Fumigant Toxicity of Two Nano-Capsulated Essential Oils with Sublethal Rate of Phosphine against Three Stored-Product Pests

N. Bayramzadeh¹, F. Mehrkhou¹*, A. A. Pourmirza¹, and M. Mahmoudian²

ABSTRACT

In this study, two essential oils including *Cuminum cyminum* (L.) and *Lavandula angustifolia* (Mill.) were nano-capsulated by solvent evaporation emulsion method and their fumigant toxicity was investigated against three important stored-products pest, *Tribolium castaneum* (Herbst), *Sitophilus granarius* (L.), and *Oryzaephilus surinamensis* (L.). Moreover, the sublethal concentrations of phosphine gas in combination with nano-capsules were evaluated to reduce their usage concentration. The synthesized nano-capsules were confirmed by Scanning Electron Microscope (SEM), Transmission Electron Microscopy (TEM) and Fourier Transform Infrared Spectroscopy (FTIR). The chemical compositions analysis of *C. cyminum* and *L. angustifolia* by GC-MS revealed that α-Pinene (44.63%) and Linalyl acetate (61.74%) were the major components of *C. cyminum* and *L. angustifolia*, respectively. The results showed that pure *C. cyminum* was more effective than *L. angustifolia* regarding the fumigant toxicity after 24 h treatment on the three mentioned stored products pests. The LC₅₀ values of pure *C. cyminum* oil after 24 h treatment were obtained as 42.51 and 78.99 μL L⁻¹ air by *S. granarius* and *T. castaneum*, respectively. However, the LC₅₀ values of *C. cyminum* oil nano-capsule form were 220.34 (S. granaries) and 374.16 μL L⁻¹ air (T. castaneum), which were determined as susceptible and resistant pests, respectively. The results indicated that the combination of nano-capsulated form of essential oil with reduced amounts of phosphine could be used as a suitable method for control of stored product pests.

Keywords: *Cuminum cyminum*, *Lavandula angustifolia*, LC₅₀, Nano formulation.

INTRODUCTION

The use of plant compounds, such as essential oils and plant extracts, is an increasing interest due to their fewer side effects on non-target organisms and low-risk to environment compared to conventional insecticides. Botanical essential oils show different types of bioactivities such as contact and fumigant toxicity, antifeedant activity and repellency (Haouas et al., 2012; Rajendran and Siriranjini, 2008; Yeom et al., 2012). The insecticidal activity of some essential oils has been studied previously (Ebadollahi et al., 2012; Fadia et al., 2015; Khelfane-Goucem et al., 2016; Chaubey, 2008, 2011). Essential oils pose a number of constraints such as high volatility, sensitivity to environmental factors including light, temperature, pH, oxygen, chemicals, heat and pressure (Woranuch and Yoksan, 2013). In order to increase the effectiveness and improve the efficacy of essential oils, new formulations based on nanotechnology have been considered seriously (Abdollahi et al., 2012; Esmaeili and Asgari, 2015; Zahir et

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al., 2012; Nenaah, 2014 b; Barzegar et al., 2016). High volatility and rapid oxidation properties of essential oils could be improved by new technologies. Controlled release behaviour and the compatibility of essential oils with the environment improved nano-encapsulated formulations of essential oil to be used more effectively during fumigation (González et al., 2014; Koul et al., 2008; Moretti et al., 2002; Negahban et al., 2012). Khoobdel et al. (2017) reported the insecticidal activity of Rosmarinus officinalis (L.) essential oil nano-capsules in effective management of Tribolium castaneum (Herbst). They found that when essential oil was prepared as nano-capsules, this technique improved pesticides controlled-release properties and reduced the applied concentrations.

Developing novel formulations such as encapsulation techniques enable loading of the essential oils in the polymeric shell which protects the bioactive compound against degradation and increases durability and stability (Kah and Hofmann, 2014; Perlatti et al., 2013; Yang et al., 2009). Ziaee et al. (2014) studied the efficiency of myristic acid-chitosan nanogel loaded with C. cymimum essential oil against Tribolium confusum (du Val) and Sitophilus granarius (L.). They suggested that encapsulation could improve the persistence of the oil. In another study, Gonzalez et al. (2014) investigated the efficiency of essential oils on biological properties of T. castaneum and Rhizopertha dominica (F.) and found that these novel systems could be used in integrated pest management programs against stored product pests.

