Insecticidal activity of spray dried microencapsulated essential oils of *Rosmarinus officinalis* and *Zataria multiflora* against *Tribolium confusum*

Seyed Mohammad Ahsaei[1], Soraya Rodríguez-Rojo[2*], Marta Salgado[2], María José Cocero[2], Khalil Talebi Jahromi[1*], Ghassem Amoabediny[3]

1. Department of Plant Protection, University College of Agriculture & Natural Resources, University of Tehran, Karaj, Iran

2. High Pressure Processes Group, Department of Chemical Engineering and Environmental Technology, University of Valladolid, Valladolid, Spain

3. School of Chemical Engineering, College of Engineering, University of Tehran, Tehran, Iran

*Corresponding author at: High Pressure Processes Group, Department of Chemical Engineering and Environmental Technology, School of Engineering–Sede Mergelina, University of Valladolid, 47011 Valladolid, Spain (S. Rodríguez-Rojo).

E-mail address: sorayarr@iq.uva.es (S. Rodríguez-Rojo)
Tel: +34 983 18 4077

*Corresponding author at: Department of Plant Protection, University College of Agriculture & Natural Resources, University of Tehran, Karaj, Iran (Khalil Talebi Jahromi).

E-mail address: khtalebi@ut.ac.ir

Abstract

Rosmarinus officinalis and *Zataria multiflora* (Lamiaceae) essential oils (EOs) contain components with insecticidal properties that can be used as pesticides for stored product pests. In the present study, they were encapsulated in octenyl succinic anhydride (OSA) - starch in order to test their insecticidal activity against *Tribolium confusum*. First an oil-in-water emulsion was prepared and afterwards, it was dried by spray-drying technique. The emulsions were characterized regarding particle size (461-854 nm), stability and encapsulated oil efficiency (68-88%). Also, solid formulations were characterized by particle size (8.29-11.35 µm), encapsulation efficiency (5-52%) and water activity (0.19-0.26). Further, the release rate at storage conditions (at 27±3 ºC and 70–75% relative humidity in the dark) was measured over a period of 40 days. The insecticidal activity against *T. confusum* was determined by specific bioassays performed at 27±3 ºC temperature and 70–75% relative humidity in the dark. Five concentrations were used for estimation of fumigant toxicity of rosemary and *Zataria* oils after 72 h exposure in adult beetles. Fumigant toxicity results revealed that microencapsulated oils were more effective than non-formulated oils against beetles in long time. Similarly, it was demonstrated that microencapsulation of the essential oils increases their persistence: non-formulated oils have not insecticidal activity after 15 days of the storage period, whereas at the same period, the mortality rate against *T. confusum* of rosemary and *Zataria* microencapsulated oils was 46.6 and 35.5%, respectively.

Keywords: essential oil, microcapsules, stored product pest, Rosemary, *Zataria*, OSA-starch

1. Introduction

The confused flour beetle, *Tribolium confusum* (Duval) (Coleoptera: Tenebrionidae) is one of the destructive secondary pests of stored grains and grain-derived products (Rees, 2007). Synthetic pesticides, both direct contact and fumigants, are typically used to the management of the stored product pests. Though, nowadays, the tendency is to avoid direct treatment to the grain. Main representative of direct contact or grain protectants are pyrethroids and organophosphates (Kljajić et al., 2014) while sulfuryl fluoride is one of the major examples of grain fumigants (Zettler and Arthur, 2000). However, the chemical pesticides have harmful impacts on human health and the environment and their repeated used has contribute to changes in the susceptibility of the insects and the development of resistances (Ali et al., 2012). Therefore, there is a serious need to find alternative agents. Insect losses in post-harvest period can be decreased by applying essential oils, instead of the use of chemical insecticides (Werdin González et al., 2013; Reyes et al., 2019). Essential oils are mixtures of volatile compounds and rapidly evaporate from the surface. It is desirable to formulate them in a cover that increases oil life, controlled release property, protects the essential oils against evaporation, oxidation, high temperature, UV light and facilitating their handling (Martín et al., 2010).

Several essential oils from the different families have fumigant toxicity against the stored product pests. Alves et al. (2019) studied the fumigant effect of lemongrass essential oil (*Cymbopogon citratus*) and citral on the reproductive cycle, sexual behavior, lipid composition and the enzymatic activity of biotransformation enzymes in controlling *Callosobruchus maculatus.* Toxicity, antifeedant, and biochemical efficacy of *Mentha piperita* L. essential oil investigated against *Sitophilus oryzae* and *Tribolium castaneum* at 24 h of exposure (Rajkumar et al., 2019). Insecticidal activity of rosemary oil investigated against red flour beetle and rice weevil (Shaaya et al., 1997; Lee et al., 2002).

Sanna Passino et al. (2004) studied insecticidal activities of *Rosmarinus officinalis* and *Thymus vulgaris* microencapsulated essential oils against *Plodia interpunctella* larvae. The toxicity of these oils was examined after diet contamination with these microcapsules and vapors exposition. They showed a different release pattern of the oils and mentioned that it could be due to the different hydrophilic characteristics. Rosemary and *Zataria* oils have effective fumigant toxicity against the insects and mites, particularly against stored product pests (Shaaya et al., 1991; Papachristos et al., 2004; Saleem et al., 2004; Khoobdel et al., 2017). The fumigant toxicity of main monoterpenes of these essential oils, carvacrol, 1,8-cineole and thymol, was assayed against *Tenebrio molitor* (L.) (Lima et al., 2011). The toxicity of each compound was carvacrol > 1,8-cineole > thymol.

By the micro and nanotechnology approaches, a slow release formulation of essential oils can be obtained (Yang et al., 2009; Anjali et al., 2010; López et al., 2014; Ziaee et al., 2014; Pavunraj et al., 2017). Microencapsulated *Schinus molle* essential oil was studied against *Haematobia irritans* (Diptera: Muscidae) as a blood-sucking pest (López et al., 2014). Arabic gum and maltodextrin were used as carriers for the preparation of microcapsules. Their results showed that microencapsulation is a suitable method for obtaining controlled release of *S. molle* essential oil.

From the different encapsulation processes (coacervation, in-situ polymerization, melt dipersion, electrospraying…), spray-drying is the method of choice at industrial scale for the encapsulation of oils (i.e. essential oil) and other food additives (preservatives, flavors vitamins,…). In this process, generally, an aqueous solution containing the active principles for formulation is uniformly mixed with the wall materials, and this mixture is then fed into a spray dryer and atomized with a nozzle or spinning wheel. Water is evaporated by the hot air contacting the atomized material, and the powder is then collected in a cyclone separator. This technology offers different advantages such as

113 inexpensive, relatively simple and continuous operation, compared to other
114 microencapsulation techniques, and it also widely applied for drying heat-sensitive
115 materials (foods, pharmaceuticals) because of the rapid evaporation of the solvent that
116 helps to keep the particles at relatively low temperature (ca. 80 ºC) (López et al., 2014;
117 Bakry et al., 2016; Zhang et al., 2017).

118 In the case of essential oils, due to its hydrophobic nature, an o/w emulsion is formed first
119 to have an adequate dispersion in the water phase. To avoid the use of chemical
120 surfactants that may threat health, a carrier or coating material with amphiphilic character,
121 good emulsifying capacity and also film-forming properties is needed to achieve high
122 encapsulation efficiency in the spray-drying process. Among the different types of wall
123 materials (proteins, synthetic polymers, …), carbohydrate polymers (gums,
124 maltodextrins, starch, chitosan, alginate and their derivatives) have been widely
125 investigated thanks to their biocompatibility, bioavailability, biodegradability, and
126 economy. In this case, modified starches have been selected since they provide high oil
127 retention owing to their good film forming properties, long shelf-life, and high
128 manufacturing efficiency (Li, 2014). Specifically, octenyl succinic anhydride (OSA) –
129 starch materials provides good emulsification efficiency and stability of different
130 essential oils (Varona et al., 2009; Rodríguez-Rojo et al., 2012), and good retention of
131 essential oil in dry capsules by spray-drying (Baranauskienė et al., 2007; Baranauskienė
132 et al., 2016) and also by other techniques such as electrospraying (Biduski et al., 2019).

133 The present study deals with the development of a formulation of rosemary and *Zataria*
134 essential oils with controlled release property by spray drying of an oil-in-water emulsion
135 of the essential oil using octenyl succinic anhydride (OSA) - starch both as surfactant and
136 coating material. These microcapsules produced with safe and non-contaminant products
137 may be suitable for agricultural applications as a pesticide against *T. confusum*.

2. Materials and methods

2.1. Materials

R. officinalis and *Z. multiflora* essential oils were purchased from COCOPE Co. (Valladolid, Spain) and Barij Essence Co. (Kashan, Iran), respectively. These oils were produced by hydrodistillation. The oils were kept in dark glass containers at 4 °C. OSA starch (Capsule®) was kindly supplied by Ingredion (Hamburg, Germany). Trans-2-Hexen-1-al 98% was provided by Sigma–Aldrich (Madrid, Spain). All other chemicals and reagents used were of analytical grade.

2.2. Insects

The colonies of *T. confusum* were established in a growth chamber in insect physiology laboratory at the University of Tehran, at 27±3 ºC temperature and 70–75% relative humidity in the dark. The pests were reared on wheat flour mixed with yeast (10:1 w:w). Adults of *T. confusum* with same-age were used in fumigant toxicity and persistence bioassays.

2.3. Essential oil composition by Gas chromatography–mass spectrometry (GC–MS) analysis

The components of oils were determined using the GC–MS technique (7890C GC/5977A MSD Agilent Technologies, Palo Alto, CA, 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 placed in oven at 40 °C for 5 min and increased to 65 °C (5 °C/min) for 7 min, then increased to 180 °C (3 °C/min), and finally 300 °C (20 °C/min) for 1 min. MSD transfer line heater temperature was 250 °C. The volume injected was 1 μL. An electron ionization system with an ionization voltage of 70 eV was used for GC–MS detection. The components were

identified by comparison of their retention times and mass spectra with those gathered in from databases (Willey Library) of the gas chromatography–mass spectrometry system. As well, trans-2-Hexen-1-al dissolved in hexane (20 (V/V%)) as an internal standard. This solution used to quantify the amounts of components of oils by the ratio of areas. The essential oil samples, either pure essential oil or extracted essential oil from formulations, were dissolved in the internal standard solution. The concentration of rosemary and *Zataria* oils was 25 (V/V%).

