $F = 43.92$; $df = 4, 20$; $p > 0.0001$) with rising concentration mortality was increased. As seen in Fig. 3, used concentrations in nanoemulsion (8000-20000ppm) were less than essential oil doses (10000-40000ppm). The highest and the lowest mortality were 88% (40000ppm) and 12.20% (10000ppm) in the oil treatment, respectively.

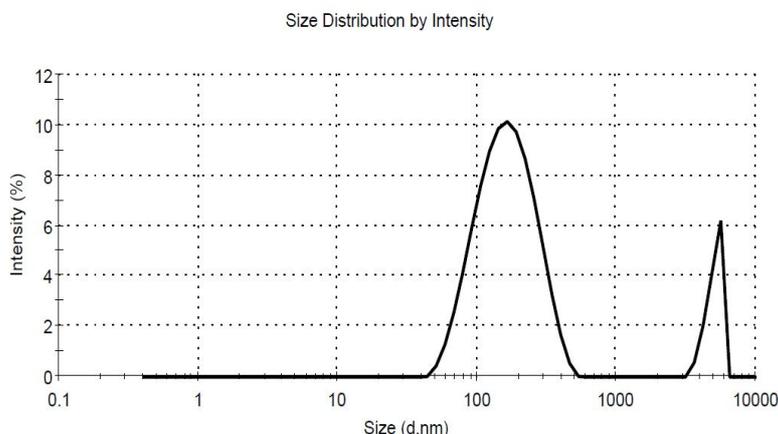


Figure 2 Size distribution of nanoemulsion from *Mentha longifolia* oil.

The highest concentration (20000ppm) of the nanoemulsion caused 79.5% mortality of *E. kuehniella* larva. At the lowest dose (8000ppm), mortality reached 20%. The mortality percentage of nanoemulsion control groups (water and nanoemulsion without oil) was the same. At 21352ppm, *M. longifolia* oil caused 50% mortality with a 24 hours exposure, while nanoemulsion LC₅₀ was 14068ppm at the same time (Table 2). The relative median potency parameter (RMP) showed that there is a significant difference between LC₅₀ values of nanoemulsion and essential oil (Table 3). These observations demonstrated that nanoemulsion in lower concentrations compared with *M. longifolia* oil has a considerably greater effect on the control of *E. kuehniella* larvae.

Durability of contact toxicity

According to Fig. 4, the results showed that nanoemulsion has longer durability compared to essential oil on *E. kuehniella* larva. Durability of nanoemulsion lasted for 33 days. In the first five days, its effect on mortality was 100%, and then it showed a reduced trend until day 33 when mortality rate was zero. On the other hand, the EO had lower durability as its effect on larva mortality was 84% on the first day. This value reached to zero by the 7th day. PT₅₀ for nanoemulsion and EO were 2.39 and 17.13 days, respectively (Table 4). The results showed that nano-formulation causes slow-release of EO and raises its period of durability on *E. kuehniella* larva.

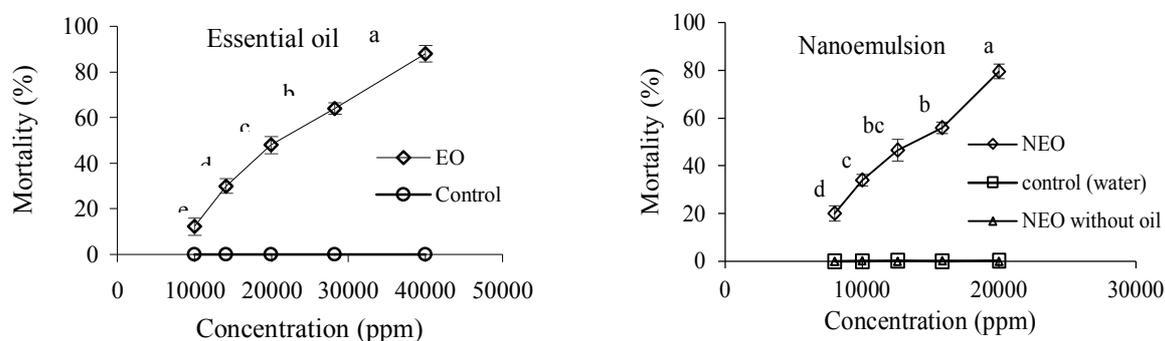


Figure 3 Mortality Percentage of *Ephestia kuehniella* larva exposed to different concentrations of *Mentha longifolia* oil and its nanoemulsion.