Phosphine (PH₃) is an organophosphorus compound that is highly toxic, free of hazardous residues, and consumed globally in stored product fumigation. Many researchers have reported the insecticidal activity of phosphine (Ahmed et al., 2002; Carpaneto et al., 2016; Collins et al., 2005; Rajendran and Muralidharan, 2001; Valmas et al., 2008). Khater (2012) stated that mixtures of botanical and chemical insecticides could be used for elevation of toxicity and possibly for decreasing the pollution burden of the environment.

In the last years, considerable attempts have been made to develop new biodegradable polymeric materials to improve both protection and controlled release properties of active compounds (Hosseini et al., 2013; Wu et al., 2012). In present work, we reported novel nano-formulation composed of polyethersulfone and encapsulated C. cymimum and Lavandula angustifolia (Mill.) essential oils, which was made by emulsion solvent evaporation technique. This technique is one of the popular methods for the encapsulation within water-insoluble polymer. The emulsion evaporation technique was developed at the end of the 1970s and has been used successfully in the preparation of microspheres made from several biocompatible polymers (Hoa et al., 2009). This method involves two steps. The first step requires emulsification of the polymer solution into an aqueous phase. During the second step, polymer solvent is evaporated, inducing polymer precipitation as nanoparticles. The nanoparticles are collected by ultracentrifugation, washed with distilled water to remove stabilizer residue, and lyophilized for storage (Song et al., 1997). Polyethersulfone was selected in the current study as the shell material, due to its biocompatibility, low toxicity, and no odor release (Konieczna et al., 2003).

Cuminum cymimum is an annual plant of the family of Apiaceae that is used as spice and ancient medicine in different countries (El-Ghorab et al., 2010; Jirovetz et al., 2005; Rebey et al., 2012). Lavandula angustifolia is a perennial strongly aromatic shrub of the family Lamiaceae, which is known for its great aroma and flavour and widely used in food, perfume, and cosmetic industries (Cavanagh and Wilkinson, 2002). The purpose of this study included: (a) To synthesize the nano-capsulated formulation of essential oils including C. cymimum and L. angustifolia, (b) To evaluate their fumigant toxicity on the three major stored product pests including T. castaneum, S. granarius.
and O. surinamensis, and (c) To improve the fumigant toxicity of nano-formulated essential oils by using sublethal rate of phosphine. The lack of such information was a justification for carrying out the present research.

MATERIALS AND METHODS

Preparation of Insects Colony and Essential Oils

The colony of three stored product pests including T. castaneum, S. granarius and O. surinamensis were prepared from Urmia University. The insects’ rearing medium included wheat flour mixed with yeast (10:1, w/w), wheat grain and barley, respectively. All species were reared at 25±2ºC and 60±5% Relative Humidity (RH) in darkness (Bagheri-Zenouz., 2011). Adults (1-3 days old) of mixed sex were used in the assays.

Preparation of Essential Oils

The essential oils of C. cyminum and L. angustifolia were purchased from Eadeh Ara Pishgaam, Tehran, Iran, the mentioned company has been reported the detail of effective ingredients and the percentage purity of essential oils, the detail of effective ingredients and the percentage purity.

For nano-capsules preparation, the following materials were used: PolyEtherSulfone (PES Ultrason E6020P; Mw= 58,000 g mol⁻¹) was used as the shell material; DiChloroMethane (DCM 99%) as a solvent for polyethersulfone was supplied by Merck; Poly-Ethylene Glycol (PEG; Mw= 600 g mol⁻¹) as a surfactant and porosity agent; and PolyVinyl Alcohol (PVA; as a surfactant) was purchased from Merck.

Nano-Capsulation of Essential Oils by PES

In this study, nano-capsules were prepared using emulsion solvent evaporation technique described by Pal et al. (2011), which comprises two steps. At first, 0.1 g PES was dissolved in 4 mL DCM, then, 0.01 g PEG and 200 μL of each oil were added separately. Later, the mixture was placed in magnetic stirring for 10 min. At the same time, the aqueous phase was prepared by dissolving 0.1 g PVA in 10 mL distilled water. The organic phase was added to the aqueous phase drop by drop slowly. The obtained mixtures were sonicated for 5 min by UP100H Ultrasonic Processors. After evaporation of the solvent, the nanoparticles were collected by centrifugation (3,000 rpm) for 10 minutes. The precipitated nano-capsules were dried in a vacuum oven at room temperature for 20 min. The resulting powder was used for bioassays.

