Potential of cyclodextrins-based formulations in the control of the date moth Ectomyelois ceratoniae (Pyralidae)

Maha Ben Abada (mahabenabada@gmail.com)
INRAT: Institut National de Recherche Agronomique de Tunis
Soumaya Haoual Hamdi
INRAT: Institut National de Recherche Agronomique de Tunis
Abir Soltani
INRAT: Institut National de Recherche Agronomique de Tunis
Emna Boushih
INRAT: Institut National de Recherche Agronomique de Tunis
Sophie Fourmentin
ULCO: Universite du Littoral Cote D'Opale
Hélène Greige Gerges
University of Lebanon: Universite Libanaise
Jouda Mediouni Ben Jemâa
INRAT: Institut National de Recherche Agronomique de Tunis

Research Article

Keywords: Cyclodextrin, Rosmarinus officinalis, Fumigation, Ectomyelois ceratoniae, Dates

Posted Date: May 22nd, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2864028/v1

Abstract

Recently essential oils (EOs) encapsulation is experiencing growing applications in the agricultural and agri-food sector. Encapsulation is reported as safe environmental technology leading to a reduction of conventional insecticides use. This study concerns the assessment of fumigant toxicity and persistence of *Rosmarinus officinalis* EO encapsulated in two cyclodextrins β-CD and HP-β-CD against larvae of the date moth, *Ectomyelois ceratoniae.* The retention capacity, encapsulation efficacy, loading capacity and release behavior of the two inclusion complexes were investigated. The results showed that the encapsulation efficiency of HP-β-CD (EE = 25.25%) was higher than that of β-CD (EE = 17.73%). Additionally, compared to crude EO, the *in vitro* release profile of the two inclusion complexes showed a slow and sustained release. (0.0009 and 0.0007 min^{-1} for β-CD and HP-β-CD, respectively). After 30 days of exposure, the larval mortality rates for HP-β-CD/EO and β-CD/EO inclusion complexes were 40.38 and 53.85%, respectively. Furthermore, the half-life of HP-β-CD and β-CD/EO inclusion complexes (9.620 and 8.045 days) was significantly longer than that of the crude EO (5.245days). This study supports the use of cyclodextrins, mainly HP-β-CD, in the date industry for the control of insect pests.

Introduction

The pyralid moth *Ectomyelois ceratoniae* Zeller (Lepodoptera: Pyralidea), is a polyphagous damaging pest wide-reaching, that attacks fruits in field and in storage. Female date moths lays eggs inside the calyx (pomegranate crown) or in the crocks of fruit peel (Dhouibi et al. 2016; Mirzabegi Kesbi et al. 2023). The pest larvae feed on the inner parts of the fruit, causing the contamination with saprophytic fungi, making the fruits unfit for human consumption and food processing, and unsuitable for marketing (Shakeri and Sadatakhavi 2004). The use of chemical insecticides is actually the main approach for the control of *E. ceratoniae.* The environmental and ecotoxicological impact of the wide use of synthetic insecticides in agriculture has led researchers to look for viable alternatives that are more eco-friendly than chemical ones (Tudi et al. 2021). In this respect, the use of botanical insecticides has attracted the interest of both researchers as well as consumers. Amongst botanical extracts used as insecticides, essential oils (EOs) appear to be an alternative or complementary method to chemically insecticides in integrated pest management programs (IPM) due to their relative cost-effectiveness and their worldwide availability (Campolo et al. 2018). EOs are natural volatile secondary metabolites known for their strong odor (Bakkali et al. 2008). Numerous studies have highlighted their fumigant (Abada et al. 2020; Haouel et al. 2010; Mediouni Ben Jemâa et al. 2013) and repellent action (Khemira et al. 2012), antifeedant activity (Ricardo et al. 2014), ability to delay emergence (Ben Abada et al. 2020), fertility and development inhibition (Marimuth at al. 1997). Despite these promising proprieties, some limitations still need to be overcome. Indeed, EO compounds are very sensitive to volatilization and chemical alterations due to light, humidity, heat, and oxygen exposure. To achieve high efficacy and stability, EOs could be encapsulated in microcapsules, which are used as delivery systems and are considered to be an encouraging strategy for the use of EOs in agriculture and particularly in insect pest management (Moretti et al. 2002). On the other hand, microencapsulation of pesticides was found to protect the sensitive components from