2.4. Emulsion preparation

A surfactant solution was initially prepared by dispersing the OSA-starch in deionized water (Milli-Q, Millipore) at 50 ºC with the aid of a magnetic stirrer (IKA, Staufen, Germany). Afterwards, the necessary amount of oil according to the experimental plan (Table 1) was gradually added to the solutions under continuous agitation for 5 min. This solution was then fed into the rotor-stator machine (IKA® LABOR PILOT 2000/4) whose capacity is 200 mL and processed during 4 min with velocity 70 Hz for fine emulsification (Varona et al., 2009). The rotor-stator machine was cooled by ethylene glycol to avoid hot spots during emulsification.

Table 1
Experimental design of o/w emulsions prepared from rosemary essential oil.

Test*	Oil concentration (%)	Surfactant/Oil ratio
1	5	1:3
2	5	1:1
3	5	3:1
4	10	1:3
5	10	1:1
6	10	3:1
7	20	1:3
8	20	1:1

*At the highest concentration of EO (20%), the surfactant/oil ratio of 3:1 could not be tested since the surfactant concentration exceeded the aqueous solubility.

2.5. Spray drying

O/W emulsions (200 ml) were processed to produce dry microcapsules with the oil encapsulated. Drying was performed in a GEA Mobile Minor™ spray dryer model MM Basic (Düsseldorf, Germany) equipped with a rotary atomizer. The atomization pressure was maintained at 0.6 MPa and the hot air flow rate was 40 kg/h. The inlet temperature was fixed at 140 °C and the emulsion was pumped into the equipment (peristaltic pump Watson Marlow 520S) with a flow rate of 1.2 L/h to achieve an outlet temperature of 85 ºC for emulsions 1 to 8 (Table 1), according to previous works of the group concerning labile compounds (Moreno et al., 2016). The spray dried powder (discarding any particles deposited on the dryer chamber) was recovered from the cyclone, transferred into sealed plastic containers, and stored at 4 °C before analysis.

Afterwards, inlet and outlet temperatures were optimized regarding total encapsulation efficiency and concentration of main rosemary essential oil components by GC-MS. Emulsion 5 was selected due to good encapsulation results (higher concentration of oil in dried microcapsules), as it will be explained later. Emulsion flowrate was varied (0.9-1.3 L/h) to achieve the desired outlet temperature for each inlet temperature according to the experimental plan (Table 2).

Table 2
Experimental design of microcapsules prepared by different inlet and outlet temperatures from rosemary essential oil.

Test	Inlet temperature (°C)	Outlet temperature (°C)
9	120	85
10	140	85
11	160	85
12	120	81
13	120	89

205 **2.6. Characterization of microcapsules**

206 **2.6.1. Particle size analysis of emulsions and microcapsules**

207 The volume particle size distribution of emulsions and solid particles were determined by
208 laser diffraction using a Mastersizer 2000 (Malvern instrument). The solutions were
209 suspended in water whereas the solid particles were suspended in an air flow at 0.2 MPa
210 using a Sirocco unit for dry via measurements. The mean particle size of emulsions and
211 solid particles was expressed using the Sauter mean diameter, D_{32} (μm), and was
212 calculated using the equation 1, where m_i is the volume of particles and d_i is the diameter:

213 $$D_{32}=\sum m_i \cdot d_i^3 / \sum m_i \cdot d_i^2 \qquad \text{(Equation 1)}$$

214 The width of particle size distribution was characterized by the Span. The span of a
215 volume-based size distribution is defined according to equation 2, where dv (0.1), dv (0.5)
216 and dv (0.9) are the particle size diameters of 10, 50 and 90 percent of the cumulative
217 distribution curve in volume, respectively:

218 $$Span = \frac{[d(0.9)-d(0.1)]}{d(0.5)} \quad \text{(Equation 2)}$$

219 **2.6.2. Morphological analysis**

220 Appearance and size of solid microcapsules containing rosemary essential oil were
221 examined under a scanning electron microscope (SEM) (KYKY-EM3200 model, KYKY,
222 Beijing, China) using an acceleration voltage of 26.0 kV. The samples were coated with
223 gold using a sputter coater.

224 **2.6.3. Determination of encapsulation efficiency**

225 The encapsulation efficiency in the emulsions and microcapsules was determined by
226 distilling 5 g of emulsion or encapsulated powder in a Clevenger-type apparatus for 3 h.
227 The oil volume collected was multiplied by a density factor (i.e. density of the rosemary

oil 0.908 g/mL) to calculate the weight of recovered oil (Baranauskienė et al., 2007). Determination was carried out in duplicate. The encapsulation efficiency was calculated according to equations 3 and 4, respectively.

$$Emulsion\ encapsulation\ efficiency = \frac{\frac{Volume\ of\ oil\ in\ microcapsules\ (ml)}{Weight\ of\ emulsion\ (g)}}{\left(\frac{\text{Volume of initial oil (ml)}}{weight\ of\ water\ (g)+weight\ of\ starch\ (g)+weight\ of\ oil\ (g)}\right)} \quad \text{(Equation 3)}$$

$$Dry\ particle\ encapsulation\ efficiency = \frac{Concentration\ of\ oil\ in\ solid\ formulation\ (g)/\ g\ particles}{\frac{Volume\ of\ oil\ in\ microcapsules\ (ml)*Density\ of\ oil}{Weight\ of\ solid\ formulation\ (g)*\left(\frac{starch(\%)}{100}+\frac{oil\ (\%)}{100}\right)}} \times 100 \text{ (Equation 4)}$$

The total encapsulation efficiency was calculated using equation 5 taking into account the final concentration of oil in the solid formulation (i.e. microcapsules) and the mass of oil per mass of starch employed in the preparation of the initial emulsion.

$$Total\ encapsulation\ efficiency = \frac{Concentration\ of\ oil\ in\ solid\ formulation\ (g)/\ g\ particles}{\frac{Weight\ of\ initial\ oil\ (g)}{Weight\ of\ initial\ oil\ (g)+Weight\ of\ starch\ (g)}} \times 100 \text{ (Equation 5)}$$

2.6.4. Determination of drying yield

The drying yield (%) was calculated according to equation 6 as the percentage of the mass of particles recovered in the cyclone respect to the mass of solid material in the volume of emulsion processed.

$$Drying\ yield = \frac{weight\ of\ particles\ (g)}{\left(\frac{\text{Volume of emulsion (ml)}\times(weight\ of\ starch(g)+\text{weight of oil (g)})}{weight\ of\ water\ (g)+weight\ of\ starch\ (g)+weight\ of\ oil\ (g)}\right)} \times 100 \quad \text{(Equation 6)}$$

2.7. Determination of emulsion stability

The stability of the emulsions was calculated by visual determination of the de-emulsified oil after 21 and 50 days of storage at 25±2 ºC in the dark: 7 mL of the emulsion were poured in a vertical glass tube with an inner diameter of 13 mm (height of emulsion: 55 mm). The height of visible supernatant oil layer was measured, and the volume of de-

emulsified oil calculated. The percent of the supernatant oil was calculated using equation 7:

$$\%V = \frac{Vt}{Vo}100 \quad \text{(Equation 7)}$$

Where Vt is the volume of de-emulsified oil and Vo is the total volume of oil in the emulsion.

2.8. Controlled release analysis of microcapsules

For controlled release analysis, 2 g of encapsulated powders from different tests were introduced to Petri dishes (9 cm diameter) and stored at 27±3 ºC and 70–75% relative humidity using sodium chloride salt, in the dark for 15 and 30 days. After these periods, the remained oil was determined by distilling 2 g of encapsulated powder in a Clevenger-type apparatus for 3 h. The percentage of released oil was calculated as ratio of the difference between the initial oil and the remained oil respect to the initial oil amount of oil encapsulated in the powder, multiply by 100.

2.9. Determination of water activity (a_w)

The water activity of solid particles was measured using water activity meter (Rotronic probe type HC2-AW- (USB)) after calibration. Spray dried samples were kept overnight in a refrigerator (4 °C); after being allowed to come at 25±2 °C, the water activity was measured for about 5 min in a temperature stable area (Baranauskienė et al., 2007).

2.10. Fumigant toxicity

Fumigant toxicity of the oil and microcapsules was tested in plastic vials (125 mL) according to Suthisut et al. (2011) method with some modifications. Fifteen adults with the same age were introduced to each vial. Bioassays did at 27±3 ºC temperature and 70–75% relative humidity in the dark. Filter paper disks (Whatman No. 1, with 2.5 cm

diameter) were placed under the surface of the screw caps with concentrations of 115.84, 142.24, 163.28, 187.52 and 203.44 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 oils. A cloth mesh was put under the caps of the vials and placed same oil concentration from microcapsules (i.e. taking account the oil encapsulation efficiency) and then sealed with air-tight lids. Two independent bioassays were performed at different times and for each concentration and control vials, five replicates were used. Empty vials and the combination of starch particles (without the oil) were used as the controls. Mortality rate was determined after 72 h exposure time.

2.11. Persistence assays

LC_{80} values (182.32 and 251.56 µL/L for rosemary and *Zataria* essential oils, respectively) obtained from fumigant toxicity bioassay were used to determine the persistence of the oil and microcapsules. From the date of the treatment, every 5 days, 15 adults were inserted to each experimental units. Then, the mortality rate was determined 72 h after exposure (Ziaee et al., 2014). The condition for the persistence experiment was according to fumigant toxicity section. Also two independent tests with five replicates were performed.