Gas Chromatography-Mass Spectrometry (GC/MS) Analysis

The qualitative and quantitative analyses of the mentioned essential oils were performed by GC/MS. Additionally, GC-Mass analysis was used to examine the composition of the encapsulated essential oils within the polymer shell just and 48 h after preparation. For this purpose, 0.1 g of the prepared nano-capsules were dissolved in a 1 mL of dichloromethane in order to extract the encapsulated content (Chan, 2011). GC/MS analyses were performed on a Thermo Finnigan capillary gas chromatograph directly coupled to the mass spectrometer system (model GC TRACE; TRACE MS plus). HP-5MS non-polar fused silica capillary column (30 mx0.250 mm, 0.25 μm film thickness) was used. Temperature profile was as follows: at first, the temperature of the oven was fixed at 40°C for 2 minutes, then, increased to 160°C with the temperature rate of 5°C min⁻¹ for 2 minutes, and finally, increased to 280°C at 5°C min⁻¹. The carrier gas was helium at a flow rate of 1 mL min⁻¹, and ionization energy was 70ev. The identification of particular compounds of the essential oils was based on the comparison of their
relative retention times with those obtained from authentic samples of NIST 98 library standard database.

Nano-Capsules Characterization

The particle size of synthesized Nano-capsulated Essential Oils (NEO) was measured by field emission scanning electron microscope (SEM: HITACHI S-4160 at 30 kV acceleration voltage). The Transmission Electron Microscopy (TEM) analyses were performed using (Philips Bio-Twin, the Netherlands) electron microscope. Transmission electron micrographs were taken at 75 kV. FTIR spectra of PES, C. cyminum oil, L. angustifolia oil, nano-capsulated C. cyminum and nano-capsulated L. angustifolia oil were recorded with NEXUS-670 spectrophotometer in the range of 500–4,000 cm⁻¹ to analyze the functional groups.

Nano-Capsulation Efficiency and Oil Loading

The oil-loaded capsules (0.1 g) were dissolved in 1 mL dichloromethane to release the essential oils. Then, 1 mL of distilled water was added to segregate precipitate and centrifuged (3,000 rpm) for 15 minutes and dried. The difference between the initial amount of precipitate and secondary precipitate gives the amount of oil encapsulated (Chan, 2011). The encapsulation efficiency, yield, and oil loading content were calculated as the following formula (Khoee and Yaghoobian, 2009).

\[
\text{Yield} \, (\%) = \frac{\text{Weight of nanoparticle}}{\text{Weight of oil, polymer and excipient}} \times 100
\]

\[
\text{Oil loading} \, (\%) = \frac{\text{Weight of oil in nanoparticle}}{\text{Weight of the nanoparticles}} \times 100
\]

\[
\text{Encapsulation efficiency} \, (\%) = \frac{\text{Weight of oil in nanoparticle}}{\text{Weight of oil fed initially}} \times 100
\]

Fumigant Toxicity

The experiment was performed according to the method explained by Nenaah (2014 a) with some modifications. The fumigant toxicity of Pure Essential Oils (PEO) and Nano-capsulated Essential Oils (NEO) were examined in 60 mL volume glass containers (4 cm diameter and 6.5 cm height). Filter papers (Whatman No. 1) were impregnated with concentrations of 0, 27.66, 55.50, 83.33, 111.00, and 138.83 μL L⁻¹ air of each oil and then were attached to the screw caps of the vials. Lids were screwed tightly and sealed. In the NEO experiments, the powders were spread in the bottom of the vials and insects were placed in the meshed bags, which were hung inside the container to avoid direct contact with powders. Adults were exposed to encapsulated essential oil at concentrations of 0, 111, 222.16, 333.33, 444.33, and 555.5 μL L⁻¹ air. Thirty 1-3 days old insects with 0.5 g prepared food were released in the vials. Control insects were kept under the identical conditions, which included two control groups: vials without essential oil and nano-capsules without oil. Experiments were carried out according to standard procedure with three replications (Robertson et al., 2007). Treated lids were maintained under identical conditions similar to rearing environment. The mortality data was counted after 24 (Ebadollahi et al., 2012) and 48 hours (Suthisut et al., 2011) of exposure.

