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Insecticidal activity of polycaprolactone nanoparticles decorated with chitosan containing two essential oils against Tribolium confusum

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ABSTRACT
Essential oils are composed of volatile components, and rapidly evaporate. It is desirable to formulate them in a cover protecting the essential oils against environmental conditions. Applying nanotechnology to preparing desirable formulations of essential oils can be provided in a cover along with controlled release, increased oil life and greater mobility. In this study, nanoparticles were prepared using polycaprolactone and chitosan aimed at encapsulation of Rosmarinus officinalis and Zataria multiflora essential oils. The nanoparticles were characterized in terms of size, polydispersity, encapsulation efficiency, and scanning electron microscope photomicrographs. The nanoparticles showed a positive surface charge ranging between $+11.60$ and $+29.20$ mV and a mean particle radius ranging between 181-407 nm. Encapsulation efficiency was obtained between 75.8-84.4% under different preparation conditions. The LC$_{50}$ values of fumigant toxicity of R. officinalis essential oil and nanoparticles were equal to 103.53 and 112.64 µL/L air, and for Z. multiflora essential oil and nanoparticles, they were equal to 195.13 and 206.66 µL/L air, respectively for 24 h exposure time against Tribolium confusum. The effectiveness of non-formulated essential oils was significantly ($P < 0.05$) lower than nanoencapsulated oils in the confused flour beetle adults for a long period under the same condition, due to the sustained release of the essentials provided by the formulation.

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KEYWORDS
Nanoparticles; encapsulation; rosemary; insecticide; essential oil

Introduction
Different species of insect pests can feed on the cereals and other stored products in packages and storage structures decreasing the food quality and quantity. The confused flour beetle, Tribolium confusum (Duval) (Coleoptera: Tenebrionidae) as a secondary pest causes injuries to some stored products such as cereals. The application of chemical insecticides is the prevail control strategy for controlling this pest, but this method has negative consequences, such as water and soil contamination, long time persistence in the environment, toxicity for animals and humans, and residues in foods (Regnault-Roger et al. 2004). These negative consequences of chemical insecticides have led to the development of a new formulation of safe alternatives such as essential oils for controlling the insect pests (Buteler et al. 2019; Cheng et al. 2019). Fumigant insecticides can be useful for treating floor, wall, crack and crevice treatments (Johnson 2013). These pesticides may be used for processing and storage areas. Essential oils are composed of volatile components, and rapidly evaporate. It is desirable to formulate them in a cover protecting the essential oils against environmental conditions such as evaporation, oxidation, high temperature, and UV light (Martín et al. 2010). Rosemary (native plant to the Mediterranean area) and Zataria (native plant to Iran, Pakistan and Afghanistan) have several traditional uses (Zargari 1990; Borrás-Linares et al. 2014). R. officinalis (Lamiaceae) and Z. multiflora (Lamiaceae) essential oils have an effective fumigant and contact toxicity against the insects and mites, particularly against stored product beetles (Shaaya et al. 1991; Papachristos et al. 2004; Saleem et al. 2004; Khoobdel et al. 2017; Campolo et al. 2018; Saedi and Pezhman 2018). Studies carried out by Saedi and Pezhman (2018) showed that, the LC$_{50}$ value of Zataria after 24 h exposure time was equal to 58.43 and 99.94 µL/L air, respectively for Bruchus lentis and Callosobruchus maculatus.

Regarding pest management, nanotechnology can be used for delivery of nanomaterials encapsulated pesticides, nanocides in order to have controlled release, as well as the stabilization of pesticides with nanotechnology (Sinha et al. 2017). Nanoprecipitation can be one of the most...
interesting methods for encapsulation of drugs since this method can be run with conventional equipment and it has the ability to be scaled up from the laboratory to industrial scale (De et al. 2014). After cellulose, chitosan is the most abundant polysaccharide in the world and derived from the deacetylation of chitin (Mazzarino et al. 2012). PCL and chitosan are biodegradable, biocompatible and non-toxic to the humans. Thus, they are interesting biomaterials for preparation and design of carriers (Mazzarino et al. 2013). Two polymer-coated particles can improve the stability or release of encapsulated active materials (Milner 1991). The positively charged surface in the chitosan-coated nanoparticles improve the stability of suspension through electrostatic forces (Lourenco et al. 1996). Chitosan can enhance plant innate immunity defenses (Fondevilla and Rubiales 2012) and exhibits antimicrobial activities (Rabea et al. 2009).