2.12. Statistical Analysis

Percent of main compounds of rosemary oil under different inlet and outlet temperatures were statistically handled by one-way analysis of variance (ANOVA) using the Tukey test at $P<0.05$.

Mortality data was adjusted for the control unit using Abbott's formula when it was more than 5% (Abbott, 1925; Albouchi et al., 2018). The significance of mean differences between all units was compared using analysis of variance (ANOVA) using the Tukey

297 test at P<0.05 through SPSS v.16.0 software. Mortality data was checked for normality
298 (Shapiro–Wilk test). Probit analysis was used to estimate LC_{50} and LC_{80} values (lethal
299 concentration for 50 and 80% of the pest population) and its 95% confidence limits using
300 Polo-Plus 2.0 software.

301

302 **3. Results and Discussion**

303 **3.1. Main components of pure essential oils**

304 Main components of the *R. officinalis* and *Z. multiflora* essential oils used in this study
305 are shown in Tables 3 and 4, respectively. The more abundant components according to
306 GC-MS were 1,8- cineole (26.12 %) in rosemary and thymol (27.95 %) and carvacrol
307 (24.63 %) in the *Zataria*. These results are in accordance with literature studies
308 concerning the main components in rosemary (Isman et al., 2008; Ephrem et al., 2014)
309 and *Zataria* essential oil (Karimian et al., 2012; Saei-Dehkordi et al., 2010). As already
310 mentioned, the main monoterpenes present in these essential oils (carvacrol, 1,8-cineole
311 and thymol) have recognized fumigant toxicity against *Tenebrio molitor* (L.) (Lima et al.,
312 2011).

313 **Table 3**
314 Main chemical constituents of the essential oil from the *Rosmarinus officinalis* leaves.

No.	Compounds	Retention time (min)	Relative percentage
1	3-carene	10.38	13.860
2	camphene	10.92	10.024
3	2-β-Pinene	12.19	5.560
4	1,8-cineole	15.69	26.121
5	camphor	23.74	15.814
7	isobornyl acetate	31.75	3.851
8	Trans-caryophyllene	37.81	4.479

315
316
317
318
319
320
321

Table 4

Main chemical constituents of the essential oil from the *Zataria multiflora* leaves.

No.	Compounds	Retention time (min)	Relative percentage
1	3-carene	10.30	4.447
3	α-terpinene	14.53	2.529
4	p-cymene	15.18	8.759
5	γ-terpinene	17.87	8.622
6	L-linalool	21.07	2.504
8	thymol	32.57	27.958
9	carvacrol	33.08	24.637
11	carvacryl acetate	35.97	2.038
12	trans-caryophyllene	37.83	3.432

3.2. Characterization of emulsions

In the first step, the influence of the process variables (starch/oil ratio and oil concentration) on the physical properties (droplet size and droplet size distribution), stability and encapsulation efficiency of the emulsion has been studied.

Prepared emulsions have a mean droplet size expressed as Sauter mean diameter (D_{32}) between 461 and 854 nm. Narrow particle size distributions were found in all the cases as indicated by the Span value, in the range from 0.978 to 1.073. As shown in Fig. 1A, the mean droplet size decreased as the starch: oil ratio increases, as expected since the concentration of surfactant (OSA-starch) increases (Varona et al., 2009). It can be also observed that there is a minor effect of the % of oil, although higher droplet size is achieved for low EO content emulsion, whereas there are no important differences between the emulsions prepared at 10 or 20%. A table with complete data of the D_{32} and Span of all emulsions is provided in Supplementary Information, together with the droplet size distribution curve of emulsion and microparticles samples.

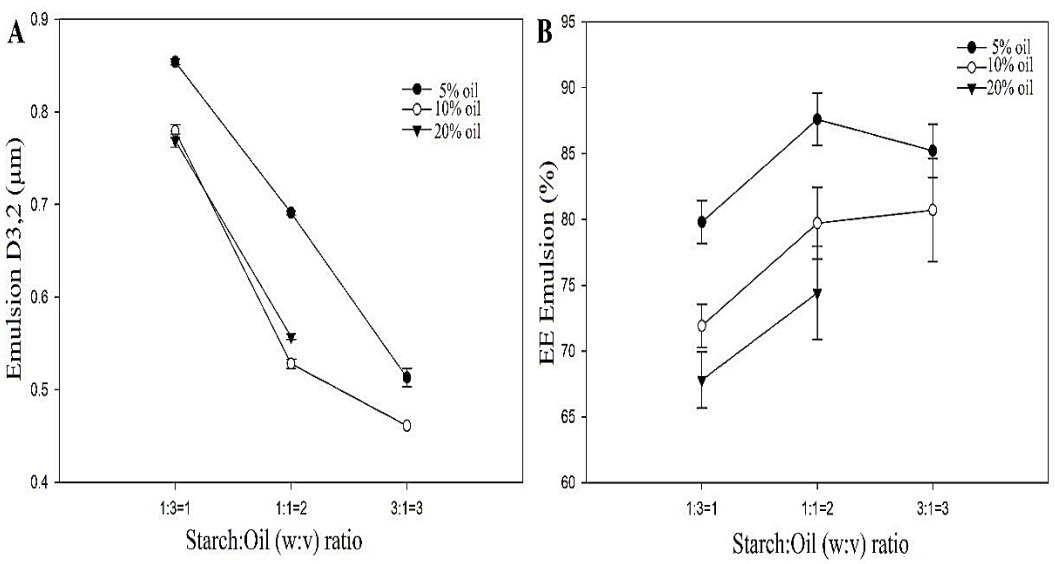

Fig. 1. Effect of starch: oil ratio and % oil (Black Circle: 5% Oil; White Circle: 10% Oil; Triangle: 20% Oil) in: A. Mean droplet size (D_{32}) of the emulsion. B. Encapsulation efficiency in the emulsions.

The encapsulation efficiency in the emulsification step (Table 5) varied from 68 to 88%. As shown in Fig.1B (Experiments 1-8), the encapsulation efficiency increases as the % oil decreases and also as the ratio of surfactant (OSA-starch): oil increases from 1: 3 to 1:1, as expected. However, no further increase is achieved for 3:1 ratio. The encapsulation efficiency decrease when the initial oil/starch ratio is increased, because the amount of oil increases and so the formed emulsion is less stable (Varona et al., 2009). The emulsifying properties of OSA-modified starch and its ability to form films at the interfaces between the emulsion phases are important factors in the emulsification efficiency (Baranauskienė et al., 2016). The reproducibility of the emulsification process regarding encapsulation efficiency is very good; emulsions from 9 to 13 are performed at the same conditions as emulsion 5, with a mean encapsulation efficiency of 79.07 ± 1.03 %.

Table 5

Properties of liquid emulsion and spray-dried microencapsulated rosemary oil at different experimental conditions.

Test	Emulsions EE (%)	Dry particles EE (%)	Total EE (%)	Concentration of oil g/g particles	Drying yield (%)	a_w
1	79.8 ± 1.6	5 ± 2	4.4 ± 0.6	0.032±0.001	12.4	0.249
2	88 ± 2	18 ± 3	14.9 ± 1.1	0.071±0.003	45.7	0.208
3	85 ± 2	45 ± 4	32.8 ± 1.6	0.076 ± 0.003	50.9	NA
4	71.9 ± 1.6	8.9±1.1	6.2 ± 0.7	0.046 ± 0.002	22.7	NA
5	80 ± 3	38 ± 3	27.4 ± 1.4	0.131 ± 0.004	41.5	0.218
6	81 ± 4	52 ± 6	30 ± 4	0.070 ± 0.002	26.9	0.248
7	68 ± 2	10.3 ± 1.2	6.7 ± 0.8	0.049 ± 0.001	25.7	0.260
8	74 ± 3	42.1 ± 1.5	26 ± 2	0.122 ± 0.003	31.5	0.252
9	77.7 ± 1.5	39.0 ± 1.8	27.6 ± 1.1	0.131 ± 0.005	51.5	0.243
10	79 ± 5	37.5 ± 1.2	27 ± 2	0.129 ± 0.003	53.5	0.221
11	79 ± 4	36.7 ± 1.5	26 ± 2	0.126 ± 0.002	43.1	0.19
12	78 ± 5	39.6 ± 1.9	28 ± 2	0.134 ± 0.006	61.5	0.253
13	80 ± 2	33.1 ± 1.75	24 ± 4	0.115 ± 0.004	58.7	0.226

EE:encapsulation efficiency; NA: not available.

Similarly, the stability of the emulsions (Table 6) was higher for low oil content emulsions (5% oil) and high surfactant: oil ratio (3:1), as expected. In this conditions 5.45% of the initial oil content was emulsified after 21 days. The maximum % of destabilized oil was achieved for the emulsions prepared at 20% oil with values between 18 – 20%. The stability of emulsion decreased when the initial oil/starch ratio is increased, because the relationship surfactant-oil decreases with increasing the amount of oil, droplet are bigger and also coalescence is more likely, producing a faster destabilization of the emulsion and so the formed emulsion is less stable (Tsech et al., 2002; Varona et al., 2009; de Paz et al., 2012). The release of microencapsulated oil controlled by the diffusion mechanism through wall of microcapsules (Baranauskienė et al., 2007). In general, no further destabilization was detected after 50 days. Nevertheless, it should be taken into account

that emulsions were dried just after preparation to get the microcapsules, before any de-stabilization took place.

Table 6
Percentage of de-emulsified oil at different experimental conditions.

Test	After preparation	After 21 days	After 50 days
1	0	9.09	10.90
2	0	9.09	9.09
3	0	5.45	5.45
4	0	12.72	14.54
5	0	12.72	14.54
6	0	12.72	12.72
7	0	20	20
8	1.16	18.18	18.18

3.3. Characterization of the microcapsules

3.3.1. Particle size and morphology of the microcapsules

The microcapsules produced after the spray drying have a mean particle size (D_{32}) between 8.29 and 11.35 µm with narrow particle size distribution, i.e. span values slightly above 1. Although it shows the expected tendency of higher mean particle size and span with increasing oil content and higher surfactant concentration. Detailed information can be found in Table S1, Supplementary Information. Also, Fig. S1.B includes the particle size distribution of the dried powder. To verify the morphology of the microcapsules, SEM was used to obtain information about shape, surface, and diameter of microcapsules. SEM photomicrograph of essential oil-loaded microcapsules reveals the presence of oval and spherical microcapsules with irregular surfaces (Fig. 2). The particles show a sphere-like shape as it is commonly observed for spray-dried products (Zhang et al., 2017). Further, they do not show cracks nor breaks which is an advantageous characteristic for oil protection thanks to the film forming ability of OSA-starch (Li, 2014).