Sublethal Insecticidal Activity of Nano-Capsulated Essential Oils with Phosphine Treatment

Phosphine was generated from aluminum phosphide 56% Detia bag (Agrodragon company, China), which is recommended for one cubic meter of indoor area 3 to 5 grams. To determine the sublethal activity of phosphine, the preliminary concentration-mortality tests were done before each experiment. The six concentrations which were used to assign LC₂₅ were 0.03, 0.07, 0.12, 0.25, 0.67, and 1.33 μL L⁻¹. The sublethal symptoms were measured as the number of adults that survived after 72 hours of phosphine treatment.
In the bioassays related to nano-capsulated essential oils in combination with phosphine gas, \( LC_{25} \) of phosphine along with \( LC_{25} \) of nano-capsules were added at the same time to the experimental units. Therefore, each experimental unit, 5 L container, included the \( LC_{25} \) phosphine and \( LC_{25} \) nano-capsulated essential oils. To avoid the direct contact of insects, one petri dish was placed within 5 L container, which contained thirty 1-3-day old adults with 2 g of rearing medium and perforated hole was imbedded on the cap for ventilation, and fumigated for 72 hours. All bioassays were replicated three times. Two control groups included nano-capsules without oil and vials without phosphine. Mortality data were recorded after 72 hours. Adults showing no response when prodded with a brush were considered as dead. In each experiment, the number of live insects was compared with the number of dead insects using the Chi-square test. The purpose of this test was to compare between observed and expected percentage mortality. This might result in different combined effects including additism (which is almost equal to the sum of the separate constituents), synergism (which is more than the sum of the separate constituents) and antagonism (which is less than the sum of the separate constituents) (Otitoloju, 2002).

**Data Analysis**

Probit analysis (Finney, 1971) was used to estimate \( LC_{25} \) and \( LC_{50} \) values, and related statistical analyses were conducted by SPSS software package (Ver. 20, 2011). The treatments were considered as significantly different when there was no overlap in the 95% fiducial limits of lethal concentration values. The comparison between essential oils regarding the loading efficiency as well as the percentage of composition in pure essential oil and nano-capsulated oils was performed by T-test. Synergy between phosphine and nano-capsulated essential oils was analyzed by comparing mortality rate induced by combinations of both agents (observed) with the sum of mortalities induced by each agent separately (expected) using a Chi-square test \((P<0.05)\) (Yii et al., 2016). For this purpose, the observed mortality was compared to the table value for 1 \( df \) (> 3.84). If the calculated Chi-square value exceeds the tabulated value, it indicates a non-additive effect (either synergistic or antagonistic) of the two agents. A significant interaction of the phosphine–nano-capsulated essential oils combination was determined through the difference of (Mortality observed–Mortality expected), where Positive= Synergistic, and Negative= Antagonistic. In contrast, if the tabulated value exceeds the calculated Chi-square value, it represents an additive effect at \( P \leq 0.05 \).

**RESULTS AND DISCUSSION**

**Essential Oils Chemical Constituents**

GC-MS analysis revealed chemical compositions. Percentage of these compounds and retention times are shown in Table 1. The major constituents belong to the monoterpenes, which may be involved in fumigant toxicity of the insects in this study (Abdelgaleil et al., 2009; Lee et al., 2003; Wang et al., 2014; Ziaee et al., 2014). The \( \alpha \)-Pinene (44.63\%) was the major component in \( C. \) cuminum oil as supported by Ebadollahi and Mahboubi (2011). However, Ziaee et al. (2014) and Vasile et al.(2017) characterized cuminaldehyde (27.02 and 28.8\%, respectively), as a major compound in \( C. \) cuminum oil. Ali et al. (2014) reported that the major compound detected in the seeds of \( C. \) cuminum was Ethaneperoxoicacid,1-cyano-1-(2-ph) (17.11\%). These differences may be due to the geographical position, plants origin, harvesting time, and extraction procedure of essential oils, which affects
their chemical constituents (Vasile et al., 2017). The nano-capsulated essential oils had no significant effects on the quality and quantity of components just and 48 hours after preparation (P > 0.05). This is in agreement with Negahban et al. (2012), who mentioned that the quantity of chemical compositions of A. sieberi essential oil have no changes during 21 days after formulation.