Previous studies available about the application of nanomaterials in the management of insect pest suggested that, the use of nanomaterials develops a new approach for production of more effective insecticides. Ahsaei et al. (2020) evaluated the insecticidal effect of spray dried particles were spread in 10 ml from remaining room temperature (Ephrem et al. 2014; Khoobdel 2017). After settling the nanoparticles, nanomaterials can be run with conventional equipment and it has the ability to be scaled up from the laboratory to industrial scale (De et al. 2014).

**Materials and methods**

**Materials**

Poly-Ɛ-(caprolactone) (PCL, with the average molecular weight of 14,000 Da) and a medium (190,000–3,10,000 Da) containing molar mass chitosan with deacetylation degree of ~85% were purchased from Sigma–Aldrich Company (USA). Polysorbate (Tween80) (hydrophilic surfactant) and Sorbitan 20 monostearate (Span20) (non-ionic surfactant) were purchased from Merck Company (Darmstadt, Germany). 

**Insect rearing**

Some colonies of *T. confusum* were held in a growth chamber in insect physiology laboratory at the University of Tehran, at 27 ± 3°C temperature and 70–75% of relative humidity in the dark. The pests were reared on wheat flour mixed with yeast (10:1 w:w).

**Extraction of the essential oil**

Rosmarinus officinalis and Zataria multiflora (Lamiaceae) essential oils used in this study were purchased from Barij Essence Company (Kashan, Iran). These essential oils were produced by hydro-distillation. They were kept in dark glass containers at 4°C.

**Gas chromatography-mass spectrometry (GC–MS) analysis**

The components of essential oils were determined using the GC–MS technique (Agilent 6890/5973 (Agilent Technologies, USA), equipped with an HP-5 MS capillary column (30 m × 0.25 mm × film thickness 0.25 μm). Helium was used as the carrier gas, at a flow rate of 0.7 mL/min with a split ratio of 1:500 and then, was placed in oven at 40°C for 5 min, and the temperature increased to 65°C (5°C/min) for 7 min, then increased to 180°C (3°C/min), and finally to 300°C (20°C/min) for 1 min. MSD transfer line heater temperature was equal to 250°C. An electron ionization system with an ionization voltage of 70 eV was used for GC–MS detection. The chemical compositions of two essential oils were identified by comparing their retention times and mass spectra with those recorded in databases (Willey Library) on the gas chromatography-mass spectrometry system.

**Preparation of nanoparticles**

The nanoparticles were prepared using the nanoprecipitation method, as previously described by Mazzarino et al. (2013) with some modifications. Briefly, an organic phase contained PCL, rosemary or *Zataria* essential oils (60 mg), Span20 in 12 mL of acetone. The organic phase was added drop wise using a syringe to an aqueous phase (24 ml, pH of 5) containing 1% acetic acid and chitosan, Tween 80 under moderate magnetic stirring. Then, organic solvent was eliminated under moderate magnetic stirring for 2 h at room temperature. The suspension of nanoparticles was centrifuged at 7830 rpm at room temperature (Ephrem et al. 2014; Khoobdel et al. 2017). After settling the nanoparticles, nanoparticles were spread in 10 ml from remaining
supernatant. The different formulations prepared are presented in Table 1.

### Characterization of nanoparticles

#### Particle size and zeta potential

The mean diameter, polydispersity index and zeta potential values of the nanoparticles were determined and evaluated by Dynamic Light Scattering (DLS) using a Malvern Zetasizer Nano Series after formation of the nanoparticles. For analysis, all suspensions were diluted in Milli-Q water to a volume ratio of 1:100 (Ephrem et al. 2014).

### Determination of encapsulation efficiency

The encapsulation efficiency percentage (EE%) of essential oils into the nanoparticles was calculated using the following equation according to the studies by Zahra et al. (2017) and Mazzarino et al. (2013) with some modifications:

\[
\text{Encapsulation efficiency} = \frac{\text{Initial oil} - \text{Free oil}}{\text{Initial oil}} \times 100
\]

Where, initial oil is the amount of initial oil used for preparation of nanoparticles and free oil is the amount of unloaded oil in the lower chamber of Millipore Amicon® after centrifugation. For separation of unentrapped oil from nanoparticles, the colloidal suspension was placed in the upper chamber of Amicon® centrifugal filter (10 kDa, Millipore, USA), and was centrifuged at 4°C and 5000 rpm for 20 min (twice). The amount of unentrapped oil was determined by measuring the absorbance of oils in the sample collected from the lower chamber of the filter using UV/VIS spectrophotometer (Optizen 3220 UV, Mecasys Co. Ltd., Korea) at 319 and 274 nm for rosemary and Zataria essential oils, respectively with acetone as solvent.