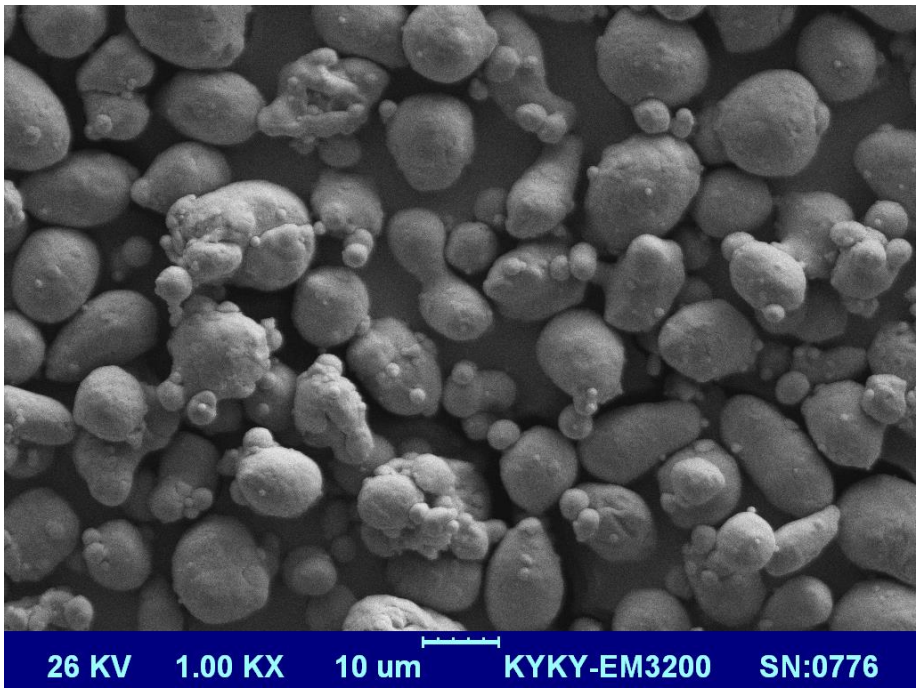

Fig. 2. SEM photomicrograph of rosemary essential oil-loaded microcapsules.

3.3.2. Encapsulation efficiency: Effect of surfactant: oil ratio and % oil

The influence of the emulsion process variables (starch/oil ratio and oil concentration; Experiments 1-8) in the microcapsules in terms of final content of oil in the particles, encapsulation efficiency of the drying step, total encapsulation efficiency was assessed. Also, the yield for the drying process and the water activity of the micro particulate powder were determined. The effect of the inlet and outlet temperature in this parameters was also studied (Experiments 9 – 13). All these data are compiled in Table 5.

As it is shown in Table 5, the total encapsulation increases as the surfactant: oil ratio increases; although differently as happen with the emulsion encapsulation efficiency where the maximum encapsulation efficiency was achieved for 1:1 ratio, there is a clear improvement when increasing from 1:1 to 3:1; and this tendency is equivalent for the encapsulation efficiency exclusively due to the drying process. This can be due to reduction of the volatilization of the essential oil by the high temperature that the higher amount of carrier (i.e. surfactant) provides (Varona et al., 2009; Turasan et al., 2015). The

encapsulation efficiency values decreased as the viscosity of the wall material decreased (Turasan et al., 2015). The microcapsules are produced in a two-step process, emulsification followed by spray drying, being this second process the one that influences more the global efficiency since it has lower efficiencies (below 45%) due to the volatility of the essential oils and temperatures of the process. Nevertheless, it is the most implemented in the industry.

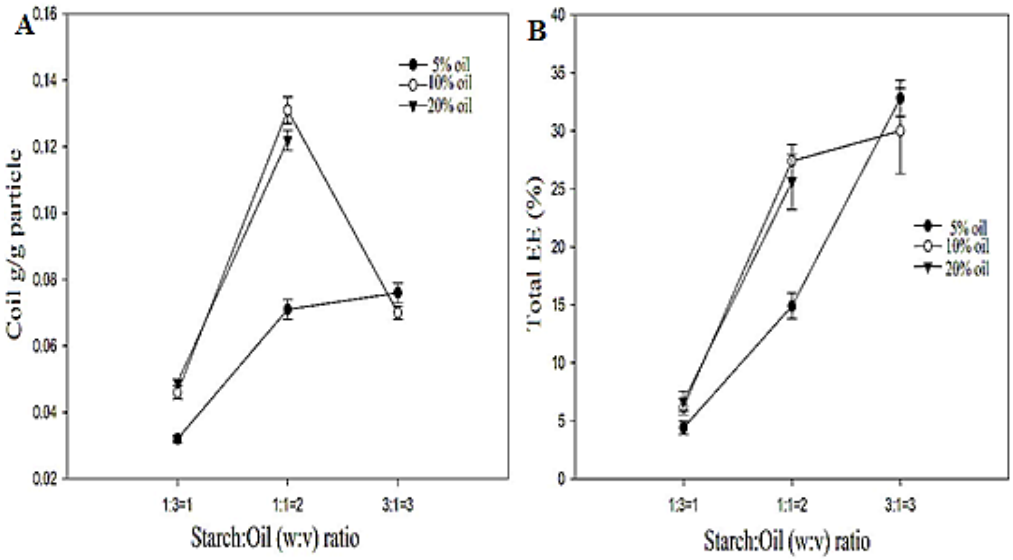

Fig. 3. Effect of starch: oil ratio and % oil (Black Circle: 5% Oil; White Circle: 10% Oil; Triangle: 20% Oil) in: A. Final concentration of oil in the dry particles. B. Total encapsulation efficiency (EE) of essential oil in dry particles.

Regarding the effect of the % oil (Fig. 3B), the Total EE% increases from 5 to 10% of oil, but no further increase is achieved for 20% oil. This trend is opposite to that of the EE of the emulsification process, probably due to the higher volatilization of the EO for these experimental conditions at which the microcapsules particle size are smaller, hence having a higher surface area (Kim & Morr, 1996; Baranauskienė et al., 2007).

Having a look to the concentration of oil in the microcapsules, the trend regarding the % oil is similar to Total EE%, being higher for the 10% oil. However, although it increases from 1:3 to 1:1 respect to the surfactant: oil ratio, a decrease in concentration is produced at 3:1 ratio, despite the increase in the Total EE%, due to a dilution effect in the matrix

of the microcapsule (i.e. there is more carrier or surfactant). The highest concentration achieved was 0.131 g/g at 10% oil and 1:1 ratio that corresponds to experiment #5. Regarding the effect of drying temperatures, the conclusion is the same at that extracted for the %EE of the drying process.

3.3.3. Encapsulation efficiency: Effect of inlet and outlet temperature

From the study of the influence of the inlet and outlet temperature in the spray-drying in inlet temperatures above 140 °C (Exp#11) and outlet temperature above 85 °C (Exp.#13) reduce the %EE of the drying step, being more significant the effect of the outlet temperature. The effect was also studied in the composition of the oil in the microcapsule in comparison with the percentage of the main components in the non-formulated oil (Table 7). The composition of 3-carene and camphene was similar, while a reduction in the relative percentage was found for camphor (>1%) and namely, for 1,8- cineole, with a decrease in almost 4 % (20% of the total initial 1,8-cineole). The significant differences ($p<0.05$) between non-formulated and formulated essential oils have been also shown in literature (Baranauskienė et al., 2007). The lower encapsulation efficiency of some compounds in the essential oil by spray drying is related to different factors, namely higher volatility and/ or high aqueous solubility (Soottitantawat et al., 2003; Baranauskienė et al., 2007). In this case, the aqueous solubility is pointed out as a possible reason since the value for 1,8- cineole and camphor is two orders of magnitude higher than that of 3-carene and camphene (https://comptox.epa.gov/dashboard/). Also, the molecular dimensions might lead to the loss of compounds because they are related to the molecules diffusion directly (Baranauskienė et al., 2007).

After preparation of microencapsulated rosemary oil under different inlet and outlet temperatures for spray drying, the relative percentage of main compounds were analyzed. The main compounds of pure and encapsulated rosemary oil were similar; however, some

changes in the percentages of 1,8- cineole, camphor, 3-carene, camphene were observed (Table 7) and there are significant differences ($P<0.05$).

Table 7
Percent of main compounds of rosemary oil under different inlet and outlet temperatures.

Compounds	Non-formulated oil	Test 9	Test 10	Test 11	Test 12	Test 13	F ve P value
3-Carene	15.73±0.03^{d}	16.44±0.02^{a}	16.28±0.02^{b}	15.92±0.02^{c}	15.87±0.01^{cd}	15.57±0.02^{e}	$F_{5,17}$=86, P<0.05
Camphene	10.02±0.02^{d}	10.63±0.02^{a}	10.56±0.01^{a}	10.39±0.03^{b}	10.36±0.02^{b}	10.25±0.02^{c}	$F_{5,17}$=136.07, P<0.05
1,8-Cineole	26.12±0.05^{a}	21.92±0.04^{d}	21.59±0.03^{e}	22.50±0.03^{b}	22.19±0.02^{c}	21.11±0.05^{f}	$F_{5,17}$=1.18, P<0.05
Camphor	15.81±0.03^{a}	14.22±0.02^{b}	13.75±0.02^{c}	14.26±0.01^{b}	14.22±0.01^{b}	14.25±0.02^{b}	$F_{5,17}$=335.69, P<0.05

For letters a-f, values within rows followed by the same letter do not differ statistically at P =0.05.