EO) Essential oil, NEO) Nanoemulsion

The mortality percentage of nanoemulsion control groups was the same.

Table 2 LC₅₀ values of *Mentha longifolia* oil and its nanoemulsion against *Ephestia kuehniella*.

Compound	n	Chi-square	Intercept	df	slope ± SE	P-value	LC ₅₀ (ppm)	95% confidence limits (ppm)	
								Lower	Upper
Essential oil	250	0.92	-16.2 ± 2.14	3	3.75 ± 0.49	0.82	21352	19010	24011
Nanoemulsion	250	1.31	-18.2 ± 3.02	3	4.38 ± 0.73	0.72	14068	12739	15719

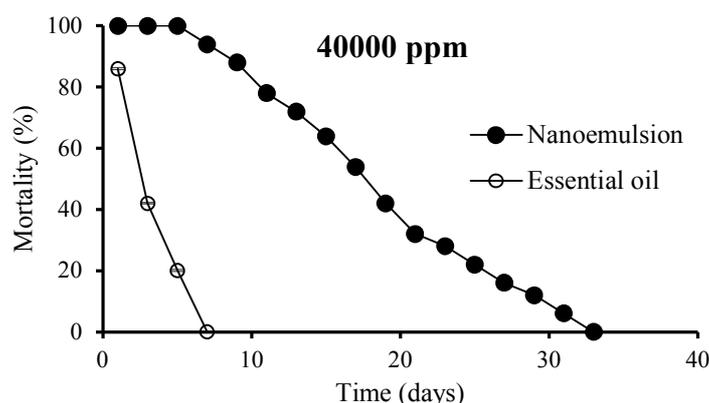
Table 3 Comparison of estimated LC₅₀ of *Mentha longifolia* oil and its nanoemulsion on *Ephestia kuehniella* larvae.

LC ₅₀ (ppm) (EO)	LC ₅₀ (ppm) nanoemulsion	RMP ¹	95% confidence limits
21352	11128	1.55	1.26-2.03

RMP = Relative Median Potency.

Table 4 PT₅₀ values of *Mentha longifolia* oil and its nanoemulsion against *Ephestia kuehniella* at 40000ppm.

Compounds	PT ₅₀ (day)	Chi-square	Intercept	df	Slope ± SE	P-value	95% confidence limits (day)	
							Lower	Upper
Essential oil	2.39	0.04	1.07 ± 0.21	1	-2.83 ± 0.43	0.83	1.94	2.91
Nanoemulsion	17.13	1.09	5.49 ± 0.59	7	-4.45 ± 0.48	0.99	16.06	18.33

**Figure 4** Mortality percentage of *Ephestia kuehniella* exposed to same concentration of *Mentha longifolia* oil and its nanoemulsion over time.

Discussion

A reduction in the quality of foodstuff would be important for control of *E. kuehniella*. In this study, an eco-friendly formulation was used that reduces EOs limitations in control of insect pests. Prepared nanoemulsion from *M. longifolia* oil could increase the toxicant effect of this oil on *E. kuehniella*. Also, it boosts the oil persistence time. It is noteworthy to mention, that there is no data about the impacts of formulations containing plant essential oils on *E. kuehniella*. In the present study, a new

method for production of nanoemulsion has been used. Adjuvant materials of formulation and essential oil that are used in this compound are completely eco-friendly. The produced nanoemulsion with this technique can be employed in storage houses to conserve foodstuff from pest damages. The mentioned nanoemulsion has been produced from nanoparticle of *M. longifolia* as active ingredient. The main constituents of *M. longifolia* oil are pulegone (28.84%), 1, 8-cineol (19.6%), p-menthan-3-one-cis (8.2%), β-pinene (6.46%) and p-menthan-3-one-trans