Structural Characterization of Nanoparticles by SEM, TEM and FTIR

SEM photographs of PES (1-a) and oil loading nano-capsules are shown in Figure 1. As clearly illustrated in Figure 1 (b and c), all of the nano-essential oil particles have spherical, smooth surface and the capsules formed and preserved their size less-than-one μm and oils were embedded in the polymer matrix. The average particle sizes of SEM for C. cyminum and L. angustifolia oil were 127.59 nm and 542.20 nm, respectively.

TEM image of neat PES (Figure 2-a) confirmed that no core-shell structure was formed, while in Figures 2-b and -c images, the appearance of nano-sized oil loaded capsules revealed an obvious core-shell structure, which proved the formation of capsules. Capsules surface showed no significant difference between the two plant oils which were loaded in the polymer.

The FTIR spectra of pure PolyEtherSulfone (PES), C. cyminum essential oil, and their nano-capsulated formulation are shown in Figure 3. Spectrum (a) displays characteristic peaks of PES molecular structure with –OH (3,431.07 cm⁻¹), three peaks between 1,600 and 1,400 cm⁻¹ were attributed to aromatic skeletal vibration and the CH3 and CH2 bands in aliphatic compounds were observed in 2,927.57 cm⁻¹ and the S=O stretching peaks were present at 1099.69 cm⁻¹, which demonstrated that our compound was PES. For C. cyminum

| Table 1. Chemical composition of essential oils.† |  
| --- | --- |
| Entry | Compositions | Retention time (min) | Pure essential oils | Nano-capsules (just after preparation) | Nano-capsules (48 hours after preparation) |
| 1 | C. cyminum | 44.63 | 4.23 | 39.42 | 39.42 |
| 2 | L. angustifolia | 23.62 | 28.93 | 22.10 | 22.10 |

† There is no significant difference between treatments regarding the percentage of composition (P < 0.05, Student’s t test).
Synergism Effects of Phosphine and Nano-Capsulated_____________________________

Figure 1. SEM images of polyethersulfone (a), nano-encapsulated C. cyminum oil (b) and nano-encapsulated L. angustifolia oil (c).

Figure 2. TEM images of polyethersulfone (a), nano-encapsulated C. cyminum oil (b) and nano-encapsulated L. angustifolia oil (c).

Figure 3. FTIR spectra of PES (a), C. cyminum oil (b) and nano-encapsulated C. cyminum oil (c).
essential oil, the peaks observed at 2,930.41, 2,119.36, 1,652.12 and 1,457.39 cm\(^{-1}\) were associated with aliphatic C-H band stretching vibrations, substituted benzene rings and the C=C/aromatic skeletal vibration respectively (curve b). The FTIR spectrum of nano-capsulated \(C.\) \textit{cyminum} oil (curve c) indicated that the active constituents of our oil were available in capsules (\(\alpha\)-Pinene, M-cymene, \(\beta\)-terpineol and \(\delta\)-Terpinene). The weak peak, which appeared at 3,011.78 cm\(^{-1}\), was attributed to aromatic C-H bond and proved the aromatic structure of the effective compounds. Figure 4 (b) shows FTIR spectrum of the \(L.\) \textit{angustifolia} oil, and (c) the nano-capsulated \(L.\) \textit{angustifolia}. The absorbance at 3,419.51 cm\(^{-1}\) could be attributed to the OH functional group present in Linalyl acetate, while the absorbance at 2,930.29 cm\(^{-1}\) may be related to aliphatic C-H bond found in Linalool and Linalyl acetate. Absorbance at 1,647.64 cm\(^{-1}\) corresponds to C=C in Linalool and Linalyl acetate. In addition, the presence of sharper peaks between 1,000-1300 cm\(^{-1}\) in the \(L.\) \textit{angustifolia} and the nano-capsulated \(L.\) \textit{angustifolia} oil in comparison to polymer spectrum indicated the C-O functional group. It seems that the peak of carbonyl group that appeared at 1,730 cm\(^{-1}\) was not observed because of the overlap with double bond peak. Evaluation of the obtained curves of pre and post-encapsulation demonstrated that, after loading oil in capsules, the main constituent of oils was preserved. According to FTIR results, the chemical composition of the essential oil in the nano-formulation was not modified from the original compound during the preparation process.

