J. Crop Prot. 2019, 8 (4): 501-520

Research Article

Efficacy of *Mentha spicata* and *Mentha pulegium* essential oil nanoformulation on mortality and physiology of *Tribolium castaneum* (Col.: Tenebrionidae)

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Abstract: Recently, the methods that improve essential oils (EOs) properties and make them appropriate to be applied as biorational pesticides have been regarded more precisely. The essential oils nanoformulation (EONF) is a promising strategy to develop and facilitate the applicability of the EOs in stored pest management. In this study, the toxicity, antifeedant and physiological effects of *Mentha spicata* L. and *Mentha pulegium* L. EOs and their NFs was investigated on the red flour beetle, *Tribolium castaneum* (Herbst). Characterization of nanocapsules using dynamic light scattering (DLS) and transmission electron microscopy (TEM) showed that the nanocapsules were spherical in shape with the average sizes of 56.91 and 98.99 nm for *M. spicata* and *M. pulegium* EONF, respectively. The encapsulation efficiency obtained was 95.47 and 86.03% for *M. spicata* and *M. pulegium* EONF, respectively. After 72 h, the LC_{50} values of the EOs and NF of *M. spicata* were 18.422 and 9.279 µl/ml and 7.939 and 6.793 µl/ml for *M. pulegium*, respectively. The results confirmed that the feeding indices of *T. castaneum* were affected by the EOs and their NFs. In addition, both the EOs and EONF decreased the relative growth rate (RGR) and relative consumption rate (RCR) and had a moderate feeding deterrent activity on the adults of *T. castaneum*. The EOs and their NFs decreased the general esterase, acetylcholine esterase, α-amylase and general protease and increased the glutathione *S*-transferases activity of *T. castaneum*. The overall findings of this research suggest that the NF of the EOs (especially *M. pulegium*) can be used for an efficient control of *T. castaneum*.

Keywords: encapsulation, enzymatic activities, essential oil, feeding indices, toxicity

Introduction

Cereal crops remain the primary food source in many countries (Alonso-Amelot and Avila-Núnez, 2011). During storage, huge amounts of these foodstuffs are destroyed each year by different stored product pests and other agents (Rajendran, 2002). Therefore, stored products protection against insect pests and diseases is necessary for modern agriculture.

The red flour beetle, *Tribolium castaneum* (Herbst) (Col.: Tenebrionidae), is a widespread destructive insect that lives in

Handling Editor: Saeid Moharramipour

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Received: 25 June 2019, Accepted: 27 October 2019
Published online: 3 December 2019

stored grains. Adults and larvae grow on various foodstuffs, such as milled cereal products, causing great losses in both the quality and quantity of these products (Rees, 2004). Infestations not only cause irreparable damage due to huge consumption of grains, but also result in elevated temperature and humidity leading to faster growth of molds such as toxigenic species (Magan *et al*., 2003). This pest is mainly controlled by the use of chemical pesticides and fumigants. However, the frequent use of pesticides has resulted in resistance, manipulation in the ecosystem and toxic effects on humans as well as other organisms (Kumar *et al*., 2011). The resistance of stored-grain insects such as *T. castaneum*, to insecticides and fumigants has also been reported in many studies (Hussain *et al*., 2005; Opit *et al*., 2012). Subsequently, there is a growing interest in finding less hazardous substitutes to control stored foodstuff insect pests.

Plant metabolites such as essential oils (EOs) have been investigated extensively in order to develop user-friendly natural alternatives that are pest specific and non-hazardous to health and ecosystem in order to reduce the need for chemical pesticides (Isman, 2006; Koul, 2012). Some of medicinal plants are rich in EOs and there are many studies about their insecticidal, physiological and behavioral activities on different insect species (Stefanazzi *et al*., 2011; Gonzalez *et al*., 2014; Bahrami *et al*., 2016; Reddy *et al*., 2016; Shahriari *et al*., 2017 and 2018). The EOs of *Tagetes terniflora* Kunth, *Cymbopogon citratus* Stapf. and *Elyonurus muticus* Sprengel have been reported to cause post-ingestive toxicity and change nutritional indices of *T. castaneum* and *Sitophilus oryzae* L. (Col.: Curculionidae) adults (Stefanazzi *et al*., 2011). The EO of *Teucrium polium* L. (Shahriari *et al*., 2017), α-pinene, trans-anethole and thymol as the EO constituents (Shahriari *et al*., 2018) and EOs of *Allium sativum* L. and *Eucalyptus globulus* Labill (Shahriari *et al*., 2019) caused oral toxicity and disruptively affected biological systems including digestive performance and detoxifying enzymes of *Ephestia kuehniella* Zeller larvae (Lep.: Pyralidae). The species of *Mentha spicata* L. and *Mentha pulegium* L. (Lamiales: Lamiaceae) have a cosmopolitan distribution (Kumar *et al*., 2011). These species are also one of the aromatic plants commonly grown in Iran (Choupani *et al*., 2019). The research over the past several years has shown that the *Mentha* species including *M. spicata* (Souza *et al*., 2016) and *M. pulegium* (Salem *et al*., 2017) possess insecticidal and repellency activity against different foodstuff pests. Some special properties of EOs (such as low water solubility, high volatility, chemical instability, short residual activity due to degradation by heat and light) hinder their use as crop protectors in storehouses (Gonzalez *et al*., 2015; Louni *et al*., 2018). An innovative strategy to overcome the above-mentioned drawbacks is to design a controlled-release system that can improve their physical stability, target specificity, bioactivity and optimize the action of the active compounds (Koul, 2012; Gonzalez *et al*., 2014). Nanoformulation (NF) of EOs offers great promise in this direction and can be used to facilitate the application of these products and improve the EOs stability, biocompatibility and efficacy (Ghormade *et al*., 2011; Perlatti *et al*., 2013).

Polymer-based NF is one of the most promising techniques to encapsulate the EOs (Kah and Hofmann, 2014; Roy *et al*., 2014). Sodium Alginate, extracted from marine brown algae, is one of the commonly used polymers which is applied as an encapsulation membrane. This biopolymer is capable of preparation a versatile non-toxic matrix in crosslink with divalent cations which is employed for different applications, particularly in drug delivery systems (Goh *et al*., 2012; Etchepare *et al*., 2015). The efficiency of some EONF against various stored pests has been studied. For instance, the lethal and sublethal activity of geranium and bergamot EOs-NPs against *T. castaneum* and *Rhyzopertha dominica* F. (Col.: Bostrichidae) was studied by Gonzalez *et al*. (2014). Their results showed that the EOs-NPs enhanced bioactivity compared to their bulk

EO. The remarkable ability of chitosan nanogels loaded by *Cuminum cyminum* L. EO to control *Sitophilus granarius* L. (Col.: Curculionidae) and *T. confusum* was claimed by Ziaee *et al.* (2014). Also, Emamjomeh *et al*. (2017) stated that NF is an appropriate method to enhance the effectiveness of *Zataria multiflora* Boiss. EOfor control of *E. Kuehniella* larvae. In another research, the higher fumigant and contact toxicity of *Rosmarinus officinalis* L. nanoencapsulation was approved compared to its bulk EO against *T. castaneum* (Khoobdel *et al*., 2017). Bayramzade *et al* (2018) proved that the nanoencapsulation of *C. cyminum* and *Lavandula angustifolia* (Mill.) EOs improved their post-ingestive toxicity against *S. granarius* adults.