### Scanning electron microscopy (SEM)

SEM imaging of the nanoparticles (in accordance with the optimized condition (formulation 1)) was performed with a Scanning Electron Microscope (SEM) (KYKY-EM3200 model, KYKY, Beijing, China) using an acceleration voltage of 26.0 kV and 25.00-fold magnification. The samples were diluted in Milli-Q® water at 5% (v/v), and the colloidal suspension was evaporated on the glass slide. Then, the samples were gold-sputtered (Christofoli et al. 2015).

### Fumigant toxicity

Fumigant toxicity of the essential oils and nanoparticles was evaluated according to the method proposed in the study by Suthisut et al. (2011) with some modifications. These tests were performed in plastic vials (125 mL, 5 cm diameter and 7.5 cm height) as experimental units and 15 adults with the same age were introduced to each vial. The condition of the experimental units was adjusted at 27 ± 3°C of temperature and 70–75% of relative humidity in the dark. The nanoparticles used in bioassays and persistence assays were prepared under the first test condition. Different concentrations of 79.41, 95.49, 114.81, 138.03, and 168.26 μL/L air for rosemary and 172.15, 190.10, 211.83, 236.04, and 264.42 μL/L air for Zataria from non-formulated essential oils were shed on filter paper disks (Whatman No. 1, with 2.5 cm diameter) under the surface of the screw caps then, a cloth mesh was put under the vials caps and was sealed with air-tight lids. All vials were fitted using an adhesive film. Also, the same concentrations from nanoparticles were used (in accordance with the first test condition according to the amount of the essential oil encapsulation efficiency). Bioassays were conducted two times and for control vials and each concentration, four replicates were used. Empty nanoparticles (without the essential oils) were used as controls. Mortality rate was determined after 24 h exposure time.

### Persistence assays

To perform persistence assays for the essential oils and nanoparticles, the method introduced in the study by Ziaee et al. (2014) was used with some modifications. The LC₈₀ value obtained from fumigant toxicity bioassays was applied to evaluate the persistence of encapsulated essential oils (suspension with pH 5 and 7). From the beginning of the treatment, in different periods, 15 adults were inserted into each experimental unit in three replicates. Then, the mortality rate was determined after 24 h exposure time. The conditions of the experimental units were adjusted at 27 ± 3°C of temperature and 70–75% of relative humidity in the dark.

### Data analysis

For bioassays, data were expressed as (mean ± SE). Analysis of Variance (ANOVA) at P < 0.05 was used using Tukey’s Honest Significant Difference
Tables S1 and S2. In the rosemary essential oil, the (24.63%) were the main components in the (10.54%). Also, thymol (27.95%) and carvacrol phor (22.25%), 3-carene (14.83%) and camphene main components were 1,8- cineol (29.48%), cam-
to 8% in the Zataria percentage between 1 to 6% in the rosemary and 1 essential oil. There are some components with a
tion (Table 2).

Results

The main components of essential oils

The main component of the Rosmarinus officinalis and Zataria multiflora essential oils is presented in Tables S1 and S2. In the rosemary essential oil, the main components were 1.8- cineol (29.48%), cam-
phor (22.25%), 3-carene (14.83%) and camphene (10.54%). Also, thymol (27.95%) and carvacrol (24.63%) were the main components in the Zataria essential oil. There are some components with a percentage between 1 to 6% in the rosemary and 1 to 8% in the Zataria essential oil. Moreover, other components were detected with a percentage lower than 1%. Main chemical components of two essential oils are provided in Supplementary Information (Tables S1 & S2).

Characterization of nanoparticles

In this study, suspensions were obtained by the nanoprecipitation method. To optimize the nanoparticle preparation, main parameters were evaluated including concentration of PCL and chitosan polymers and concentration of surfactants. The nanoparticles were prepared using polycaprolactone as a biodegradable polymer and chitosan aimed at encapsulation of two essential oils. Our results showed that, the size and zeta potential of the suspensions can be affected by the polymer concentra-
pensions can be affected by the polymer concentra-
showed that, the size and zeta potential of the sus-
encapsulation of two essential oils. Our results
were used to estimate LC25, LC50, and LC75 values with
their fiducial limits using Polo Plus software 2.0.