**Nano-Capsulated Essential Oils Efficiency**

The nano-capsulated essential oils characterization including oil loading, encapsulation efficiency, and encapsulation yield of essential oils are presented in Table 2. Encapsulation efficiency depends on various variables. The maintenance of the active agent inside the membrane shell is ruled by factors related to the chemical nature of the core, including its molecular weight, chemical functionality, polarity and volatility, shell material properties, surfactants properties, and the selected encapsulation technique (Martins \textit{et al.}, 2014). In this study, the obtained data showed that the maximum encapsulation efficiencies belonged to \(C.\) \textit{cyminum} essential oil. In addition, by comparing the two essential oils, it was found that the oil loading and encapsulation yield in \(C.\) \textit{cyminum} was higher than \(L.\) \textit{angustifolia}. The oil loading content for \(C.\) \textit{cyminum} (73.03±0.14%) was more...
Table 2. Encapsulation efficiency, yield, and oil loading content of nano-capsulated essential oils. 

<table>
<thead>
<tr>
<th>Essential oil</th>
<th>Encapsulation yield (%)</th>
<th>Encapsulation efficiency (%)</th>
<th>Oil loading content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. cymimum</em></td>
<td>89.59±0.23^b</td>
<td>89.95±0.06^b</td>
<td>73.03±0.14^b</td>
</tr>
<tr>
<td><em>L. angustifolia</em></td>
<td>88.75±0.08^a</td>
<td>88.42±0.13^b</td>
<td>66.49±0.19^b</td>
</tr>
</tbody>
</table>

^a Mean±Standard error within each column followed by different letter are significantly different (P< 0.05, Student’s t test).

than *L. angustifolia*. These results suggest that the content of essential oils could influence encapsulation efficiencies. Ephrem et al. (2014) suggested that high encapsulation efficiency can be caused by the high hydrophobicity of the components.

**Fumigant Toxicity**

Probit analysis of the concentration-mortality response of the three studied insects to pure essential oils after 24 and 48 hours is presented in Table 3. As the values of LC50 show, the lowest and highest LC50 values were obtained by *S. granarius* and *T. castaneum*, when exposed to either *C. cymimum* (42.51 and 78.99 μL L−1 air) or *L. angustifolia* (68.78 and 106.47 μL L−1 air) oils after 24 h, respectively. These results imply that, in the same condition, *C. cymimum* oil was more effective in comparison to *L. angustifolia* oil after 24 hours (Table 3). The LC50 values with fiducial limits for 48 h treatment revealed that there were no differences among essential oils and the susceptibility of insects in most cases, except *O. surinamensis*. The LC50 of nano-capsulated essential oils are presented in Table 4. *C. cymimum* nano-capsulated was more toxic to *O. surinamensis* and *T. castaneum* after 24 hours exposure. In all treatments, there was no difference in toxicity and susceptibility to the tested nano-capsulated oils at 48 hours. (Table 4).

Essential oils affect the respiratory system, cuticle, and digestive system of insects via fumigant toxicity, contact effects, and antifeedant producer (Prates et al., 1998). Fumigant toxicity of both plant oils and their nano-capsules showed that *C. cymimum* and *L. angustifolia* were toxic to adults of the tested pests. Among the three stored product pests, the highest and lowest susceptibility were observed after 24 hours exposure of *S. granarius* and *T. castaneum* to the tested oils, respectively. The insecticidal toxicity of *C. cymimum* and *L. angustifolia* essential oil have been studied against different pests (Chaubey, 2008, 2011; Manzoomi et al., 2010; Rozman et al., 2007). Concerning the insecticidal activity of *L. angustifolia* oil, related to the presence of Linalyl acetate and Linalool, Sfara et al. (2009) and Ayvaz et al. (2010), noted that the presence of these compounds had the fumigant and repellency effects against the stored-product insects. Manzoomi et al. (2010) studied fumigant toxicity of essential oil from *Lavandula officinalis* (L) against *Callosobruchus maculatus* (F) who reported that LC50 value for *L. officinalis* was 41.52 μL L−1. These findings are similar to the result of the present study for insecticidal activity regarding another species of *Lavandula* spp., i.e. *L. angustifolia*, which was toxic against stored product beetles. Essential oil concentrations, exposure time, and type of essential oils had different effects on insects. The contact toxicity of two essential oils such as *Carum copticum* (L.) and *C. cymimum* against *S. granarius* and *T. confusum* was studied by Ziaee and Moharramipour (2013). They stated that adults of *T. confusum* were more resistant than *S. granarius*, in line with the red flour beetle, *T. castaneum*, in the current study.