The objective of this study was to investigate the toxicity, antifeedant and physiological effects of *M. spicata* and *M. pulegium* EOs and their NFs against *T. castaneum* adults. The lack of information specifically about the impact of EONF on the enzymatic activity of stored products pests was a justification for carrying out the present research.

Materials and Methods

Insect rearing

The rearing stock of *T. castaneum* was obtained from the Insectarium of Agricultural Research Center in Department of Plant Protection, Urmia University, Urmia, Iran. The adults of *T. castaneum* were reared in plastic containers (15×30 cm) containing wheat flour mixed with yeast (10:1 w/w). The cultures were kept in a growth chamber at 27 ± 2 °C and 60 ± 5% RH in constant darkness.

Collection and extraction of the plants EO

The studied plants have been selected due to their importance as aromatic plants which are widely cultivated in Iran. Aerial parts of *M. spicata* and *M. pulegium* were collected from medicinal plants farm of the Faculty of Agriculture (59°40'E, 36°14'N) during the summer season 2016 and were identified at Department of Botany, Ferdowsi University of Mashhad, Mashhad, Iran. The plants were dried naturally at room temperature until they were crisp. Then, they were subjected to hydro-distillation using a modified Clevenger-type apparatus. The EOs were extracted as follows: 50 g of each dried plant; 600 ml distilled water and 4 h distillation. After extraction, anhydrous sodium sulfate was used to dehydrate the EO. The isolated oils were stored at 4 °C in a refrigerator. The EO yields of aerial parts of *M. spicata* and *M. pulegium* were 1.5 and 1.1 (ml/100 g dry matter), respectively. In addition, the density of *M. spicata* and *M. pulegium* EOs were estimated as 0.85 and 0.90 (g/ml), respectively.

GC/MS analysis

Gas chromatography-mass spectroscopy (GS-MS) analysis was carried out by an Agilent 7890 gas chromatograph coupled with a 5975A mass spectrometer using a flame ionization detector (FID) and BP-5 MS (non-polar) capillary column (30 m × 0.25 mm; 0.25 µm thickness). The commencing oven temperature was set at 80 °C and kept for 3 min, and then increased with 8 °C/min intervals to reach up to 180 °C for 10 min. Other operating conditions were a carrier gas of He, at a flow rate of 1 ml per min, the electron impact (EI) for ionization was 70 eV. The injector was set in a split mode, with the mass-to-charge ratio (m/z) of 40–500 *m/z*. Quantitative data were obtained by comparing their mass spectra and linear retention indices to those published in the literature (Adams, 1995) and presented in the MS computer library.

Preparation of essential oils nanoformulations (EONF)

The EONF were prepared according to the method described previously by Lertsutthiwong *et al*. (2008) with some modifications using the o/w emulsification technique and finally cross-linked by $CaCl_2$. Initially, Sodium alginate (Merck, Germany) as polymer was dissolved in double-distilled water to produce 1% (w/v)

Sodium alginate solution and then the solution was left standing for 24 h to disengage any possible bubble before use. Afterward, 5 g of each EOs (equivalent to 5.88 and 5.56 ml of *M. spicata* and *M. pulegium* EO, respectively) and 5 g of Tween 80 (Polysorbate 80, Merck, Germany), as a surfactant, were stirred at a speed of 200 rpm for 5 min by a magnetic stirrer. The initial EO-Tween solution was dropped into a 300 ml beaker containing 90 g of 1% (w/v) sodium alginate aqueous solution under the continuous stirring with an overhead stirrer (Heidolph-TORQUE, Germany) at a constant speed of 1200 rpm for 20 min. The EO was dropwisely added to the alginate solution during mixing until the desired oil loading was obtained. Thereafter, the fast cooling of the emulsions was attained by placing the beaker in an ice bath. Finally, an appropriate volume of calcium chloride (Merck, Germany) (0.5 mg/ml) as cross-linking agent was injected into the resulting solution using a syringe while mixing with an Ultra-Turrax (T 25, Ika-Werke, Germany) at speed of 10000 to 20000 rpm for 20 min (7 min at 10000 rpm, 7 min at 15000 rpm and 6 min at 20000 rpm). The suspensions were kept 24 h at room temperature to equilibrate. The nanocapsules containing oil were used as a diffuse form in the aqueous solution.

Encapsulation efficiency (EE)

The encapsulation efficiency (EE) was determined after separating the encapsulated EOs from the non-encapsulated ones (free EOs) in the NF suspensions according to the method of Khoobdel *et al*. (2017) with a slight modification. The filtration-centrifugation technique was used to measure the concentration of the free EO in the diffusion medium of each EONF. On account of the complexity of EOs constituents, the most important component of *M. spicata* and *M. pulegium* EOs, that is, pulegone (67.03%) and menthol (31.75%), respectively, were chosen as the indexed constituents to calculate the amount of free oil in the studied EONF (Lai *et al*., 2007; Nasseri *et al*., 2016). Briefly, 1 ml of each EONF was added to the upper chamber of Ultrafiltration tubes (Amicon® Ultra – PLHK Ultracel-PL Membrane, 100 kDa, Merck Millipore, Germany) and was centrifuged at 10,000 rpm for 30 min at 4 °C. After centrifuging, a filtrate with free EOs and a concentrate with encapsulated EOs were obtained. The transparent solution of each amicon was injected to GC-MS to estimate the amount of pulegone and menthol in the filtrate of *M. spicata* NF and *M. pulegium* NF, respectively, after proper dilution with menthol (Merck, Germany). The encapsulation efficiency was determined using the difference between the total concentration of the EO which was initially used in NF and the amount of the free EO based on the method described by Christofoli *et al*. (2015).

Morphological characteristics

The particles size and polydispersity index (PDI) were measured using dynamic light scattering (DLS) instrument. DLS analysis was done at 25 °C using a Nanophox 90-264v model apparatus (Nuremberg, Germany) equipped with a 623 nm He-Ne laser.

Morphological characteristics of the EONF were studied by high-resolution transmission electron microscopy (TEM, Zeiss-EM 10C-100KV, Oberkochen, Germany). At first, one droplet of aqueous solution of the sample was prepared and deposited on the holey carbon-coated on 300 mesh copper grid and was allowed to dry at the ambient temperature and was scanned (Baboota *et al*., 2007) afterward.