undesirable conditions, ensure their controlled release, and restore their functional performance and toxicity (Estrada-Cano et al. 2017; Gupta et al. 2016; Sherje et al. 2017; Zhu et al. 2021). Encapsulation is the process of enveloping molecules to create a functional barrier between a bioactive core and the wall material in order to minimize physical and chemical interactions between the core and the external molecules (Kalemba and Kunicka 2003). The microparticles commonly used as wall materials to protect, the volatiles from degradation are liposomes (Hammoud et al. 2020), polymer particles, solid lipid nanoparticles (Zielińska et al. 2020), chitosan-gum Arabic microcapsules (Soltani et al. 2022; Xu et al. 2012) and cyclodextrins (Abada et al. 2019; Ciobanu et al. 2013; Kfoury et al. 2019).

Cyclodextrins (CDs) have a unique structure with a hydrophobic cavity and a hydrophilic surface. Their cavity is suitable for the inclusion of a wide variety of guests (Dodziuk 2006; Szejtli and Sadatakhavi 2004). The commonly used native CDs are alpha CD (α-CD), beta CD (β-CD), and gamma CD (γ-CD) which contain respectively six, seven, and eight glucose subunits, respectively (Kfoury et al. 2019). The use of CDs to encapsulate EO can improve the solubility of insoluble components, protect oils from environmental conditions, control sublimation, and volatility, modify the taste of flavors, entrap odors, and control the release of guests (Kfoury et al. 2019, Rakmai et al. 2017). Among native CDs, β-CD is the most widely used due to its suitable cavity size, its availability and its reasonable price (Waleczek et al. 2003). However, its low water solubility (1.8g in 100 ml of water at 25°C) required some chemical modification such as the alkylation of the hydroxyl groups of the β-CD. For example, the hydroxypropyl-β-CD derivative (HP-β-CD), which has a high aqueous solubility (in the order of 60g in 100ml of water at 25°C), shows strong complexing ability with EOs (Ciobanu et al. 2013; Kfoury et al. 2014).

This paper reports the characterization of HP-β-CD and β-CD/*R. officinalis* EO inclusion complexes and to investigate their toxicity against the date moth *E. ceratoniae* fifth instars larvae. In addition, this study aimed to evaluate the persistence of the insecticidal activity of the two inclusions complexes compared to the free EO.

Material and methods

Chemicals

β-CD was purchased from Wacker-Chemie (Lyon, France) and HP-β- CD (DS = 5.6) was obtained from Roquette (Lestrem, France).

Insect rearing

To initiate *E. ceratoniae* rearing, infested dates were collected from oasis in south Tunisia. Rearing was established under the following controlled laboratory conditions: 25 ± 1°C, 65 ± 5% relative humidity, and 15:9 (L:D) photoperiod. The insects' rearing medium included wheat bran (60%) mixed with sucrose (12%), salt mixture (2%), yeast (1.3%), lysine (1.23%), methyl paraben (0.13%), vitamin (0.67%),

aureomycine (0.67%), glycerine (150 ml) and distilled water (150ml) (Mediouni and Dhouibi 2007). The fifth instar larvae (last instar larvae) were used for bioassays.

Plant material and essential oils extraction

Plant material and essential oils extraction
Rosmarinus officinalis, growing wild in Tunisia, was collected from Fernana: Jendouba (36° 39' 41.2" N 8° 41' 05.7" E) (a sub-humid area) in March 2020. Fresh branches and leaves were kept at ambient temperature (20–25°C) until dryness. The EO was extracted from dried plant leaves using a modified Clevenger hydrodistillation method.

GC-MS Analysis

A sample of the EO was diluted in n-hexane and analysed by gas chromatography coupled to a mass spectrometer (GC-MS). GC-MS analysis was performed on an Agilent- Technologies 6890 N Network equipped with a flame ionization detector and HP-5MS capillary column (30 m× 0.25 mm, film thickness 0.25 µm; Agilent- Technologie, Littlr Falls, CA, USA). The column temperature was programmed at 80°C for 3 min and increased at a rate of 4°C/min until 220°C. The injector temperature were set respectively at 290°C. The carrier gas was Helium at flow rate of 1.0 ml/min. All quantifications were performed using a built-in data-handling program supplied by the gas chromatograph manufacturer. The composition was determined as a relative percentage of the total peak area. EO constituents were identified by comparing their retention time to n-alkanes (C_5-C_{36}), with those reported in the literature under the same operating conditions. The components were further identified and authenticated by means of their mass spectra in comparison with the Wiley version 7.0 library. Relative percentages of the individual compounds of the EO were obtained by averaging the GC peak area (%) reports.