(5.86%). Biological properties of EO are mainly due to the main components that typically are in high values. In this respect, the insecticidal effect of 1,8 cineol has been proved by Kordali *et al.* (2006). Elansary *et al.* (2013) reported similar combination of *M. longifolia* oil. They showed that pulegone (56.43%) had the highest value among the other compounds. Because fumigant toxicity of *M. longifolia* oil is stronger than its contact toxicity (Shahmirzaei *et al.*, 2016), using nanoemulsion technique can increase the contact activity of EO and its durability, through slow-release of active ingredients over time. The average nanoparticle size has been estimated around 234nm by using GMS in formulation. There are similar studies where GMS accompany with compounds except essential oil as nanocapsules core has been used for production of nanoparticles (Tiyaboonchai *et al.*, 2007; Nayak *et al.*, 2010). Negahban *et al.* (2013a) estimated the average nanoparticle size encapsulated *A. sieberi* oil by using poly urea formaldehyde polymer about 95.5nm. In addition to the used polymer, other factors such as the applied method, synthesis conditions and active ingredient type play an important role in nanoparticle size (Uppal *et al.*, 2010; Dang *et al.*, 2012; Allahvaisi *et al.*, 2017). GMS is used for the first time in nanoemulsion formulation containing EO. Our data indicated that this polymer has a high capability to load biopesticides like EO.

The results showed that nanoemulsion (LC₅₀ = 14068ppm) compared to EO (LC₅₀ = 21352ppm) has stronger contact toxicity and effect on mortality rate of *E. kuehniella* 5th instar larva. There are some data about different formulations on various pests. Lai *et al.* (2006) demonstrated high physical stability of solid lipid oil of *A. arborescens* L. on *B. tabaci* (Gennadius) in different temperatures for a period of 60 days. In the period of keeping, oil type and its quality and quantity was constant. Negahban *et al.* (2013 a & b) concluded that nanocapsulated oil of *A. sieberi* has a stronger impact than oil of this plant on repellency and nutrition values of *P. xylostella* (Lep.: Plutellidae). These observations are in good

agreement with present results. Similar conclusions were obtained about effect of nanocapsulated essential oil from *C. copticum* on *P. xylostella* (Jamal *et al.*, 2013). Moreover, in present study durability rate of nanoemulsion (PT₅₀ = 17.13 day) compared with oil (PT₅₀ = 2.39 day) was longer. The highest insect mortality of nanoemulsion was determined during the first five days. This trend declined slowly until day 33 when no mortality was observed. *Mentha longifolia* oil had the greatest release volume in the primary hours (24 h), and it was reduced quickly. The mortality rate reached to zero after day 7. Therefore, nanoemulsion method can improve performance of *M. longifolia* oil and maintains its properties in a long-term. Passino *et al.* (2004) stated that the highest release rate of microcapsulation from *R. officinalis* oil (75%) accrued within 25 days. In other study, prepared nanoemulsions from different EOs on *Aedes aegypti* (L.) had a better physical stability and longer durability than the oils by themselves (Nuchuchua *et al.*, 2009). Laing *et al.* (2012) increased stability and bioavailability of peppermint oil starch based on the nanoemulsion. Their results confirm our findings. *Mentha longifolia* is considered as a prevalent plant in most areas of Iran (Kamkar *et al.*, 2012). Insecticidal properties of *M. longifolia* oil have been demonstrated by other researchers, like fumigant toxicity and repellency on *T. confusum* (Saeidi and Moharramipour, 2013), contact activity against *Sitophilus oryzae* L. (Motamedi *et al.*, 2011), and oviposition deterrency against *Callosobruchus maculatus* F. (Bruchidae) (Shakarami *et al.*, 2010). There is no information about preparing formulation from *M. longifolia* essential oil and its effect on *E. kuehniella* until now. According to the results, production of nanoemulsion with this new technique results in considerable decrease of the required EO concentrations. Moreover, nanoemulsion can increase stability and durability of *M. longifolia* essential oil. In fact, this technique owing to its controlled release of oil improve the control operation in the long