Bioassays

Ingestion bioassay

Ingestion bioassays for EOs and EONF against *T. castaneum* adults were done following the methods of Popović *et al*. (2013) with some modifications. The EOs were diluted in ethanol, whereas in the EONF, the distilled water was applied as a solvent. Based on the concentration -setting pre-tests, six concentrations causing the mortality range between 10-90% were calculated for each EOs and their NF (Robertson *et al*., 2007). The ranges of

concentrations for the EOs of *M. spicata* and *M. pulegium* were 12.10-25.10 and 3.75-18.00 µl/ml, respectively. The concentration ranges for the EONF of *M. spicata* and *M. pulegium* were 6.00-18.00 and 6.00-18.00 µl/ml, respectively. The bioassays were carried out using 20 similar-aged unsexed adults (2-4 days old) which were starved for 48 h before use. Flour discs were prepared according to the method of Huang *et al*. (1997) with some modifications. Aliquots of 200 µl of wheat flour suspension in water (10 g in 50 ml) were spread on a nylon sheet to convert the suspension to disc. The weight of flour discs was between 35-40 mg. The discs were left to dry in the fume hood for 12 h, then they were equilibrated at 27 ± 2 °C and 65% R.H. for 24 h. Flour discs were treated with 20 µl of different concentrations of the EOs or EONF using the micropipette. The solvent was allowed to evaporate at ambient temperature for 20 min. Then, the adults were released in a glass vial, fed with flour discs containing different concentrations of the treatments. For the control group in the EOs, the flour discs treated with ethanol were used. In the EONF control treatment, the flour discs treated with NF without oil were used. A no-choice method was adopted in this experiment through which the control and treated discs were placed individually in vials. The mortality was recorded after 72 h. Adults showing no response when probed with a brush were considered as dead. The experiment was set in six replicates for each treatment and control at 27 ± 2 °C and 60 ± 5% R. H in constant darkness.

Feeding indices bioassay

To determine the effects of the EOs and their NF on the feeding indices, the feeding LC_{10}, LC_{15} and LC_{25} values of all treatments which were already estimated from the ingestion bioassay were added to the diet. The flour discs were treated with different feeding LC values of the EOs or EONF (20 µl) using micropipette. For the control treatment, the flour discs were treated with ethanol and NF without EO. After evaporation of the solvent for 20 min, the discs were placed in glass vials. Ten group-weighed, unsexed adults were added to each preweighed vial containing the discs. For each concentration and the control, two flour discs were given to the insects. After 72 h, the glass vials with flour discs and live insects were separately weighed again and the nutritional indices were determined. For each concentration, six replicates were prepared. The nutritional indices were calculated according to Huang *et al.* (2000) formula:

$$\text{Relative Growth Rate (RGR)} = \frac{(A-B)}{(B \times day)}$$

$$\text{Relative Consumption Rate (RCR)} = \frac{D}{(B \times day)}$$

$$\text{Efficacy of Conversion of Ingested Food (\%)} = \left(\frac{RGR}{RCR}\right) \times 100$$

$$\text{Feeding Deterrence Index (FDI) (\%)} = \left(\frac{C-T}{C}\right) \times 100$$

where *A* is the weight of the survived insects after the test (mg) divided by the survived insect number after test, *B* is the weight of the insects before the test (mg) divided by initial number of the insects, *D* is the food biomass ingested (mg) divided by the survived insect number after the test, *C* is the food weight which was consumed in the control (mg) and *T* is the food weight consumed in the treatment (mg) (Isman, 2006).

Biochemical bioassay

Adult beetles that were exposed to the feeding LC_{50} value of each EO or its NF were used for enzyme assays. The survived insects were homogenized before (control) and after (treatment) using the EOs and EONF LC_{50} value in a buffer at 4 °C, 72 h after the exposure. The homogenate mixture was centrifuged (12000 g for 10 min at 4 °C). The resulting supernatants were transferred to a new tube and frozen at -20 °C for further use as an enzyme source (Shojaei *et al*., 2017; Hu *et al*., 2019).

Detoxification enzymes bioassay

Evaluation of glutathione S-transferase activity was done according to Habig *et al*., (1974) with some modifications. In each well of a 96-well

microplate, 20 µl of the enzyme sample was added to 200 ml of a solution containing GSH (10 mM) and CDNB (63 mM) at a 1:10 ratio. The GST activity was determined by the change in absorbance as measured every 30 seconds for 5 min at 340 nm using microplate reader (Biotek Elx800). Van Asperen method (1962) was used to determine the general esterase activity in which 30 mM α -naphthyl (α-NA) acetate and β-naphthyl acetate (β-NA) were used as substrate. Enzyme samples (12.5 µl), plus substrate (112.5 µl) and 50 µl of fast blue RR (dissolved in distilled water) were poured in microplate wells. Finally, absorbance reading was performed at 450 and 540 nm for α-NA and β-NA every 30 seconds for 20 min, continuously using a microplate reader (BioTek Synergy HT, Vermont, USA). Acetylcholinesterase (AChE) activity was measured according to Ellman *et al*. (1961) method, using Acetylthiocholine iodide as a substrate with slight modifications. Briefly, 40 µl enzyme samples, 140 µl phosphate buffer and 40 µl substrate (2.5 mM) were added to a microplate. The enzyme activity was determined continuously by monitoring the change in absorbance at 405 nm for 20 min at 1 min intervals at 25 °C using a microplate reader (BioTek Synergy HT, Vermont, USA).

Digestive enzymes bioassays

The α-amylase activity in the treated and control adults of *T. castaneum* was assayed by the dinitrosalicylic acid (DNS) procedure according to the method of Bernfeld (1955), using 1% soluble starch (Merck, Darmstadt, Germany) as a substrate. 10 µl of the enzyme were incubated for 30 min at 35 °C in 50 µl phosphate buffer (pH = 7) and 40 µl soluble starch. The reaction was stopped by adding 100 µl DNS and the subsequent heating in hot water for 10 min. Absorbance was then measured at 540 nm using a microplate reader (BioTek Synergy HT, Vermont, USA). The general proteinase activity was determined based on a method by Elpidina *et al*., (2001) using azocasein as the substrate. The reaction mixture consisted of 30 µl of 2% azocasein solution in 90 µl phosphate buffer (0.1 M) and 15 µl enzyme. The reaction mixture was incubated at 37 °C for 60 min. The proteolysis was stopped by adding 30 µl of 30% trichloroacetic acid (TCA). Some precipitation was achieved by cooling at 4 °C for 60 min and the reaction mixture was centrifuged at 16000 g for 10 min. An equal volume of NaOH (1M) was added to the supernatant and the absorbance was recorded at440 nm using a microplate reader (BioTek Synergy HT, Vermont, USA).

Data analysis

The bioassay data were used to estimate the lethal concentrations, 95% confidence limits and relative median potency between the EOs and EONF using Polo-PC software (Probit analysis using Maximum Likelihood Programme software). The enzyme assays and the nutritional indices were subjected to the Kolmogorov-Smirnov test for normality test, before the one-way ANOVA test and the differences among the treatments were compared using Tukey's HSD test at $p < 0.05$.

Results

Chemical composition of the EOs

The quantitative *M. spicata* and *M. pulegium* EOs compositions are presented in Table 1. The results showed that 20 components were identified from *M. spicata* EO and its major constituents were Menthol (32.75%), Menthone (32.4%), Menthofuran (12.75%), 1,8-cineole (5.05%) and Camphane (5.04%). Also, as shown in Table 1, 18 components were identified from *M. pulegium* EO with the major constituents as Pulegone (67.03%), L-menthone (14.1%), 1,8-cineole (7.47%) and Piperitenone (1.14%).

Characterization of nanocapsules

Morphological characteristics

The sizes, polydispersity index (PDI) and the encapsulation efficiency (EE) for *M. spicata* and *M. pulegium* EONF are shown in Table 2. The average size obtained for *M. spicata* and *M. pulegium* EONF were 56.91 and 98.99 nm, respectively. The result indicated that the PDI

for *M. spicata* and *M. pulegium* EONF were 0.140 and 0.256, respectively. The rather low value of PDI for the *M. spicata* EONF demonstrated the homogeneity of this formulation. According to the results, the encapsulation efficiency (EE) was 95 and 86% for *M. spicata* and *M. pulegium* EONF, respectively. Transmission Electron Microscopy (TEM) observations indicated that the nanocapsules of both of the EOs were spherical in shape (Fig. 1). The TEM analysis verified that the dimensions of both nanocapsules were less than 100 nm, which was consistent with our DLS findings (Table 2).