Screening tests

To establish the lethal concentrations CL_{50} values of *R. officinalis* EO, four concentrations 15, 30, 60 and 90 µl/l air were tested after 15 exposure days. The trial were carried out in 1L bottle glass containing 20 dates infested with *E. ceratoniae* fifth instar larvae. The EO was deposited using a micropipette on 5cm filter paper discs (Whatman N°1 paper). Non-treated insects (control) were kept under the same conditions. Tree replicate were performed for each concentration. Mortality data were corrected according to Abbot's formula (Abbott 1925). Probit analysis (Finney 1971) was used to calculate LC_{50}.

Preparation of inclusion complexes

β-CD/EO and HP-β-CD/EO inclusion complexes were prepared as described previously by Kfoury et al. (2015) with slight modifications. β-CD/EO and HP-β-CD/EO complexes were prepared at a 1:1 ratio. The

mixture of CD and EO was dissolved in 40ml of distilled water. The solutions were stirred for 24h (33 rpm at 25°C) and filtered through 0.45µm filters to remove undissolved compounds. The obtained filtrates were frozen at -80°C for 24h then freeze-dried in a freeze dryer for 12 h to obtain the resulting solid inclusion complexes.

Determination of retention ability, encapsulation efficacy and loading capacity

An accurate amount of *R. officinalis* EO dissolved in 10 ml water or aqueous CD solutions and 10 mg of inclusion complex dissolved in 10 ml ethanol, were placed in 22 ml headspace glass vials. Vials were then closed by silicone septa and aluminum foil. After equilibration between the aqueous and gaseous phases (30 min at 25 ± 0.1°C), 1ml of vapor presents above the solution was withdrawn from the vial using a gas-tight syringe and injected directly in the chromatographic column via a transfer line (250°C) for chromatographic analysis. All measurements were performed with an Agilent headspace auto sampler and a Perkin Elmer Auto system XL equipped with a flame ionization detector using a DB624 column gas chromatography. Temperature conditions were set as follows initial temperature of 50°C for 2 min, increased to 190°C at 5°C/min for a total duration of 30 min. Nitrogen was used as carrier vector.

The percentage of retention (r) of *R. officinalis* essential oil by the two CDs was determined by for a 2 mM CDs solution and expressed as follows:

$$r\,(\%) = \left(1 - \frac{\sum \mathrm{ACD}}{\sum \mathrm{A0}}\right) \times 10$$

1

where $\sum A_0$ and $\sum A_{CD}$ stand for the sum of peak areas of the EO in the absence and the presence of CDs, respectively. For each component, measurements were done in triplicate

The encapsulation efficacy and loading capacity were determined according to the following equations:

$$EE\,(\%) = \left(\frac{m_{exp}}{m_i}\right) * 100 (2\,)$$

$$LC\,(\%) = \left(\frac{m_{exp}}{m_c}\right) * 100\,(3)$$

Where m_{exp} is the mass of the EO in the inclusion complex; m_i is the amount of the EO initially used to prepare the inclusion complex and m_c is the mass of inclusion complex.

Release studies

The multiple headspace extraction method (MHE) was used to study the dynamic release of the EO and it components as described by Kolb and Ettre (2006). In this method successive extractions of the headspace vial are carried out. At each extraction, 1ml of the vial headspace was withdrawn and the amount of each EO compound present in the gas phase was determined by GC analysis. The successive extractions of headspace led to a reduction of the amount of volatiles present in the gas phase. The rate of this decrease reflected the retention efficiency of CD toward each essential oil component in solution. GC setting were set as previously. Aliquots of essential oil were added to water or CDs solutions (2mM) previously placed in 22 ml headspace glass vials. Vials were sealed and ten successive extractions were carried out at 60°C. At each interval time (45min), the remaining percentage of each free or encapsulated EO compound was determined using the following equation:

$$\text{Remaning}\ (\%) = \frac{A_t}{A_0} \times 100\ (4)$$

Where A_t and A_0 stand for the peak area of each EO component at time t and time 0.

Fumigant toxicity of the inclusions complexes

To evaluate the fumigant efficacy of the free and the encapsulated *R .officinalis* EO against *E. ceratoniae* fifth instars larvae, infested dates were treated with the LC_{50} value determined in screening test and with the amount of formulation equivalent to the LC_{50} value. Free EO was deposit on 5 cm diameter Whatman filter papers using a micropipette and HP-β-CD and β- CD/EO inclusion complexes were placed in mosquito net and attached to glass bottles caps. Untreated insects were kept under the same conditions. Each treatment and control was replicated three times. Mortality was carried out according to Abbott's formula (Abbott 1925).