Ziaee et al. (2014) applied the Myristic Acid-chitosan (MA-chitosan) as a nanogel which loaded with *C. cymimum* for the management of two stored product beetle pests, and they stated that nano-formulation improved the persistence of the oil. Loha et al. (2012) evaluated the bio-efficacy of nano-formulations of β-cyfluthrin against *C. maculatus*. They indicated that release from the commercial formulation was faster than nano-formulations, such that EC50 values of the commercial formulation were lower than nano-formulation and prolonged insecticidal activity was seen in the developed formulation rather than the commercial form. However, Ziaee et al.
Table 3. \( LC_{50} \) (μL L\(^{-1}\) air) values of three stored-product insect pests exposed to pure essential oils after 24 and 48 hours.\(^a\)

<table>
<thead>
<tr>
<th>Essential oils</th>
<th>Insects</th>
<th>Period (h)</th>
<th>( LC_{50} ) (95% Fiducial limits)</th>
<th>Slope±SE</th>
<th>( \chi^2 ) (df= 3)</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C.) cyminum</td>
<td>( S.) granarius</td>
<td>24</td>
<td>42.51 (17.55-60.44)</td>
<td>2.28 ± .26</td>
<td>8.27</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>38.5 (18.78-52.96)</td>
<td>2.58 ± .27</td>
<td>7.43</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>( O.) surinamensis</td>
<td>24</td>
<td>56.17 (47.49-64.5)</td>
<td>2.06 ± .25</td>
<td>4.38</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>42.98 (24.36-57.36)</td>
<td>2.34 ± .26</td>
<td>5.59</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>( T.) castaneum</td>
<td>24</td>
<td>78.99 (68.51-91.79)</td>
<td>1.98 ± .26</td>
<td>4.81</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>58.18 (33.15-81.93)</td>
<td>1.91 ± .25</td>
<td>5.89</td>
<td>0.90</td>
</tr>
<tr>
<td>( L.) angustifolia</td>
<td>( S.) granarius</td>
<td>24</td>
<td>68.78 (62.07-75.76)</td>
<td>3.04 ± .29</td>
<td>3.34</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>55.03 (40.46-68.55)</td>
<td>3.05 ± .28</td>
<td>5.66</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>( O.) surinamensis</td>
<td>24</td>
<td>99.34 (87.67-115.66)</td>
<td>3.32 ± .28</td>
<td>2.84</td>
<td>0.96</td>
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<tr>
<td></td>
<td></td>
<td>48</td>
<td>65.05 (58.35-71.9)</td>
<td>2.93 ± .28</td>
<td>4.02</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>( T.) castaneum</td>
<td>24</td>
<td>106.47 (95.78-121.07)</td>
<td>2.89 ± .32</td>
<td>2.52</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>83.53 (61.66-120.85)</td>
<td>2.42 ± .27</td>
<td>6.46</td>
<td>0.92</td>
</tr>
</tbody>
</table>

\(^a\)Each datum represents the mean of three replicates, each set up with 30 individuals (n= 90).