Table 1 Chemical constituents of *Mentha spicata* and *Mentha pulegium* essential oils**.**

Compounds	RI[1]	RT[2]	Content (%)	
			M. spicata	*M. pulegium*
α-pinene	934	5.28	0.58	0.81
Camphene	949	5.57	-	0.14
Sabinene	970	6.05	0.86	0.80
1-Octen-3-ol	975	6.08	0.47	-
β-pinene	978	6.14	-	1.25
β-myrcene	990	6.38	-	0.51
3-octanol	993	6.43	-	0.36
Para-cymene	1025	7.14	0.49	-
Limonene	1030	7.24	1.75	1.19
1,8-cineole	1032	7.30	5.05	7.47
Linalool l	1049	8.80	0.41	-
Menthone	1157	10.15	32.40	14.10
Isomenthone	1167	10.37	12.75	-
Borneol	1169	10.43	-	0.64
L- (-)-Menthol	1172	10.55	-	0.36
Menthol	1178	10.63	32.75	-
Terpinene-4-ol	1179	10.64-10.71	1.42	-
α -terpineol	1193	10.97	-	0.51
Pulegone	1242	12.18	0.97	67.03
Piperitone	1257	12.45	0.58	0.23
Camphane	1294	13.32	5.04	-
Piperitenone	1344	14.39	-	1.47
Trans-caryophyllene	1425	16.15	1.64	0.54
Germacrene-d	1486	17.43	1.66	-
Viridiflorol	1599	19.67	0.48	-

[1]: Retention index.
[2]: Retention time.

Table 2 Nanoformulated characteristics and their related properties of *Mentha spicata* and *Mentha pulegium* essential oils using dynamic light scattering (DLS) instrument.

Essential oils	Content of EO (W/W%)	Particle size (nm)	Polydispersity index (PDI)	Encapsulation Efficiency (%)
M. spicata	5	56.91 ± 4.65	0.140 ± 0.023	95.47
M. pulegium	5	98.99 ± 8.20	0.256 ± 0.045	86.03

EO: Essential oil.

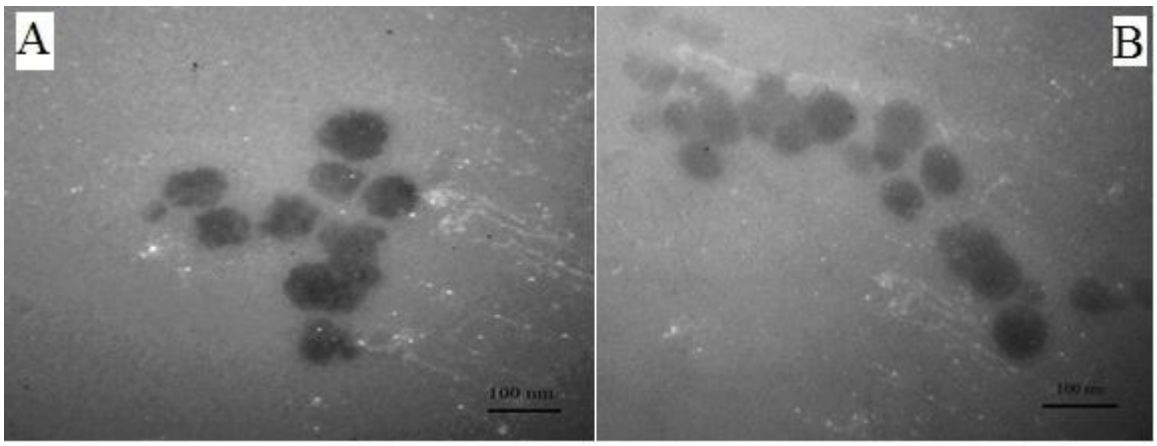

Figure 1 TEM images of nanoencapsuled essential oils of (A) *Mentha pulegium* and (B) *Mentha spicata*.

Bioassays

Ingestion bioassays

The results of ingestion bioassay of the EOs on *T. castaneum* are shown in Table 3. The LC_{50} value estimated for *M. spicata* EO was 18.422 µl/ml while the LC_{50} of its NF was 9.279 µl/ml. The LC_{50} values for the EO and EONF of *M. pulegium* were 7.939 and 6.793 µl/ml, respectively. Based on LC_{50} value, *M. pulegium* EONF is the most effective treatment against *T. castaneum*. Moreover, comparisons of toxicity on *T. castaneum* adults among two EOs using relative median potency illustrated that the toxicity of *M. pulegium* oil was significantly higher than that of *M. spicata* oil (Table 4). Also, comparisons of LC_{50} among each EO and its NF indicated that the LC_{50} values of the EONF in both plants were significantly lower than those of the EOs as such. So, the NFs are more effective than their bulk counterparts (Table 4).

Feeding indices bioassay

The effects of the EOs and EONF on the nutritional indices of *T. castaneum* adults were studied. The calculation of all indices was based on the surviving insects. The results of the effect of the EOs and EONF on the feeding indices including the relative growth rate (*RGR*), the relative consumption rate (*RCR*), the efficiency of conversion of the ingested food (*ECI*) and the feeding deterrence indices (*FDI*) of *T. castaneum* adults are presented in Table 5. To the best of our knowledge, in all treatments except *M. spicata* EO, the values of *RGR* were reduced significantly in a concentration-dependent manner and the least values of *RGR* was observed at the highest concentration (LC_{25}) of treatments. The *RCR* increased with the increasing concentration in all treatments except with *M. spicata* EO and where the lowest *RGR* was recorded at the highest concentration (LC_{25}) of the treatments. The highest reduction rate of the *RGR* and *RCR* was observed in *M. pulegium* EONF. Additionally, *M. pulegium* EONF is the only treatment that could produce a significant decrease in the *ECI* of the treated adults at the highest concentration (LC_{25}) and no obvious decrease in the *ECI* was found in other treatments. According to our findings, the values of *FDI* increased in all treatments in a concentration-dependent manner. The results showed that both the EOs and EOs- NFs had a moderate feeding deterrent activity on the adult of *T. castaneum*. In addition, the *FDI* index increased from about 2% and 4% at the lowest concentration (LC_{10}) to 17% and 30% when fed on the disc treated by the highest concentration (LC_{25}) of *M. spicata* EO and its NF, respectively. The *FDI* values obtained were about 15% and 17% when adult fed on the discs were treated by LC_{10} value of *M. pulegium* EO and its NF, which enhanced to 39% and 49% respectively by LC_{25} value. Moreover, the results of comparing the effects of the EOs and their NFs (using statistical analysis) on feeding the indices showed that *M. pulegium* EONF (LC_{25}) decreased *RGR* ($F_{13,83}$ = 4.301, $p < 0.001$), *RCR* ($F_{13,83}$ = 5.292, $p < 0.001$)

and *ECI* ($F_{13,83}$ = 2.377, $p < 0.001$) significantly compared to the other treatments. Also, the effect of LC_{25} of *M. pulegium* EONF on *FDI* was significantly more than *M. spicata* EONF and both EOs ($F_{11,71}$ = 5.839, $p < 0.001$; data not shown).