3.3.4. Water activity of the microcapsules

The water activity value (Table 5, Experiments 1-8) was low with values in the range from 0.19 to 0.26. No important effect was noticed for the effect of inlet and outlet temperatures in the drying step, only a reduction was found for the highest inlet temperature (Exp. #11) but at the cost of reducing encapsulation efficiency due to higher volatilization of EO. Water activity of microcapsules is an important factor related to preservation of microcapsules of essential oil. Baranauskiene et al. (2007) concluded that loss of peppermint essential oil volatiles microencapsulated into different types of OSA-starch during storage was faster at high a_w level (0.43 to 0.75). At this high values, the matrix starts to plasticize resulting in higher mobility of flavor molecules and hence, the increase of release rates. In this work, no effect of the water activity in the release rate was found since the values were low and close in all samples (0.190-0.260) and also, the increase after storage was similar and below 0.43, in the range from 0.302 to 0.368 (data not shown).

3.3.5. Drying yield of the microcapsules

The drying yield varies from 12.4 to 50.9% (Experiments 1–8). In general it increases with the increase in surfactant: oil ratio as the solid proportion of the emulsion increases, making the particles formation easier. Also, an increase is detected from 5 to 10% oil, but if the amount of oil in the initial emulsion is too high (20%) it is reduced. The effect of the temperature is not clear (Experiments 9-13), being in all the case about 50%. Nevertheless, it should be mentioned that relative small batches were processed and only the powder in the cyclone was recovered. This variable was recorded to have a global reference of the performance of the process, having in mind that a drying yield of 50% is taken to be the benchmark for successful spray drying (Moreno et al., 2016). During the drying process, remaining of particles on the dryer chamber wall (Bhandari et al., 1997) and reduction of surfactant (Jayasundera et al., 2011; Fang & Bhandari, 2012) may reduce powder recovery.

Finally, it can be said that the reproducibility of the process in very good as it can be concluded from Exp.#5 and Exp. #10 from Table 5. Main variation is registered for the drying yield, but as mentioned previously, it can be due to the fact that relatively small batches are processed (ca. 200 mL of emulsion) and that only particles in the cyclone are recovered.

3.3.6. Release rate of the microcapsules

The release rate of the encapsulated EO was also evaluated as an important parameter regarding the fumigant activity, as a controlled release is aimed to prolong the effect in time with respect to non-encapsulated EO. The results of release oil after 15 and 30 days (Table 8) show that the release is mainly governed by surfactant: oil ratio. The release is lower for the high surfactant: oil ratio, as expected since the amount of carrier around the

oil is higher reducing its volatilization. The effect of % oil is smaller, although an increase in the release ratio with this parameter is detected, mainly for the 20% oil formulations. The release is faster in the first 15 days with a % increase in the release between 20-30% of the initial release oil after 30 days, except for the cases where the maximal release (90%) is achieved. These results are similar to Varona et al. (2009) and Turasan et al. (2015) results. They demonstrated the increase in oil: surfactant ratio and % oil increased the release ratio. The release rate is influenced by wall material and the nature of the encapsulated essential oil. Pascual-Villalobos and López (2013) showed that alginate microcapsules released completely the linalool at 25 ˚C after 72 h while starch capsules released only 30% of the compound. Regarding composition, it has been shown (Sanna Passino et al., 2004) that rosemary essential oil released faster than thyme essential oil (ca. 75% versus 25% after 25 days for gelatin capsules) due to the higher amount of the higher hydrocarbon fraction of the former and its lower amount of phenolic polar compounds in comparison with thyme EO. Another parameter than can influence the release rate is the water activity since the water uptake at the relative high humidity conditions of the test, can destroy the capsule structure (Rosenberg et al., 1990).

Also, in the experiments with low oil concentrations and oil/starch ratio, the release rate of oil was lower than 40 and 49%, but when the concentration of oil and oil/starch ratio were higher, as much as 80 and 90% of the encapsulated oil was released after 15 days and 30 days, respectively (Table 8).

Table 8

The percent of released oil (Mean ± SE*) of spray-dried microencapsulated rosemary oil after 15 and 30 days.

Test**	Surfactant/Oil ratio	Oil concentration (%)	After 15 days	After 30 days
1	1:3	5	65.27±4.32^{b}	90.07±0.99^{a}
2	1:1	5	55.09±1.66^{bc}	70.18±1.16^{c}
3	3:1	5	39.85±2.04^{d}	48.89±3.07^{d}
4	1:3	10	68.04±2.17^{ab}	84.33±1.65^{ab}
5	1:1	10	62.38±1.44^{b}	77.14±2.47^{abc}
6	3:1	10	42.36±2.62^{cd}	54.16±1.44^{d}
7	1:3	20	80.04±1.55^{a}	90.60±2.55^{a}
8	1:1	20	65.15±1.47^{b}	83.52±4.59^{abc}
9			56.08±1.24^{bc}	72.45±5.19^{bc}
10			56.21±2.44^{bc}	70.10±2.45^{c}
11	1:1	10	63.85±2.50^{b}	81.14±1.46^{abc}
12			67.59±4.59^{ab}	86.78±2.54^{a}
13			60.49±4.66^{b}	77.82±0.73^{abc}
F ve P value			$F_{12,38}$=14.74, P<0.05	$F_{12,38}$=21.93, P<0.05

*For letters a-d, values within columns followed by the same letter do not differ statistically at P =0.05.

3.4. Formulation of *Zataria* essential oil

The formulation of *Zataria* EO was carried out at the conditions that provided the highest amount of oil in the solid microcapsules for the rosemary essential oil. Therefore, emulsions were prepared according to Test 5 conditions (10% EO and 1:1 surfactant: oil ratio). Emulsions of *Zataria* EO had a mean droplet size of 489±0.007 and 1.017±0.006 nm for the D_{32} and Span respectively, slightly smaller than that of rosemary EO at the same conditions. The encapsulation efficiency of *Zataria* emulsion was 75.5%, slightly lower than that of rosemary.

The drying was carried out at the conditions of Test 12 (120 ºC as inlet temperature and 81 ºC as outlet temperature). The microcapsules had a mean particle diameters 10.38±0.40 and 4.67±0.468 µm for the D_{32} and Span respectively, also in the same range

539 as those of rosemary EO. The encapsulation efficiency of *Zataria* emulsion in the dry
540 particles (%), Total EE (%), Drying yield (%), and Concentration of oil g/g particles were
541 were 39.1 %, 26.9 %, 59.9 %, and 0.128 g/g, respectively. The a_w value was 0.211.
542 Finally, the release rate (%) of the encapsulated *Zataria* EO was also 65.73 after 15 days.
543 All the values were similar to those provided by the formulation of rosemary essential oil
544 at the same conditions, as expected.

545 **3.5. Fumigant toxicity and persistence of oil and microcapsules**

546 Fumigant toxicity and persistence assays were performed with rosemary and *Zataria* EOs
547 microcapsules prepared according to Test 12. These experimental conditions were
548 selected as they provided the highest EO concentration in the microcapsules.

549 Mean mortality percentage of *T. confusum* exposed to the rosemary and *Zataria* essential
550 oils and their microcapsules are presented in Fig. 4. In all cases, mortality percentage of
551 *T. confusum* was significantly higher ($P<0.05$) when exposed to non-formulated than
552 encapsulated oils after 72 h of the exposure period for the rosemary (ANOVA,
553 $F_{9,99} = 111.55$, $P < 0.05$) and *Zataria* (ANOVA, $F_{9,99} = 60.53$, $P < 0.05$). This was an
554 expected results, since the air concentration of volatiles is lower in encapsulated oil due
555 to the controlled release of the oil. Also, the mortality showed a linear increased in both
556 cases with the concentration of EO. The LC_{50} values were 122.8 and 216.1 µL/L for pure
557 and microcapsules of rosemary with adults, respectively. Also, the LC_{50} values were
558 178.4 and 274.2 µL/L for pure and microcapsules of *Zataria* with adults, respectively
559 (Ahsaei et al., unpublished data).

560 The toxicity and repellency of rosemary oil against *T. confusum* has been previously
561 examined in literature by Saeidi and Moharramipour (2013). They provided a LC_{50} value
562 of 22.14 µL rosemary oil /L air after 24 hours of treatment. The difference with the value

reported in this work can be related difference in essential oil composition due to climate conditions (heat, photoperiod, and humidity), plant species and soil acidity that can affect the secondary metabolism of the plant (Müller-Riebau et al., 1997; Regnault-Roger et al., 2012). These variabilities have important consequences on the biological activity of different essential oils (Regnault-Roger et al., 2012).

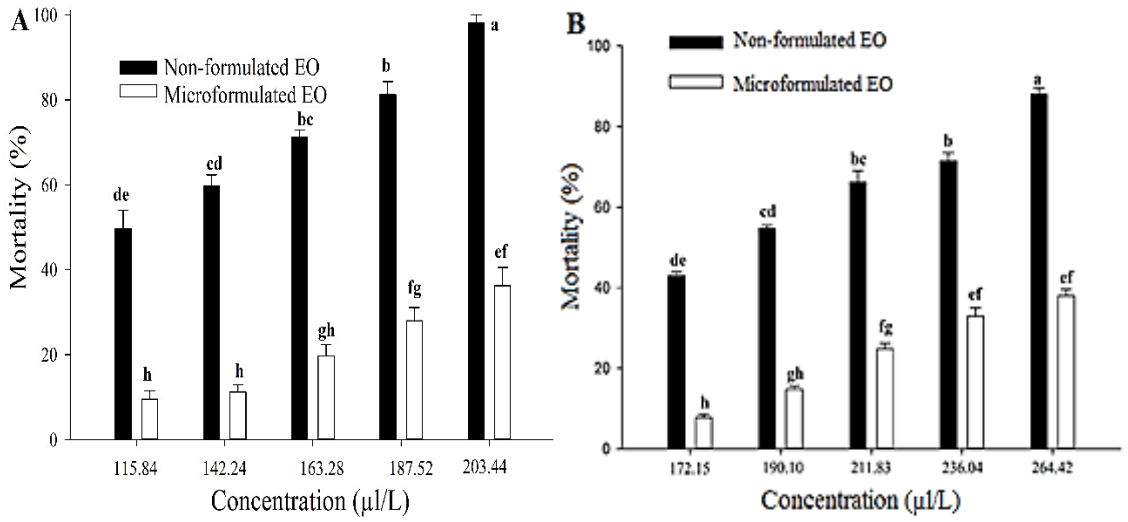

Fig. 4. Fumigant toxicity (Mean±SE) of different concentrations of the non-formulated and microencapsulated A) Rosemary B) *Zataria* oil after 72 h exposure in *Tribolium confusum* adults. Means followed by the same letter in each graph are not significantly different using Tukey's test at $p<0.05$.