Table 4. \( LC_{50} \) (μL L\(^{-1}\) air) values of three stored-product insect pests exposed to nano-capsulated essential oils after 24 and 48 hours.\(^a\)

<table>
<thead>
<tr>
<th>Essential oils</th>
<th>Insects</th>
<th>Period (h)</th>
<th>( LC_{50} ) (95% Fiducial limits)</th>
<th>Slope±SE</th>
<th>( \chi^2 ) (df= 3)</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C.) cyminum</td>
<td>( S.) granarius</td>
<td>24</td>
<td>220.34 (189.53-249.84)</td>
<td>2.32 ± .26</td>
<td>4.09</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>182.5 (100.73-246.41)</td>
<td>2.39 ± .26</td>
<td>6.66</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>( O.) surinamensis</td>
<td>24</td>
<td>266.56 (233.89-300.85)</td>
<td>2.34 ± .26</td>
<td>5.21</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>230.46 (138.66-316.81)</td>
<td>2.32 ± .26</td>
<td>7.34</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>( T.) castaneum</td>
<td>24</td>
<td>374.166 (332.94-428.04)</td>
<td>2.46 ± .28</td>
<td>4.84</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>278.85 (194.45-383.78)</td>
<td>2.14 ± .26</td>
<td>5.48</td>
<td>0.93</td>
</tr>
<tr>
<td>( L.) angustifolia</td>
<td>( S.) granarius</td>
<td>24</td>
<td>275.16 (211.66-345.23)</td>
<td>3.08 ± .29</td>
<td>5.96</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>220.75 (151-284.68)</td>
<td>2.85 ± .27</td>
<td>6.79</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>( O.) surinamensis</td>
<td>24</td>
<td>405.43 (358.81-470.8)</td>
<td>2.40 ± .29</td>
<td>3.07</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>273.95 (242.98-306.65)</td>
<td>2.54 ± .27</td>
<td>2.09</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>( T.) castaneum</td>
<td>24</td>
<td>554.97 (476.17-694.96)</td>
<td>2.38 ± .32</td>
<td>5.24</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48</td>
<td>366.51 (265.11-602.8)</td>
<td>2.35 ± .28</td>
<td>7.40</td>
<td>0.91</td>
</tr>
</tbody>
</table>

\(^a\)Each datum represents the mean of three replicates, each set up with 30 individuals (n= 90).

(2014) stated that fumigant toxicity of oil loaded nanogels was higher than the pure oil even 48 hours after exposure. These differences could be due to the type of nano-formulation and the type of polymers used.

**Sublethal Effect of Phosphine Gas with Nano-Capsulated Essential Oils**

The sublethal fumigant toxicity test has been designed to decrease the nano-capsulated essential oil concentrations and phosphine. In this bioassay, the sublethal toxicity of nano-essential oils and phosphine gas at their \( LC_{25} \) values were determined against three tested insects (Table 5). The mixture of \( C.\) cyminum oil nano-capsules and phosphine gas caused synergistic effects on three insects. Furthermore, in combination of \( L.\) angustifolia oil nano-capsules and phosphine, additive effects were observed in \( S.\) granarius and \( T.\) castaneum. Phosphine treatment in mixture with heat, \( CO_2 \), controlled atmospheres and \( N_2 \) has been considered a suitable controlling method against stored product pests.
Synergism Effects of Phosphine and Nano-Capsulated Essential Oils against Stored Grain Pests

The obtained results proved that mixture of phosphine with nano-capsulated EO resulted in decreasing the lethal concentrations to achieve higher mortality rates compared to phosphine alone treatments and a substantial synergistic interaction between mixtures was observed. Based on our knowledge, the current study is the first attempt in determining the insecticidal efficacy of phosphine in mixture with nano-encapsulated essential oils against stored grain pests.

CONCLUSIONS

In conclusion, our results showed that pure C. cyminum was more effective than L. angustifolia regarding the fumigant toxicity after 24 hours treatment on three stored products beetles. However, the nano-capsulated form of C. cyminum was more effective than L. angustifolia on two studied pests, i.e. O. surinamensis and T. castaneum. Our findings are the first report of the studied essential oils which are nano-capsulated using emulsion solvent evaporation technique by polyethersulfone polymer. Since in most cases, the synergism effects were observed in sublethal toxicity of phosphine and nano-capsulated forms of essential oils, we could conclude that the combination would help us to use either the reduced concentration of phosphine or improve essential oils as nano-capsules.

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عنوان حساس‌ترین و مقاوم‌ترین حشره تیمار شده با این اساس، تعيين شدند. نتایج آزمایشات مربوط به اثر تلفيقی فرم نانوکپسول اساس با مقادير گاز فسفين مناسب در کنترل آفات اتجاوز مورد استفاده قرار گيرد.