term. Since the 5th instar larva of *E. kuehniella* is a resistant pest, it is reasonable to claim that nanoemulsion has an acceptable control on it. In conclusion, owing to the existence of different aromatic plants in Iran, we can use species with higher toxicity that would be more economical to produce nanoemulsions.

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اثر حشره‌کشی نانوامولسیون حاوی اسانس پونه *Mentha longifolia* علیه شب‌پره آرد *Ephestia kuehniella*

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چکیده: در سال‌های اخیر فرمولاسیون‌های مختلفی مانند نانوامولسیون‌ها جهت رساندن ترکیبات آفتکش‌ها و افزایش عملکردهای بیولوژیکی آن‌ها روی هدف به‌طور گسترده استفاده شده‌اند. در این مطالعه سمیت تماسی اسانس پونه *Mentha longifolia* L. در مقایسه با نانوامولسیون آن روی شب‌پره آرد *Ephestia kuehniella* Zeller بررسی شده است. آزمایشات در دمای 1 ± 27 درجه سلسیوس، رطوبت نسبی 5 ± 65 درصد تحت شرایط تاریکی انجام شد. ترکیبات شیمیایی اسانس گیاهی نیز مطالعه شد. ترکیبات عمده شامل pulegone (۲۸/۸۴ درصد)، 1,8-cineol (۱۹/۶ درصد)، -p-menthan-3-one-cis (۸/۲ درصد)، β -pinene (۶/۴۶ درصد) و p-menthan-3-one-trans (۵/۸۶ درصد) بود. نتایج نشان داد که نرخ مرگ‌ومیر لاروهای سن پنجم شب‌پره آرد با افزایش غلظت (۱۰۰۰۰-۴۰۰۰۰ پی‌پی‌ام) برای اسانس و (۸۰۰۰-۲۰۰۰۰ پی‌پی‌ام) برای نانوامولسیون افزایش یافت. میزان LC_{50} اسانس ۲۱۳۵۲ پی‌پی‌ام بود، در حالی که این مقدار برای نانوامولسیون ۱۴۰۶۸ پی‌پی‌ام بود. مطابق با نتایج، اثر نانوامولسیون بسیار بیش‌تر از اسانس بود. اسانس پونه دوام کم‌تری ($PT_{50} = 2.39$ days) در مقایسه با نانوامولسیون ($PT_{50} = 17.13$ days) در بالاترین غلظت به‌دست آمده از اسانس داشت. سایز ذرات نانو زمانی که الکترون میکروسکوپ پرتو گذر (TEM) به‌کار برده شد حدود ۱۴-۳۶ نانومتر بود. مرفولوژی سطح نانوامولسیون نیز به‌وسیله TEM بررسی شد. میانگین سایز ذرات با استفاده از دستگاه laser light scattering (SEMATEch) ۲۳۴ نانومتر تخمین زده شد. نتایج کلی نشان می‌دهد که با استفاده از فرمولاسیون نانوامولسیون اثر سمیت تماسی و دوام اسانس افزایش می‌یابد. بنابراین فرمولاسیون دارای رهايش تدریجی نانوامولسیون احتمالاً یک دسته‌بندی جدید از آفتکش‌های زیستی را نشان می‌دهد و باید در مدیریت تلفیقی آفات (IPM) در نظر گرفته شود.

واژگان کلیدی: سمیت تماسی، شب‌پره آرد، اسانس، پونه، نانوامولسیون