Detoxifying enzymes

Table 6 shows the detoxifying enzymes activity of *T. castaneum* adult fed on the flour disc containing the LC_{50} value of *M. spicata* and *M. pulegium* EOs and their NF. As shown, all the treatments (EOs and EONF) inhibited the general esterase activity (with both substrates) in adults, such that the highest esterase activity was seen in the control and had significant differences with other treatments. However, there is no considerable difference in general esterase values between the treated adults by EOs and EONF. The same trend was observed in the activity of Acetylcholinesterase (AChE). The AChE statistically decreased in adults fed on the EOs and EONF treated discs in comparison with the control. Nonetheless, the EONF had greater impacts on reducing the AChE activity compared to the EOs. Based on the data of Table 6, the least activity in AChE was recorded in the adults treated with *M. pulegium* EONF. In contrast, the GST activity was increased significantly in the adults treated with either the EOs or EONF compared to the control. The majority of the GST activity enzyme was presented in the adults treated with EONF. Referring to the results, in both plants, the GST activity in adults treated with the EONF was more than those treated by the pure EOs (Table 6).

Digestive enzymes

Table 7 shows the effects of the EOs and their NFs on the digestive enzyme activity of *T. castaneum* adults. As it is evident, the adults fed on the discs containing LC_{50} value of the EOs and EONF showed significantly lower activity of α-amylase compared to the control group. However, the least activity was observed in the adults treated with *M. pulegium* EONF. The same trend was observed for the general protease activity. The result showed a noticeable reduction in the general protease activity in the adults fed on the discs treated with the LC_{50} value of EOs and EONF. Moreover, the obtained data demonstrated that *M. pulegium* EONF caused the most reduction in the α-amylase and general protease activity of *T. castaneum* adults (Table 7).

Table 3 Mortality of *Tribolium castaneum* adults exposed to flour discs treated with nanoformulation of *Mentha spicata* and *Mentha pulegium* essential oil.

Treatments	No. of adults used	Slope (± *SE*)	Intercept (± *SE*)	χ^2 (df = 4)	Lethal concentrations (95% Confidence limits) (µl/ml)	
					LC_{50}	LC_{90}
M. spicata (EO)	720	7.00 (± 0.52)	-29.87 (± 2.12)	1.85 (4)	18.422 17.818-19.076	28.073 26.278-30.590
M. spicata (EONF)	720	5.14 (± 0.39)	-20.41 (± 1.58)	10.84 (4)	9.279 7.982-10.470	16.468 14.000-22.095
M. pulegium (EO)	720	3.73 (± 0.25)	-14.58 (± 0.99)	5.32 (4)	7.939 7.119-8.831	17.478 14.785-22.313
M. pulegium (EONF)	720	9.85 (± 0.75)	-37.77 (± 2.92)	6.07 (4)	6.793 6.394-7.135	9.164 8.597-10.094

EO: Essential oil.
EONF: Essential oil nanoformulation.

Table 4 The LC_{50}s relative median potency for the comparison of toxicity between *Mentha spicata* and *Mentha pulegium* essential oil and their nanoformulation against adults of *Tribolium castaneum*.

Comparison of LC_{50}s	Relative Median Potency	95% confidence limits
MSEO vs MPEO	2.32	2.15-2.50*
MSEO vs MSNF	1.99	1.85-2.11*
MPEO vs MPNF	1.17	1.10-1.25*

*: Significant differences at $P < 0.05$, MSEO: *M. spicata* essential oil, MPEO: *M. pulegium* essential oil, MSNF: *M. spicata* nanoformulation, MPNF: *M. pulegium* nanoformulation.

Table 5 Nutritional and feeding deterrence indices of *Tribolium castaneum* adults exposed to *Mentha spicata* and *Mentha pulegium* essential oils and their nanoformulation.

Treatment	Concentration (µl/ml)	RGR (mg/mg/ day)	RCR (mg/mg/ day)	ECI (%)	FDI (%)
M. spicata EO	Control (0)	0.0296 ± 0.0013a	0.1370 ± 0.0166a	23.06 ± 2.64a	-
	LC_{10} (12.00)	0.0314 ± 0.0041a	0.1329 ± 0.0060a	23.45 ± 2.65a	2.39 ± 2.62b
	LC_{15} (13.10)	0.0275 ± 0.0055a	0.1213 ± 0.0049a	22.11 ± 3.83a	10.29 ± 3.77ab
	LC_{25} (14.75)	0.0237 ± 0.0062a	0.1083 ± 0.0057a	20.93 ± 4.98a	17.78 ± 4.92a
	F (*P* value)	0.507 (0.682)	1.811 (0.178)	0.094 (0.962)	3.919 (0 < 0.05)
M. pulegium EO	Control (0)	0.0296 ± 0.0013a	0.1370 ± 0.0166a	23.06 ± 2.64a	-
	LC_{10} (3.60)	0.0229 ± 0.0022ab	0.1126 ± 0.0083ab	20.15 ± 0.67a	15.34 ± 6.56b
	LC_{15} (4.20)	0.0214 ± 0.0062ab	0.1149 ± 0.0127ab	18.63 ± 5.13a	16.31 ± 8.68b
	LC_{25} (5.25)	0.0141 ± 0.0024b	0.0801 ± 0.0077b	17.40 ± 2.07a	39.51 ± 5.66a
	F (*P* value)	3.197 (0 < 0.05)	3.917 (0 < 0.05)	0.628 (0.606)	3.735 (0 < 0.05)
M. spicata EONF	Control (0)	0.0277 ± 0.0013a	0.1410 ± 0.0102a	20.22 ± 1.86a	-
	LC_{10} (5.20)	0.0247 ± 0.0030a	0.1408 ± 0.0101a	17.41 ± 1.24a	-4.77 ± 7.80b
	LC_{15} (5.80)	0.0255 ± 0.0031a	0.1270 ± 0.0125ab	19.93 ± 0.63a	3.69 ± 8.66b
	LC_{25} (6.85)	0.0168 ± 0.0022b	0.0962 ± 0.0113b	17.18 ± 1.07a	30.15 ± 7.74a
	F (*P* value)	3.484 (0 < 0.05)	3.629 (0 < 0.05)	1.593 (0.222)	5.086 (0 < 0.05)
M. pulegium EONF	Control (0)	0.0277 ± 0.0013a	0.1410 ± 0.0102a	20.22 ± 1.86a	-
	LC_{10} (5.00)	0.0186 ± 0.0018b	0.1130 ± 0.0077a	16.30 ± 0.57a	17.59 ± 5.46b
	LC_{15} (5.30)	0.0130 ± 0.0022b	0.0760 ± 0.0144b	17.63 ± 1.06a	42.21 ± 10.30ab
	LC_{25} (5.80)	0.0049 ± 0.0025c	0.0691 ± 0.0106b	6.20 ± 3.59b	49.39 ± 8.06a
	F (*P* value)	12.584 (0 < 0.001)	9.379 (0 < 0.001)	6.493 (0 < 0.01)	4.154 (0 < 0.05)

EO: Essential oil, EONF: Essential oil nanoformulation, RGR: Relative growth rate, RCR: Relative consumption rate, ECI: Efficiency of conversion of the ingested food, FDI: Feeding deterrence indices, Within each EO or EONF, means with the same letters in each column are not significantly different (Tukey's test, P < 0.05).