The results of this work for toxicity of microcapsules are in agreement with Werdin González et al. (2014) and Sanna Passino et al. (2004) results. These results show that microencapsulation can provide a formulation of two essential oils with control the release of effective ingredients and protect them against evaporation. Werdin González et al. (2014) prepared and characterized poly(ethylene glycol) (PEG) nanoparticles containing geranium and bergamot essential oils and evaluated different biological assays against adults of *Rhizopertha dominica* and *T. castaneum* compared with the essential oils alone. In the evaluation of fumigant activity, geranium and bergamot essential oils produced 100% mortality after 24 h exposure whiles the nanoparticles of these essential oils did not have any effects after 120 h exposure. They suggested that the

583 nanoformulation reduces volatility from the essential oils and release is slower than non-
584 formulated essential oils. Their results demonstrated that the nanoformulation enhanced
585 the essential oil contact activity after 72 h exposure. Sanna Passino et al. (2004) evaluated
586 insecticidal effects of microencapsulated *R. officinalis* and *T. vulgaris* essential oils
587 against *P. interpunctella* larvae. The toxicity of the oil was observed after diet
588 contamination with the microcapsules and vapors exposition. LC_{50} values were 1.3 and
589 2.1 mg/g for *Thymus* and *Rosmarinus* microcapsules with I-II instar larvae, respectively;
590 the corresponding values obtained were 83.5 mg/g and 141 mg/g with III-IV instar larvae,
591 respectively. By increasing the microcapsules concentration in the diet, mortality was
592 increased in both treatments. The microcapsules of oils had a different release pattern.

593 The persistence of rosemary and *Zataria* non-formulated essential oil and
594 microencapsulated oils in over time is presented in Fig. 5. The effectiveness of non-
595 formulated essential oils decreased with increasing the storage time, whereas this
596 effectiveness for microencapsulated oils increased in the same condition in *T. confusum*
597 adults. After 5 days of the storage period, non-formulated oils caused 44% mortality for
598 rosemary and 24% mortality for *Zataria*, while the mortality rate reached 0% when oils
599 were stored for 15 days. In contrast, for rosemary microencapsulated oil, the rate of
600 mortality reached 11.11, 46.66, and 77.77% after 5, 15 and 40 days of the storage period,
601 respectively (ANOVA, $F_{15,79} = 13.97$, $P < 0.05$). Also, for *Zataria* microencapsulated oil,
602 the mortality rate reached 8.88, 35.55, and 75.55% after 5, 15 and 40 days of the storage
603 period, respectively (ANOVA, $F_{15,79} = 18.94$, $P < 0.05$) (Fig. 5).

604 López et al. (2014) investigated the insecticidal activity of microencapsulated *S. molle*
605 essential oil against *H. irritans* (Dip.: Muscidae). They applied gum arabic and
606 maltodextrin as carriers for the preparation of microcapsules with different ratio of this
607 essential oil. Insecticidal activity for microcapsules was 32 and 73% of dead flies at 2 and

4 h of exposure time, whereas free oil caused 96% of dead flies at 2 h. Their results showed that microencapsulation provides a formulation of essential oils for obtaining controlled release of active substances. In the same way, insecticidal activity of *Cuminum cyminum* oil indicated that non-formulated oil completely lost its impact after 12 days against *T. confusum*, whereas at the same period, the oil-loaded nanogels lost about 15 % of its activity (Ziaee et al., 2014). The *Thymus* microcapsules caused the death of ≃ 25% of the treated insects after 25 days while *Rosmarinus* microcapsules had 75% mortality in the same time due to the faster release of this oil (Sanna Passino et al., 2004).

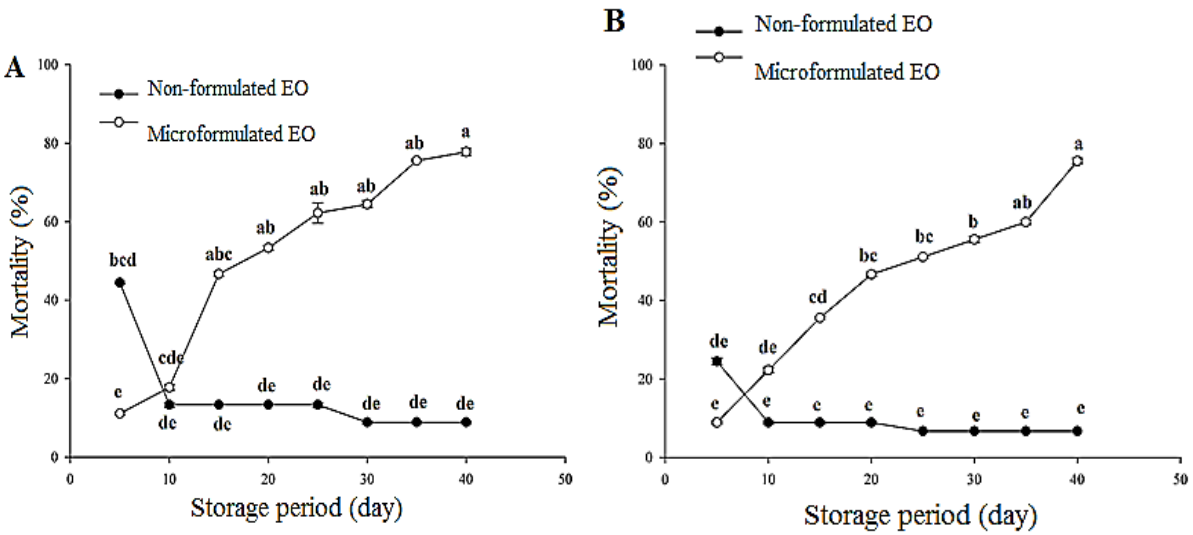

Fig. 5. Persistence (Mean±SE) of A) rosemary and B) *Zataria* non-formulated and microencapsulated essential oils after 72 h exposure in *Tribolium confusum* adults. Means followed by the same letter in each graph are not significantly different using Tukey's test at $p<0.05$.

4. Conclusion

The advance in the applications of essential oils for agrochemical use requires the development of adequate formulations that avoids their degradation and provide a correct release. In the present study, a formulation of rosemary and *Zataria* essential oils was prepared by spray drying of an oil-in-water emulsion of the essential oil using octenyl succinic anhydride (OSA) - starch both as surfactant and coating material for control of stored product pest. The encapsulation efficiency of depended directly on the surfactant: oil ratio, and the highest values was achieved was 32.8%. The maximum concentration

of encapsulated oil was 0.134 g oil/ g particle. The LC_{50} values were 122.8 and 216.1 µL/L for pure and microcapsules of rosemary with adults, respectively. Also, The LC_{50} values were 178.4 and 274.2 µL/L for pure and microcapsules of *Zataria* with adults, respectively. The *Zataria* microcapsules caused the death of ca. 35% of the treated insects after 15 days while *Rosmarinus* microcapsules had 46% mortality in the same time. The non-formulated oils had ca. 10% or less mortality in the treated insects after 25 days. Controlled release formulations can provide optimized release of amount of pesticides to maximize their biological activity for a longer time.

Author contributions

SMA, SRR and KTJ designed the research. SMA and MS conducted the experimental work. SMA and SRR performed the critical analysis of data and writing. All authors revised and approved the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

The authors acknowledge Junta de Castilla y León (Spain) with Project VA040U16 and University of Teheran (Iran) for funding. Seyed Mohammad Ahsaei thanks the Ministry of Science, Research and Technology of the Islamic Republic of Iran and Iran Nanotechnology Innovation Council (Project 97699) for his mobility scholarship. Soraya Rodríguez Rojo acknowledges Junta de Castilla y León and FEDER 2014-2020 for her postdoctoral contract under Project VA040U16. The authors express their special thanks

651 to Dr. Cristina Andrés and Reinaldo Vallejo for assistance with laboratory experiments
652 and data analysis.

653

654 **References**

655 Abbott, W.S., 1925. A method for computing the effectiveness of an insecticide. J. Econ.
656 Entomol. 18, 265–267. http://dx.doi.org/10.1093/jee/18.2.265a.

657 Albouchi, F., Ghazouani, N., Souissi, R., Abderrabba, M., Boukhris-Bouhachem, S.,
658 2018. Aphidicidal activities of *Melaleuca styphelioides* Sm. essential oils on three citrus
659 aphids: *Aphis gossypii* Glover*; Aphis spiraecola* Patch and *Myzus persicae* (Sulzer). S.
660 Afr. J. Bot. 117, 149–154. https://doi.org/10.1016/j.sajb.2018.05.005.

661 Ali, A., Ahmad, F., Biondi, A., Wang, Y., Desneux, N., 2012. Potential for using *Datura*
662 *alba* leaf extracts against two major stored grain pests, the khapra beetle *Trogoderma*
663 *granarium* and the rice weevil *Sitophillus oryzae*. J. Pest Sci. 85(3), 359-366.
664 https://doi.org/85, 359– 366. 10.1016/j.sajb.2018.05.005.