Fumigant toxicity

Probit analysis results for fumigant toxicity of the rosemary and Zataria essential oils and their nanoparticles are presented in Table 3. These results indicated that, pure rosemary essential oil (LC50 value was equal to 103.53 µL/L) was more toxic than pure Zataria essential oil (LC50 value was equal to 195.13 µL/L) against adults of T. confusum after 24-h exposure time. Also, the LC50 value of nanoformulated rosemary essential oil (LC50 value was equal to 112.64 µL/L) was higher than non-formulated essential oil (LC50 value was equal to 103.53 µL/L). The results of fumigant toxicity for Zataria essential oil were similar to those of rosemary essential oil. LC50 value of nanoformulated Zataria essential oil (LC50 value was equal to 206.66 µL/L) was higher than non-formulated essential oil (LC50 value was equal to 195.13 µL/L). Therefore, non-formulated essential oils had a more volatility than nanoformu-
lated after 24 h exposure time.

Table 2. Particle diameter, polydispersity index (Pdi), zeta potential and Encapsulation efficiency (Mean ± SD) for various nanoparticles from rosemary and Zataria essential oils.

<table>
<thead>
<tr>
<th>Essential oil</th>
<th>Run</th>
<th>Particle diameter (nm)</th>
<th>Pdi</th>
<th>Zeta potential (mV)</th>
<th>Encapsulation efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosemary</td>
<td>1</td>
<td>382 ± 52 ( ^a )</td>
<td>0.41</td>
<td>28.30 ± 0.28 ( ^a )</td>
<td>84.43 ± 1.76 ( ^a )</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>215 ± 13 ( ^a )</td>
<td>0.31</td>
<td>17.90 ± 0.56 ( ^b )</td>
<td>83.33 ± 3.23 ( ^a )</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>181 ± 6 ( ^b )</td>
<td>0.34</td>
<td>11.60 ± 1.48 ( ^c )</td>
<td>82.90 ± 4.25 ( ^a )</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>396 ± 25 ( ^b )</td>
<td>0.41</td>
<td>27.00 ± 1.13 ( ^a )</td>
<td>82.76 ± 1.70 ( ^a )</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>407 ± 35 ( ^a )</td>
<td>0.56</td>
<td>25.80 ± 1.55 ( ^a )</td>
<td>83.53 ± 2.65 ( ^a )</td>
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<tr>
<td>Zataria</td>
<td>1</td>
<td>388 ± 13 ( ^a )</td>
<td>0.40</td>
<td>29.20 ± 0.56 ( ^a )</td>
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<td></td>
<td>5</td>
<td>398 ± 42 ( ^a )</td>
<td>0.38</td>
<td>27.40 ± 2.54 ( ^a )</td>
<td>75.86 ± 0.95 ( ^a )</td>
</tr>
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* Different letters indicate significant differences according to Tukey test at \( a = 0.05 \).

The results of fumigant toxicity for rosemary and Zataria essential oils were similar to those of rosemary essential oil (LC50 value was equal to 103.53 µL/L) was more toxic than pure Zataria essential oil (LC50 value was equal to 195.13 µL/L) against adults of T. confusum after 24-h exposure time. Also, the LC50 value of nanoformulated rosemary essential oil (LC50 value was equal to 112.64 µL/L) was higher than non-formulated essential oil (LC50 value was equal to 103.53 µL/L). The results of fumigant toxicity for Zataria essential oil were similar to those of rosemary essential oil. LC50 value of nanoformulated Zataria essential oil (LC50 value was equal to 206.66 µL/L) was higher than non-formulated essential oil (LC50 value was equal to 195.13 µL/L). Therefore, non-formulated essential oils had a more volatility than nanoformulated after 24h exposure time.