Table 6 Detoxifying enzymes activity of *Tribolium castaneum* at 72 h after treatment with LC_{50} value of *Mentha spicata* and *Mentha pulegium* essential oils (EO) and their nanoformulations (NF).

Enzyme	Enzymes activity (µmol/min/mg protein)					F	*P*-value
	Control	*M. spicata* EO	*M. pulegium* EO	*M. spicata* EONF	*M. pulegium* EONF		
Esterase (α-NA)	0.056 ± 0.0022a	0.036 ± 0.0033b	0.0310 ± 0.0034b	0.0410 ± 0.0058b	0.039 ± 0.0054b	4.932	0.0190
Esterase (β-NA)	0.043 ± 0.0010a	0.033 ± 0.0006b	0.0310 ± 0.0018b	0.0260 ± 0.0017b	0.025 ± 0.0058b	6.671	0.0070
AChE	0.032 ± 0.0011a	0.023 ± 0.0020b	0.0134 ± 0.0010c	0.009 ± 0.0015cd	0.007 ± 0.0016d	50.698	0.0001
GST	0.142 ± 0.0107a	0.504 ± 0.0050c	0.4010 ± 0.0163b	0.6730 ± 0.0173d	0.633 ± 0.0193d	210.569	0.0010

Means with the same letters in the same row are not significantly different (Tukey's test, P < 0.05).

Table 7 Digestive enzymes activity (U/mg protein) of *Tribolium castaneum* at 72 h after treatment with LC_{50} value of essential oils (Eos) and their nanoformulations (NF).

Enzyme	Control	*M. spicata* EO	*M. pulegium* EO	*M. spicata* EONF	*M. pulegium* EONF	F	*P*-value
α-amylase	0.329 ± 0.0289a	0.235 ± 0.0222b	0.173 ± 0.0176bc	0.181 ± 0.0353bc	0.131 ± 0.0252c	8.292	0.003
General protease	0.074 ± 0.0062a	0.049 ± 0.0033b	0.047 ± 0.0067b	0.032 ± 0.0054bc	0.027 ± 0.0060c	10.534	0.001

Means with the same letters in the same row are not significantly different from each other (Tukey-test, P < 0.05).

Discussion

According to GC-MS analysis, a total of 18 and 20 components were recognized in *M. pulegium* and *M. spicata* EOs, where Pulegone (67.03%) and Menthol (32.75%) were identified as the main ingredients, respectively. It should be noted that the chemical composition of *M. spicata* and *M. pulegium* EOs has been studied previously and there are differences in the quantities of chemicals reported. For example, Boukhebti *et al*. (2011) showed that *M. pulegium* EO from the Amoucha (northeast Algeria) was rich in pulegone (38.81%), Menthone (19.24%) and Piperitenone (16.53%). Dhifi *et al*. (2013) revealed that L-menthone (32.74%), Pulegone (26.67%) and Menthol (11.42%) were the main constituents of *M. spicata* EO from Tunisia. In other research, Brahmi *et al*. (2016) reported that Pulegone (70.4%), Neo-Menthol (13.4%) and Neo-menthol acetate (3.5%) were the major compounds of *M. pulegium* EO. These differences could be due to some various factors such as isolation and analysis methods, environmental situation, harvesting time, soil composition, geographical position, and plants genetic makeup (Heydarzade and Moravvej, 2012; Tarigan *et al*., 2016; Ebadollahi *et al*., 2017).

According to the current study, the alginate nanocapsules based on EOs were synthesized using a multi-stage protocol of O/W emulsification, gelification and solidification. It seems that the diffusion of EOs in an aqueous alginate solution alongside Tween 80 resulted in the instantaneous formation of micelles containing oil core, followed by solidification of the polymeric shell with the addition of cross linker ($CaCl_2$) (Lertsutthiwong *et al*., 2008). In other words, after the formation of a layer around the oil droplets by used surfactant (Tween 80), its hydrophilic parts linked to the polymeric shell subsequent to the $CaCl_2$ cross-linking. This resulted in smaller capsules being produced by moving insoluble polymer to oil/water interface due to a reduction in the interfacial tension and formation capsules (Fessi *et al*., 1989; Lertsutthiwong *et al*., 2008). The size and distribution of nanocapsules are the most important quality-related factors that impact other EONFs properties (Özden, and Bayindirli, 2002; Nasseri *et al*., 2016). According to other studies, the formulation compounds (presence of surfactant), production technique (such as the amount of materials, homogeneity and agitation speed) as well as environmental conditions (homogenization time and solution temperature) could affect particle size and distribution (Özden and Bayindirli, 2002; Chang and Dobashi, 2003; Lertsutthiwong *et al*., 2008; Jiamrungraksa and Charuchinda, 2010; Soliman *et al*., 2013; Etchepare *et al*., 2015; Nasseri *et al*., 2016). Nanocapsule size distribution was measured by PDI index, which nanocapsules lower than 0.3 represent relatively narrow size distribution. The rather low value of PDI for the *M. spicata* EONF demonstrated the homogeneity of this formulation. Also, the low level of this parameter indicates that our formulations did not contain particles of various sizes. Another quality-related characteristic of the NF is the encapsulation efficiency (EE) through which the capacity of alginate nanoparticles to encapsulate EOs was determined. In this work, the EE was determined by an indirect method since it was impossible to destroy the capsules. As mentioned, because of the complexity of the EOs composition, Pulegone (the major component of *M. spicata*) and Menthol (the major component of *M. pulegium*) were selected as the leads (indexed constituents) to calculate the EE. Based on the data shown, the EE of *M. spicata* and *M. pulegium* were about 95% and 86% which are very good results for the nanocapsules EOs loading of 5%. It could be concluded that the method and materials used were suitable for nanoencapsulation of the EOs. Also, according to the results obtained, it seems that *M. spicata* EONF had better characteristics as compared to *M. pulegium* EONF since it had smaller particle size, lower PDI and higher EE. On the other hand, *M. pulegium* EONF showed higher toxicity and physiological effects on *T. castaneum* adult compared to *M. spicata* EONF. This could be attributed to the more intrinsic toxicity of *M. pulegium* EO than that of *M. spicata* EO. The bioactivity differences between the EOs isolated from different plant species

could result from the type, quantity and interaction of the components (Heydarzade and Moravvej, 2012; Popovic *et al*., 2013; Ebadollahi *et al*., 2014; Tarigan *et al*., 2016). According to the results, the difference in LC_{50} values between *M. spicata* EO with its NF is much more than *M. pulegium* EO and its NF. The main reason for this remarkable enhanced toxicity is the particular characteristics of *M. spicata* EONF.