665 Alves, M.D.S., Campos, I.M., de Brito, D.D.M.C., Cardoso, C.M., Pontes, E.G., de
666 Souza, M.A.A., 2019. Efficacy of lemongrass essential oil and citral in controlling
667 *Callosobruchus maculatus* (Coleoptera: Chrysomelidae), a post-harvest cowpea insect
668 pest. Crop Prot. 119, 191-196. https://doi.org/10.1016/j.cropro.2019.02.007.

669 Anjali, C.H., Sudheer, K.S., Margulis-Goshen, K., Magdassi, S., Mukherjee, A.,
670 Chandrasekaran, N., 2010. Formulation of water-dispersible nanopermethrin for
671 larvicidal applications. Ecotoxicol. Environ. Saf. 73, 1932–1936.
672 https://doi.org/10.1016/j.ecoenv.2010.08.039.

673 Bakry, A.M., Abbas, S., Ali, B., Majeed, H., Abouelwafa, M.Y., Mousa, A., Liang, L.,
674 2016. Microencapsulation of oils: A comprehensive review of benefits, techniques, and
675 applications. Comp. Rev. Food Sci. Food Saf. 15, 143-182. https://doi.org/10.1111/1541-
676 4337.12179.

677 Baranauskienė, R., Bylaite, E., Zukauskaite, J., Venskutonis, R.P., 2007. Flavor retention
678 of peppermint (*Mentha piperita* L.) essential oil spray-dried in modified starches during
679 encapsulation and storage. J. Agric. Food Chem. 55, 3027-3036.
680 https://doi.org/10.1021/jf062508c.

681 Baranauskienė, R., Rutkaitė, R., Pečiulytė, L., Kazernavičiūtėa, R., Venskutonis, P.R.,
682 2016. Preparation and characterization of single and dual propylene oxide and octenyl
683 succinic anhydride modified starch carriers for the microencapsulation of essential oils.
684 Food Funct. 7, 3555. https://doi.org/10.1039/c6fo00775a.

685 Bhandari, B.R., Datta, N., Howes, T., 1997. Problems associated with spray drying of
686 sugar-rich foods. Dry. Technol. 15, 671-684.
687 https://doi.org/10.1080/07373939708917253.

688 Biduski, B., Kringel, D.H., Colussi, R., Hackbart, H.C.D.S., Lim, L.T., Dias, A.R.G.,
689 Zavareze, E.D.R., 2019. Electrosprayed octenyl succinic anhydride starch capsules for

690 rosemary essential oil encapsulation. Int. J. Biol. Macromol. 132, 300-307.
691 https://doi.org/10.1016/j.ijbiomac.2019.03.203.

692 de Paz, E., Martín, Á., Estrella, A., Rodríguez-Rojo, S., Matias, A.A., Duarte, C.M.M.,
693 Cocero, M.J., 2012. Formulation of b-carotene by precipitation from pressurized ethyl
694 acetate-on-water emulsions for application as natural colorant. Food Hydrocoll. 26, 17-
695 27. https://doi.org/10.1016/j.foodhyd.2011.02.031.

696 Ephrem, E., Greige-Gerges, H., Fessi, H., Charcosset, C., 2014. Optimisation of rosemary
697 oil encapsulation in polycaprolactone and scale-up of the process. J. Microencapsul.
698 31(8), 746–753. https://doi.org/10.3109/02652048.2014.918669.

699 Fang, Z., Bhandari, B., 2012. Comparing the efficiency of protein and maltodextrin on
700 spray drying of bayberry juice. Food Res. Int. 48, 478-483.
701 https://doi.org/10.1016/j.foodres.2012.05.025.

702 Isman, M.B., Wilson, J.A., Bradbury, R., 2008. Insecticidal activities of commercial
703 rosemary oils (*Rosmarinus officinalis*) against larvae of *Pseudaletia unipuncta* and
704 *Trichoplusia ni* in relation to their chemical compositions. Pharm. Biol. 46(1-2), 82-87.
705 https://doi.org/10.1080/13880200701734661.

706 Jayasundera, M., Adhikari, B., Adhikari, R., Aldred, P., 2011. The effect of protein types
707 and low molecular weight surfactants on spray drying of sugar-rich foods. Food
708 Hydrocoll. 25, 459-469. https://doi.org/10.1016/j.foodhyd.2010.07.021.

709 Karimian, P., Kavoosi, G., Saharkhiz, M.J., 2012. Antioxidant, nitric oxide scavenging
710 and malondialdehyde scavenging activities of essential oil from different chemotypes of
711 *Zataria multiflora*. Nat. Prod. Res. 26, 2144–2147.
712 https://doi.org/10.1080/14786419.2011.631136.

713 Kim, Y.D., Morr, C.V., 1996. Microencapsulation properties of gum arabic and several
714 food proteins: Spray-dried orange oil emulsion particles. J. Agric. Food Chem. 44, 1314-
715 1320. https://doi.org/10.1021/jf950391e.

716 Khoobdel, M., Ahsaei, S.M., Farzaneh, M., 2017. Insecticidal activity of
717 polycaprolactone nanocapsules loaded with *Rosmarinus officinalis* essential oil in
718 *Tribolium castaneum* (Herbst). Entomol. Res. 47, 175–184. https://doi.org/10.1111/1748-
719 5967.12212.

720 Kljajić, P., Kavallieratos, N.G., Athanassiou, C.G., Andrić, G. Is combining different
721 grain protectants a solution to problems caused by resistant populations of stored-product
722 insects? Arthur, F.H; Kengkanpanich , R.; Chayaprasert, W.; Suthisut, D. (Eds.)
723 Proceedings of the 11th International Working Conference on Stored Product Protection
724 24-28 November 2014 Chiang Mai, Thailand.

725 Lee, B.H., Lee, S.E., Annis, P.C., Pratt, S.J., Park, B., Tumaalii, F., 2002. Fumigant
726 toxicity of essential oils and monoterpenes against the red flour beetle, *Tribolium*
727 *castaneum* Herbst. J. Asia-Pac. Entomol. 5(2), 237-240. https://doi.org/10.1016/S1226-
728 8615(08)60158-2.

729 Li, J.Z., 2014. The Use of Starch-Based Materials for Microencapsulation, In: Gaonkar,
730 A.G., Vasisht, N., Khare, A.R., Sobel, R. (Eds.), Microencapsulation in the Food
731 Industry, A practical implementation guide. Academic Press, Cambridge, pp. 195-210.

Lima, R.K., Cardoso, M.D.G., Moraes, J.C., Carvalho, S.M., Rodrigues, V.G., Guimarães, L.G.L., 2011. Chemical composition and fumigant effect of essentialoil of *Lippia sidoides* Cham. and monoterpenes against *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae). Ciênc. Agrotec. 35(4), 664-671. http://dx.doi.org/10.1590/S1413-70542011000400004.

López, A., Castro, S., Andina, M.J., Ures, X., Munguía, B., Llabot, J.M., Elder, H., Dellacassa, E., Palma, S., Domínguez, L., 2014. Insecticidal activity of microencapsulated *Schinus molle* essential oil. Ind. Crop Prod. 53, 209-216. https://doi.org/10.1016/j.indcrop.2013.12.038.

Martin, A., Varona, S., Navarrete, A., Cocero, M.J., 2010. Encapsulation and coprecipitation processes with supercritical fluids: applications with essential oil. Open Chem. Eng. J. 4, 31–41. https://doi.org/10.2174/1874123101004010031.

Moreno, T., de Paz, E., Navarro, I., Rodríguez-Rojo, S., Matías, A., Duarte, C., Sanz-Buenhombre, M., Cocero, M.J., 2016. Spray drying formulation of polyphenols-rich grape marc extract: evaluation of operating conditions and different natural carriers. Food Bioprocess Technol. 9, 2046. https://doi.org/10.1007/s11947-016-1792-0.

Müller-Riebau, F.J., Berger, B.M., Yegen, O., Cakir, C., 1997. Seasonal variations in the chemical compositions of essential oils of selected aromatic plants growing wild in Turkey. J. Agric. Food Chem. 45, 4821–4825. https://doi.org/10.1021/jf970110y.

Papachristos, D.P., Karamanoli, K.I., Stamopoulos, D.C., Menkissoglu-Spiroudi, U., 2004. The relationship between the chemical composition of three essential oils and their insecticidal activity against *Acanthoscelides obtectus* (Say). Pest Manag. Sci. 60, 514–520. https://doi.org/10.1002/ps.798.

Pascual-Villalobos, M.J., López, M.D., 2013. New application of guayule resin in controlled release formulations. Ind. Crop Prod. 43, 44–49. https://doi.org/10.1016/j.indcrop.2012.07.001.

Pavunraj, M., Baskar, K., Duraipandiyan, V., Al-Dhabi, N.A., Rajendran, V., Benelli, G., 2017. Toxicity of Ag nanoparticles synthesized using stearic acid from *Catharanthus roseus* leaf extract against *Earias vittella* and mosquito vectors (*Culex quinquefasciatus* and *Aedes aegypti*). J. Cluster Sci. 28(2), 1-16. https://doi.org/10.1007/s10876-017-1235-8.

Rajkumar, V., Gunasekarana, C., Christy, I.K., Dharmaraj, J., Chinnaraj, P., Paul, C.A., 2019. Toxicity, antifeedant and biochemical efficacy of *Mentha piperita* L. essential oil and their major constituents against stored grain pest. Pestic. Biochem. Physiol. 156, 138-144. https://doi.org/10.1016/j.pestbp.2019.02.016.

Rees, D., 2007. Insects of stored grain: a pocket reference. Csiro Publishing, Brooklyn.

Regnault-Roger, C., Vincent, Ch., Thor Arnason, J., 2012. Essential oils in insect control: Low-risk products in a high-stakes world. Annu. Rev. Entomol. 57, 405–424. https://doi.org/10.1146/annurev-ento-120710-100554.