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Persistence bioassays

The results of persistence bioassays of rosemary and *Zataria* non-formulated and nanoencapsulated essential oils over time are presented in Figures 2 and 3. The effectiveness of non-formulated and nanoformulated (suspension with pH 5 and 7) essential oils decreased by increasing the storage time in *T. confusum* adults. After 5 days of storage period, non-formulated essential oils caused 37.77% mortality for rosemary and 24.44% mortality for *Zataria*, while the mortality reached 24.44 and...
8.88% when oils were stored for 15 days. In the same trend, for rosemary nanoencapsulated essential oil (suspension with pH 5), the mortality reached to 55.55, 46.66 and 26.66%, respectively after 5, 10 and 25 days of storage period. Also, for rosemary nanoencapsulated essential oil (suspension with pH 7), the mortality reached to 71.11, 55.55 and 40.00%, respectively after 5, 10 and 25 days of storage period (F14,72 = 6.35, p = 0.00). In case of Zataria essential oil, the mortality reached to 53.33, 42.22 and 22.22%, respectively after 5, 10 and 25 days of storage period for nanoencapsulated essential oil (suspension with pH 5). The mortality reached to 64.44, 53.33 and 31.11%, respectively after 5, 10 and 25 days of storage period for Zataria nanoencapsulated essential oil (suspension with pH 5) (F14,72 = 9.39, p = 0.00). These results demonstrated that, the release of oil in nanoformulated essential oils is slower than non-formulated essential oil.

Discussion

In this study, it was found that, the encapsulation of rosemary or Zataria essential oil into a liquid controlled-release nanoformulation enhanced their stability and insecticidal activities through the fumigant toxicity. In this study, the main components were 1,8-cineol (29.48%) and camphor (22.25%) in the rosemary, and thymol (27.95%) and carvacrol (24.63%) for Zataria essential oils. Isman et al. (2008) stated that, 1, 8-cineole, camphor and camphene are main components in the rosemary essential oil. Also, in a similar research, the main components of the Zataria essential oil were reported to be thymol and carvacrol (Saei-Dehkordi et al. 2010; Karimian et al. 2012).

To select the ideal polymer carrier in nanoparticles, there are some criteria such as being non-expensive, water-soluble, biodegradable, easy to synthesize and characterize, not toxic, biocompatible, and non-immunogenic (Krishna et al. 2006). Span 20 is a lipophilic surfactant and Tween 80 is a hydrophilic surfactant. Stable emulsions can provide with an emulsifier or combination of emulsifiers having hydrophilic–lipophilic balance (HLB) values close to that required for the oil phase (Aulton 1995). The required HLB to have a stable essential oil emulsion is between 12 and 15 (Rodríguez-Rojo et al. 2012). In this study, with the increase in chitosan concentration, the zeta potential, mean size, and encapsulation efficiency of the nanoparticles increased. The nanoparticles showed a positive surface charge ranging between +11.60 and +29.20 mV and a mean particle radius ranging between 181-407 nm. The role of polymer concentration in determining particle size and zeta potential is more critical rather than surfactant concentration in this work. The surfactant concentration may provide additional suspension stability to that due to the positive charge of the chitosan. Encapsulation efficiency was between 75.8-84.4% under different preparation conditions. However, the encapsulation efficiency results do not show a significant difference. The existence of amino groups with a positive charge in chitosan polymer increases the zeta potential of nanoparticles (Mazzarino et al. 2012). The optimal formulation of nanoparticles for rosemary essential oil was obtained using Span 20 and Tween 80 (Ephrem et al. 2014). Several studies showed
that, nanoparticulate systems can be applied as active carriers because of their ability to release drugs (Cruz et al. 2006). Regarding the nanoprecipitation method, there are some elements having a crucial role in determining nanoparticle size, including the nature and ratio of internal/external phases, the nature, and concentration of the polymer, surfactants and solvent polarities (Santos-Magalhães et al. 2000; Zili et al. 2005). Mora-Huertas et al. (2010) mentioned that, the chemical nature of the stabilizing agent, polymer, and pH of the medium are determinant elements in zeta-potential. Also, due to the presence of terminal carboxyl groups in polyester polymers such as polycaprolactone, negative surface charge will be obtained (Joo et al. 2008). The highest encapsulation efficiency may be related to the first run for two essential oils with optimum hydrophilic-lipophilic balance values and more stability. When, the nanoprecipitation method was applied for preparation of nanoparticles, dispersion pH-values of nanoparticles were within a range of 3.0–7.5 (approximately 4 in this study) (Mora-Huertas et al. 2010). It has been stated that, nanoprecipitation, emulsion–diffusion and layer-by-layer methods show the highest encapsulation. In case of nanoprecipitation and emulsion–diffusion methods, the maximum solubility of the active substance has been found as one of the important criteria for essential oil selection. SEM images of oil-loaded nanoparticles indicated that, nanoparticles have smooth and spherical appearance. Similar results for PCL nanoparticles have been previously reported in the studies by Cazo et al. (2012) and Christofoli et al. (2015).