TEM images indicated that alginate nanocapsules were spherical in shape with comparable diameters as shown by DLS. Similar finding was achieved by Lertsutthiwong *et al*. (2008) who indicated that alginate nanocapsules containing turmeric oil were spherical and their approximate size was below 100 nm. The spherical structure of nanocapsules provides some advantages including minimal interactions and contact with the aqueous dispersion medium, regulated release properties, protection of encapsulated sensitive and active compounds as well as the necessity to a smaller quantity of surface-active stabilizing agents compared to other forms of nanoparticles (Bunjes, 2005; Layegh *et al*, 2013). The results indicated that the EOs from *M. pulegium* and *M. spicata* and their NFs had significant toxicity against *T. castaneum*. Previously, insecticidal impacts of the EOs isolated from *Mentha* species have been reported against some economical insect pests such as *R. dominica* (Brahmi *et al*., 2016), *S. oryzae* (Benayad *et al*., 2012), *S. granarius* (Abdelli *et al*., 2016) which support the results of the present study. As shown in the results, the toxicity of each EONF to *T. castaneum* was considerably higher than its EO, indicating that the encapsulation of the EOs enhanced their potential to control *T. castaneum*. The higher bioactivity of the EONF compared to their bulk counterparts was supported by other researches (Khanahmadi *et al*., 2011; Negahban *et al*., 2013; Adel *et al*., 2015; Gonzalez *et al*., 2014; Ebadollahi *et al*., 2017; Khoobdel *et al*., 2017). In accordance with our findings, Gonzalez *et al*., (2014) compared the lethal and sublethal activity of geranium and bergamot EOs-NPs with their bulk counterparts against *T. castaneum* and *R. dominica*. Their finding demonstrated that, due to faster and stronger penetration of nanoparticles in the insect tissue, EOs-NPs enhanced bioavailability and bioactivity compared to their bulk material. Similar to our results, Adel *et al* (2015) indicated that the geranium EO loaded- SLNs had a greater impact on the developmental phases of immature stages and a higher mortality percentage on *Phthorimaea operculella* Zeller as compared to its free EO. Khoobdel *et al*. (2017) assessed the insecticidal activity of rosemary EO and its nanocapsules on *T. castaneum*. Their data indicated that the mortality rate of rosemary oil-loaded nanocapsules in all the concentrations studied was higher than those of the non-formulated oil. It is proposed that higher efficiency of the encapsulated EOs-loaded formulations could be due to their small size, water solubility and bioavailability compared to non-formulated EOs (Kumar *et al*., 2014; Gonzalez *et al*., 2014; Louni *et al*., 2018; Adel *et al*., 2018). Because of a large specific surface, nanoparticles had higher adhesion and faster penetration into insect's body and a greater chance of being taken up by biological tissues (Margulis-Goshen and Magdassi, 2012; Gonzalez *et al*., 2014; Nasseri *et al*., 2016). Furthermore, the nanocapsules protect the EOs' active ingredient from enzymatic degradation enabling more bioactive compounds to arrive the target sites (Regnault-Roger *et al*., 2012; Gonzalez *et al*., 2014 and 2015; Adel *et al*., 2018).

To understand the ability of the EOs and EONF in pest management, it is critical to determine their impacts on the insects' nutrition and physiology. According to nutritional indices, the insect growth rate was determined by the quantity of food consumption. The amount of food consumed also varies depending on the food quality. If the insect eats less or avoids eating, it means that this food acts as a deterrent by affecting the insect's peripheral sensilla and so it might not be ingested and absorbed if consumed by the insect. It could be stated that this food can induce ingestion toxicity and prevent the insect from gaining weight or even cause weight loss (Isman, 2006). Our findings suggested that both the EOs and EONF affected the physiological and feeding parameters of *T.*

castaneum adults, generally inhibited the growth rate and food consumption rate and provoked the feeding deterrence in this species. Effects of the EOs and EONF on the feeding parameters of the stored pests have been assessed by various researchers (Huang *et al*., 2000 and 2002; González *et al*., 2014; Abou-Taleb *et al*., 2016). Our results are in agreement with those of Stefanazzi *et al*., (2011) who reported that *C. citratus* and *E. muticus* EOs reduced the relative growth rate and the relative consumption rate and had a moderate feeding deterrent effect on *T. castaneum* adult. Similar to our results, Gonzalez *et al*., (2014) demonstrated that NF of geranium and bergamot EOs significantly increased the modification of the nutritional physiology of *T. castaneum* as compared to their bulk EOs. According to their results, the PEG formulation of EOs improved their antifeedant activity and caused a considerable reduction in the growth rate and food consumption of *T. castaneum* adults. Many plant compounds deter feeding by modifying insect behavior through acting as agonists and antagonists of Octopamine (Hummelbrunner and Isman, 2001). In the present study, the results showed that the highest effect was associated with *M. pulegium* EONF as it affected all the nutritional indices significantly. It is suggested that the differences observed between the EOs and the EONF could be explained by considering that EOs will evaporate faster than the encapsulated EOs will, indicating that stability of the EONF is more and can affect for longer period of time. Furthermore, Gonzalez *et al.* (2014) suggested that the uncommon physiochemical characteristics of nanocapsules could be due to their different penetration pattern and detoxification process in the insect body. The small size of the EONF cause enhanced mobility, resulting in better distribution and penetration in the peritrophic matrix of the insect (Nel *et al*., 2009; Margulis-Goshen and Magdassi, 2012).

Our results demonstrated that the EOs and EONF had a significant effect not only on the nutritional indices of *T. castaneum* adults but also on the enzymatic responses of this insect. The effect of different EOs or EONF on the enzymatic response of insects have already been reported in other studies (Ebadollahi *et al.,* 2013; Tarigan *et al*., 2016; Shojaei *et al*., 2017; Shahriari *et al*., 2018 and 2019; Hu *et al*., 2019). Detoxification enzymes are recognized as the prevalent enzymatic defense against xenobiotic substances that are responsible for preserving the insect physiological functions (Hu *et al*., 2019). Esterases (ESTs) consist of a large group of multi-functional enzymes in the insects which contribute to the metabolism and detoxification of many agrochemicals through hydrolyzing their ester bonds (Tarigan *et al*., 2016; Hu *et al*., 2019). It can be concluded that the insect detoxification system would be restricted if EST activity was inhibited. Our finding clearly demonstrated that both the tested EOs and EONF acted as an inhibitor of the general esterase (with both substrates) in the treated adults. A similar supportive observation has been reported by Tarigan *et al*., (2016), which indicated that the cardamom, cinnamon and nutmeg oils decreased the esterase activity in the third instar of both *T. castaneum* and *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae). Another supporting result was noted by Ebadollahi *et al.* (2013) who suggested that *A. foeniculum* EO inhibited the EST activity of *T. castaneum* 3rd instar larvae.