Reyes, E.I.M., Farias, E.S., Silva, E.M.P., Filomeno, C.A., Plata, M.A.B., Picanço, M.C., Barbosa, L.C.A., 2019. *Eucalyptus resinifera* essential oils have fumigant and repellent action against *Hypothenemus hampei*. Crop Prot. 116, 49-55. https://doi.org/10.1016/j.cropro.2018.09.018

Rodríguez-Rojo, S., Varona, S., Núñez, M., Cocero, M.J., 2012. Characterization of rosemary essential oil for biodegradable emulsions. Ind. Crop Prod. 37(1), 137-140. https://doi.org/10.1016/j.indcrop.2011.11.026.

Rosenberg, M., Kopelman, I.J., Talmon, Y., 1990. Factors affecting retention in spray-drying microencapsulation of volatile materials. J. Agric. Food Chem. 38, 1288-1294. https://doi.org/10.1021/jf00095a030.

Saei-Dehkordi, S.S., Tajik, H., Moradi, M., Khalighi-Sigaroodi, F., 2010. Chemical composition of essential oils in *Zataria multiflora* Boiss from different parts of Iran and their radical scavenging and antimicrobial activity. Food Chem. Toxicol. 48, 1562–1567. https://doi.org/10.1016/j.fct.2010.03.025.

Saeidi, M., Moharramipour, S., 2013. Insecticidal and repellent activities of *Artemisia khorassanica, Rosmarinus officinalis* and *Mentha longifolia* essential oils on *Tribolium confusum*. J. Crop Prot. 2 (1), 23-31.

Saleem, M., Nazli, R., Afza, N., Sami, A., Ali, M.S., 2004. Biological significance of essential oil of *Zataria multiflora* boiss. Nat. Prod. Res. 18(6), 493-497. https://doi.org/10.1080/14786410310001608064.

Sanna Passino, G., Bazzoni, E., Moretti, M.D.L., 2004. Microencapsulated essential oils active against indianmeal moth. Bol. San. Veg. Plagas. 30, 125-132.

Shaaya, E., Ravid, U., Paster, N., Juven, B., Lisman, U., Pissarev, V., 1991. Fumigant toxicity of essential oils against four major stored-product insects. J. Chem. Ecol. 7, 499–504. https://doi.org/10.1007/BF00982120.

Shaaya, E., Kostjukovski, M., Eilberg, J., Sukprakarn, C., 1997. Plant oils as fumigants and contact insecticides for the control of stored-product insects. J. Stored Prod. Res. 33, 7-15. https://doi.org/10.1016/S0022-474X(96)00032-X.

Soottitantawat, A., Yoshii, H., Furuta, T., Ohkawara, M., Linko, P., 2003. Microencapsulation by spray drying: Influence of emulsion size on the retention of volatile compounds. J. Food Sci. 68, 2256-2262. https://doi.org/10.1111/j.1365-2621.2003.tb05756.x.

Suthisut, D., Fields, P.G., Chandrapatya, A., 2011. Fumigant toxicity of essential oils from three Thai plants (Zingiberaceae) and their major compounds against *Sitophilus zeamais*, *Tribolium castaneum* and two parasitoids. J. Stored Prod. Res. 47, 222-230. https://doi.org/10.1016/j.jspr.2011.03.002.

Tsech, S., Gerhards, C., Schubert, H., 2002. Stabilization of emulsions by OSA starches. J. Food Eng. 54, 167-174. https://doi.org/10.1016/S0260-8774(01)00206-0.

Turasan, H., Sahin, S., Sumnu, G., 2015. Encapsulation of rosemary essential oil. Food Sci. Technol. 64, 112–119. https://doi.org/10.1016/j.lwt.2015.05.036.

Varona, S., Martín, A., Cocero, M.J., 2009. Formulation of a natural biocide based on lavandin essential oil by emulsification using modified starches. Chem. Eng. Process. 48(6), 1121-1128. https://doi.org/10.1016/j.cep.2009.03.002.

Werdin González, J.O., Laumann, R.A., da Silveira, S., Moraes, M.C.B., Borges, M., Ferrero, A.A., 2013. Lethal and sublethal effects of four essential oils on the egg

parasitoids *Trissolcus basalis*. Chemosphere. 92, 608–615. https://doi.org/10.1016/j.chemosphere.2013.03.066.

Werdin González, J.O., Gutiérrez, M.M., Ferrero, A.A., Band, B.F., 2014. Essential oils nanoformulations for stored-product pest control – Characterization and biological properties. Chemosphere. 100, 130-138. https://doi.org/10.1016/j.chemosphere.2013.11.056.

Yang, F.L., Li, X.G., Zhu, F., Lei, C.L., 2009. Structural characterization of nanoparticles loaded with garlic essential oil and their insecticidal activity against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). J. Agric. Food Chem. 57, 10156–10162. https://doi.org/10.1021/jf9023118.

Zhang, S., Chen, J., Yin, X., Wang, X., Qiu, B., Zhu, L., Li, Q., 2017. Microencapsulation of tea tree oil by spray-drying with methyl cellulose as the emulsifier and wall material together with chitosan/alginate. J. Appl. Polym. Sci. 13, 1–10. https://doi.org/10.1002/app.44662.

Zettler, J.L., Arthur, F.H. 2000. Chemical control of stored product insects with fumigants and residual treatments. Crop Prot. 19 (8–10) September 2000, Pages 577-582. https://doi.org/10.1016/S0261-2194(00)00075-2

Ziaee, M., Moharramipour, S., Mohsenifar, A., 2014. MA-chitosan nanogel loaded with *Cuminum cyminum* essential oil for efficient management of two stored product beetle pests. J. Pest Sci. 87(4), 691-699. https://doi.org/10.1007/s10340-014-0590-6.

SUPPLEMENTARY INFORMATION

Table S1

Effect of starch: oil ratio and % oil in mean droplet size ($D_{3,2}$) and Span of the emulsions and microcapsules.

Test**	Emulsion		Microcapsules	
	D3,2 (µm)	Span (µm)	D3,2 (µm)	Span (µm)
1	0.854±0.003	1.032±0.001	8.46±0.04	1.068±0.000
2	0.691±0.002	1.024±0.007	9.66±0.04	1.313±0.032
3	0.513±0.010	1.072±0.010	9.03±0.08	1.370±0.010
4	0.779±0.007	1.019±0.005	8.29±0.06	1.240±0.007
5	0.528±0.005	1.083±0.000	8.98±0.06	1.311±0.008
6	0.461±0.000	0.978±0.001	9.69±0.10	1.313±0.019
7	0.769±0.007	1.032±0.006	10.50±0.16	1.981±0.157
8	0.557±0.003	1.035±0.001	11.35±0.19	1.966±0.372
9	0.531±0.007	1.014±0.002	9.84±0.12	1.172±0.010
10	0.537±0.004	1.026±0.008	10.48±0.09	1.220±0.018
11	0.521±0.005	1.073±0.004	10.44±0.13	1.290±0.025
12	0.536±0.003	1.091±0.000	9.7±0.2	1.194±0.012
13	0.525±0.009	1.055±0.001	9.85±0.08	1.140±0.008

** Test 1: 5% Essential oil (EO) and ratio of polymer to EO 1:3, Test 2: 5% EO and ratio of polymer to EO 1:1, Test 3: 5% EO and ratio of polymer to EO 3:1, Test 4: 10% EO and polymer ratio to EO 1:3, Test 5: 10% EO and ratio of polymer to EO 1:1, Test 6: 10% EO and polymer ratio to EO 3:1, Test 7: 20% EO and ratio of polymer to EO 1:3, Test 8: 20% EO and ratio of polymer to EO 1:1. The inlet and outlet temperatures of the spray dryer were 140 and 85°C in the tests 1-8. Tests 9 to 13 were prepared with the condition of test 5, while the inlet and outlet temperatures were 120, 140, 160, 120, 120 °C, and 85, 85, 85, 81 and 89 °C, respectively.

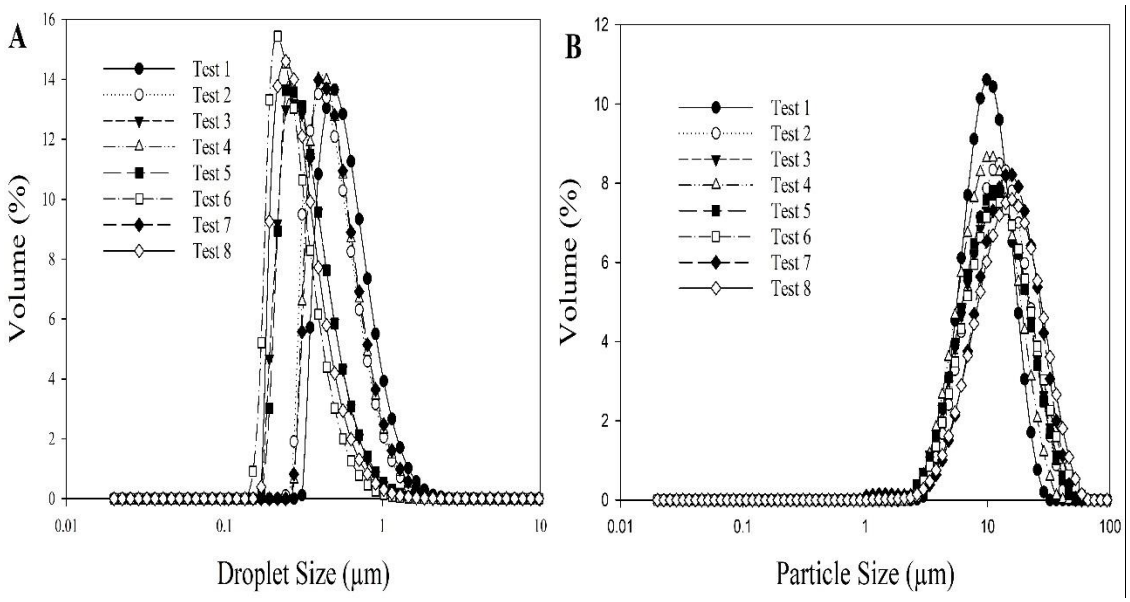

Fig. S1. A) Droplet and B) particle-size distribution curves of the emulsions and microcapsules.