Fumigant toxicity results showed that, non-formulated essential oils had a more volatility than nanoformulated after 24h exposure time. The results of fumigant toxicity for Zataria essential oil were similar to those of rosemary essential oil. The results of the present study are in agreement with those of the studies by Christofoli et al. (2015) and Werdin González et al. (2014). They showed that, nanoencapsulation can provide controlled release for two essential oils than the bulk material and protect them against evaporation. Christofoli et al. (2015) investigated the insecticidal activity of both in pure and nanoencapsulated essential oils from Z. rhoifolium in Bemisia tabaci. They prepared nanoparticles using the polycaprolactone, and revealed the presence of spherical nanoparticles. The results of biological assays for pure and nanoencapsulated essential oils showed significant reductions in the number of nymphs and eggs compared to the control (Christofoli et al. 2015). Werdin González et al. (2014) evaluated different biological assays of poly(ethylene glycol) nanoparticles containing geranium and bergamot essential oils against Rhizopertha dominica and T. castaneum. Both of essential oils caused 100% of mortality after 24h exposure time while the nanoparticles of these essential oils did not have any effects after 120h. Werdin González et al. (2014) suggested that, the nanoparticles reduces volatility of those essential oils and their release is slower than pure essential oils. Insecticidal activity of rosemary essential oil and its PCL nanoparticles against Tribolium castaneum has been investigated (Khoobdel et al. 2017). Nanoparticles showed an average size \((145 \pm 15 \text{nm})\) (± standard error) with a uniform polydispersity index, and a high encapsulation efficiency \((78.20 \pm 0.93\%\). The nanoparticles can enhance controlled release property of the active components (Khoobdel et al. 2017). The nanoparticles can be more effective compared with their bulk counterparts in the red and confused flour beetles for a long period. It seems that by adding chitosan to the PCL–rosemary essential oil nanoparticles as in this work, the release rate of the formulation can be slower than the PCL–rosemary essential oil nanoparticles. Yang et al. (2009) observed that, the insecticidal efficacy of polyethylene glycol-coated nanoparticles loaded with garlic essential oil was about 80% after 5 months against T. castaneum. They indicated that, nanoparticles have slow and persistent release of active components. Yang et al. (2009) and Ziaee et al. (2014) also showed that the toxicity of pure and encapsulated essential oils was negatively related to the time and positively related to the dose. Insecticidal activity was significantly higher \((P<0.05)\) when exposed to pure essential oils than microencapsulated rosemary and Zataria essential oils after 72h of the exposure period against T. confusum (Ahsaei et al. 2020). They suggested that the controlled release property in the encapsulated essential oils would be the reason for
this phenomenon. Our results are in agreement with these results. The effectiveness of non-formulated essential oils was significantly (P < 0.05) lower than nanoencapsulated oils (suspension with pH 7) in *T. confusum* adults for a long period under the same condition. The release of essential oils in nanoparticles is slower than non-formulated essential oils. The *Thymus* and *Rosmarinus* microcapsules caused the death of ≥25 and 75 percent of the treated insects after 25 days against *Plodia interpunctella*, respectively (Sanna Passino et al. 2004). The rosemary and *Zataria* microcapsules caused the death of ≥ 46 and 35 percent of the treated insects after 15 days, respectively while the pure essential oils showed about 10% mortality after 25 days (Ahsaei et al. 2020). In future works, it may be interesting to check the fumigant toxicity of main individual compounds or the synergic effect between them all in the essential oils.

**Conclusion**

Given the potential applications of essential oil nanoparticles in agrochemical formulations, in the present study, a formulation of rosemary and *Zataria* essential oils was prepared by nanoprecipitation method using chitosan and polycaprolactone polymers for controlling a species of stored product pest. The nanoparticles showed a positive surface charge and a mean particle radius ranging between 181 and 407 nm. Encapsulation efficiency was between 75.8-84.4% under different preparation conditions. The effectiveness of non-formulated essential oils was significantly (P < 0.05) lower than nanoencapsulated oils (suspension with pH of 7) in *T. confusum* adults for a long period under the same condition. These results suggested that, nanoencapsulation has a good potential to provide controlled release for two essential oils than the bulk material and protect them against evaporation. However, additional experiments are required for elucidating its potential toxicity in different environmental conditions and different insect species.

**Disclosure statement**

The authors declare that they have no conflict of interest.

**Author contribution statement**

SMA and KTJ conceived the original idea and wrote the initial version. SMA, KTJ and GHA collected and analyzed the data. All the authors contributed in the final version of the manuscript.

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**Data availability statement**

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary information file.

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