Acetylcholine esterase (AChE) is a crucial hydrolytic enzyme in insect nervous structure which is a significant target site for many plants compounds. AChE inhibition triggers the accumulation of acetylcholine in synapses leading to disturbance in the neuromuscular system, palsy and insect mortality (Isman, 2006; Shahriari *et al*., 2018). Our results stated the significant inhibition of AChE in adults treated by both the EOs and EONF with the highest inhibitory effect induced by EONF. The experimental results of the current study were in line with various studies that proved the ability of plant products to inhibit AChE activity (Shahriari *et al*., 2018; Hu *et al*., 2019). Hu *et al*., (2019) have demonstrated that *Artemisia brachyloba* Franchet EO and α-terpineol inhibited ESTs and AChE activities in *T. castaneum* adults. Based on the results by

Shahriari *et al*. (2018 and 2019), feeding *E. Kuehniella* larvae on diets containing LC_{50} value of *A. sativum* EO, *E. globulus* EOs and EO constituents (α-pinene, trans-anethole, and thymol) considerably reduced the AChE activity compared to control group. GSTs include a category of detoxifying enzymes which perform a noticeable role in conferring resistance against insecticides and also protecting insects from oxidative stress and secondary plant metabolites through the nucleophilic attack of reduced glutathione on the substrate (Tripathy, *et al*., 2016; Shojaei *et al*., 2017; Hu *et al*., 2019). The findings by the current study showed that both the tested EOs and EONFs promoted the activity of the GST in the treated adults. Shojaei *et al* (2017) reported higher GST activity in *Tribolium confusum* adults treated with different LC values of *Artemisia dracunculus* EO in a dose-dependent manner which in turn, supports the results of the present study. Enhancement of the GST was also observed in the larvae of lesser mulberry pyralid, *Glyphodes pyloalis* treated with *Rosemarinus officinalis* (Yazdani *et al*., 2013). Based on the present research, we find that the EST and AChE, unlike the GST, may not play a role in detoxifying the EOs and EONF.

According to our findings, the α-amylase and general protease activity were decreased in the adults fed on the treated discs compared to the control group and greater inhibition was observed in the EONF in all treatments. Reduction in digestive enzymes activity by EOs has been demonstrated by several studies (Jbilou and Sayah, 2007; Jbilou *et al*., 2008; Shahriari *et al*., 2017 and 2019). Similarly, Shahriari *et al*. (2017) reported that *Teucrium polium* EO and α-pinene decreased the general protease and α-amylase activity in the treated *E. kuehniella* larvae versus the control group. The effect of *Centaurium erythraea*, *Peganum harmala*, *Ajuga iva*, *Aristolochia baetica*, *Pteridium aquilinum* and *Raphanus raphanistrum* extracts on the α-amylase activity of *T. castaneum* has been studied by Jbilou *et al*. (2008). Their result demonstrated that all plant extracts inhibited the α-amylase activity. The reduction of digestive enzymes activity by the EOs could be attributed to the impact of the plant's defense compounds, including inhibitors on the alimentary canal which disturb insect's digestive physiology. It is suggested that the plant metabolites result in reduced digestive enzymes synthesis by reducing metabolism rate, alteration of the structure of some gut hydrolases and cytotoxic impacts on midgut epithelial cells (Franco *et al*., 2002; Jbilou *et al*., 2008; Shahriari *et al*., 2017 and 2019).

The overall results of the current research indicated that the EOs and EONF affected detoxifying and digestive enzymes of *T. castaneum* adults considerably, meanwhile this effect was more evident in the EONF. It is suggested that the distinctive characteristic of the EONF could result from improved mobility and bioavailability, higher chemical activity, expanded penetration profile, reduced detoxification proportion and resistance to hydrolysis compared to their bulk EOs (Yang *et al*., 2009; Gonzalez *et al*., 2014 and 2015).

To sum up, the NF is a promising approach to develop and facilitate the applicability of the EOs as botanical pesticides as well as improving the stability, bioactivity, and bioavailability of the EOs. In the present study, the alginate nanocapsules containing EOs with reasonable physical characteristics were prepared to provide a suitable alternative to manage the stored product pests. It has also been demonstrated that the EONF (particularly *M. pulegium*) were efficient in controlling *T. castaneum* adults and can be used as a biorational product to control other stored grains insects. However, in order to confirm their economic values as a natural pesticide, further research is required to assess their effectiveness under an actual store condition.

Acknowledgments

We are grateful to Dr. Sadigh-Eteghad (Tabriz University of Medical Science), En. Razmi (Agriculture Faculty of Urmia University) and En. Badali (Faculty of Veterinary Medicine, Urmia University) for their valuable assistance

in this research. We would like to acknowledge the financial support provided to this research by the Faculty of Agriculture, Urmia University, Urmia, Iran.

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تأثیر نانوفرمولاسیون اسانس گیاهان نعناع *Mentha spicata* و پونه *Mentha pulegium* روی مرگ‌ومیر و فیزیولوژی شپشه آرد *Tribolium castaneum* (Col.: Tenebrionidae)

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چکیده: روش‌های مورد استفاده برای بهبود ویژگی‌های اسانس‌ها و آماده‌سازی آن‌ها برای کاربرد به‌عنوان حشره‌کش‌های زیست بنیاد اخیراً مورد توجه قرار گرفته است. نانوفرمولاسیون کردن اسانس‌ها به‌عنوان یک استراتژی امیدوارکننده برای توسعه و تسهیل کاربرد اسانس‌ها در مدیریت آفات انباری شناخته می‌شود. هدف مطالعه حاضر بررسی سمّیت، اثرات ضدتغذیه‌ای و فیزیولوژیکی اسانس نعناع و پونه و نیز نانوفرمولاسیون آن‌ها علیه شپشه آرد *Tribolium castaneum* (Herbst) می‌باشد. مطالعه ویژگی‌های نانوکپسول‌ها با استفاده از روش Dynamic Light Scattering و میکروسکوپ الکترونی عبوری (TEM) نشان داد که نانوکپسول‌ها در هر دو فرمولاسیون، کروی شکل بودند و اندازه متوسط آن‌ها در نانوفرمولاسیون اسانس نعناع و پونه به‌ترتیب برابر ۵۶/۹۱ و ۹۸/۹۹ نانومتر بود. بازدهی کپسوله‌سازی برای نانوفرمولاسیون اسانس نعناع و پونه به‌ترتیب برابر با ۹۵/۴۷ و ۸۶/۰۳ درصد به‌دست آمد. پس از ۷۲ ساعت، مقادیر LC_{50} اسانس و نانوفرمولاسیون گیاه نعناع به‌ترتیب برابر با ۱۸/۴۲۲ و ۹/۲۷۹ میکرولیتر در میلی‌لیتر و برای اسانس و نانوفرمولاسیون پونه به‌ترتیب برابر با ۷/۹۳۹ و ۶/۷۹۳ میکرولیتر در میلی‌لیتر بود. نتایج به‌دست آمده نشان داد که اسانس‌های مورد بررسی و نانوفرمولاسیون آن‌ها روی شاخص‌های تغذیه شپشه آرد به‌صورت معنی‌داری مؤثر بودند. علاوه براین، هر دو اسانس مورد مطالعه و نانوفرمولاسیون آنها، نرخ رشد نسبی و نرخ مصرف نسبی را به‌طور معنی‌داری کاهش دادند و اثر بازدارندگی تغذیه‌ای متوسطی روی حشرات کامل شپشه آرد داشتند. هم‌چنین اسانس‌ها و نانوفرمولاسیون‌های آنها منجر به کاهش فعالیت آنزیم‌های استراز عمومی، استیل کولین استراز، آلفا-آمیلاز و پروتئاز کل و افزایش فعالیت آنزیم گلوتاتیون اس ترانسفراز در حشرات کامل شپشه آرد شدند. یافته‌های کلی این مطالعه نشان می‌دهد که نانوفرمولاسیون‌های تهیه شده از اسانس‌های مورد بررسی به‌خصوص اسانس پونه می‌تواند به‌منظور کنترل و مدیریت بهتر شپشه آرد مورد استفاده قرار گیرد.

واژگان کلیدی: کپسوله‌سازی، فعالیت آنزیمی، اسانس، شاخص‌های تغذیه‌ای، سمّیت