Persistence experiments

The persistence of *R. officinalis* EO and the of two inclusion complexes was followed as described by Negahban (2012). Indeed, new larvae were introduced after 3, 5, 7, 9 and 30 days (5 replicates per time interval).

Statistical analysis

Data were analyzed using SPSS version 20 (IBM corporation NY, USA). Results were subjected to variance analysis (Pintore et al. 2002) and Duncan's test was employed to notice significant differences at the level 0.05%. Values presented the means of three replications and expressed as the mean ± SD. For each parameter (Mortality percentage and persistence), data were subjected to two- ways ANOVA, with exposure time and treatments type as main fixed factors plus their interactions. Besides, mortality and persistence data were subjected to Probit analysis to obtain the lethal concentration (LC) and lethal time

(LT). In addition, correlation analyses were performed between the matrix proprieties and the efficiently of encapsulated EO. The correlation analyses (Pearson's correlation coefficient) between the encapsulation matrices and the efficacy of the encapsulated oils (mortality and persistence) were determined.

Results

Chemical composition of the essential oil

The yield of oil obtained from the aerial parts of *R. officinalis* isolated by hydrodistillation was 1.34%. The chemical composition of the EO was summarized in Table 1. Nine volatile compounds were identified, covering 95% of the peak areas. The main components of rosemary oil were 1,8-cineole (48.45%), camphor (13.10%), α-pinene (12.66%) and borneol (12.42%).

Table 1
Chemical formulas, retention times (RT) of the identified volatiles and their percentage in *Rosmarinus officinalis* essential oil

	Components	Formula	RT	Percentage (%)	KI
1	α-pinene	$C_{10}H_{16}$	5.46	**12.66**	936
2	Camphene	$C_{10}H_{16}$	5.868	3.96	
3	β pinene	$C_{10}H_{16}$	6.55	1.53	983
4	p-cymene	$C_{10}H_{14}$	7.83	1.58	1031
5	1,8-cineole	$C_{10}H_{18}O$	8.09	**48.45**	1040
6	Borneol	$C_{10}H_{18}O$	12.25	**12.42**	1176
7	(+)-camphor	$C_{10}H_{16}O$	19.77	**13.10**	
8	β-caryophyllene	$C_{15}H_{24}$	20.43	1.30	1431
9	Unidentified components			5	

KI: Kovalts Index as determined on a GC-MS column using the homologous series of n-hydrocarbons. In bold: main components

Retention capacity

The retention capacities of *R. officinalis* by a 2mM CDs solution (β-CD and HP-β-CD) were determined using Eq. 1. and are given in Table 2. Retention values indicated that rosemary EO was efficiently retained by CDs. This demonstrated the tendency of EO components to form inclusion complexes with CDs. The retention capacity of β-CD (98.06%) is slightly more efficient to than that of HP-β-CD (97.82%). Statistical

analyses showed a significant difference between the retention capacities of the two CDs ($F = 216$, $P < 0.001$, ddl = 1).

Table 2
Retention capacity (%) of *Rosmarinus officinalis* essential oil in aqueous solution of 2Mm HP-β-CD and β-CD

	HP-β-CD	β-CD
Retention capacity (%)	97.82 a	98.06 b

Encapsulation efficacy

The encapsulation efficiency and loading capacity of *R. officinalis* EO in CDs (HP-β-CD and β-CD) were determined using Eqs. 2 and 3, and presented in Tables 3 and 4. Results indicated that the EE of rosemary EO in β-CD (EE = 17.73%) was lower than that in HP-β-CD (EE = 25.25%). This observation could be explain by the precipitation of the CD/EO inclusion complex. Indeed, the amount of the EO in the precipitated fraction of β-CD was 32.5%. In addition, our results indicated that the loading capacity depends on the chemical compound and the coating matrix. Observation of Table 4 showed that most of the volatile components of *R. officinalis* encapsulated in β-CD were retained by the precipitated fraction with a loading capacity ranged between 5.63 and 36.30 $\mu g.mg^{-1}$powder. Even though, most of the volatile compounds (α-pinene, Camphene, β-pinene, p-cimene, 1,8-cineole, borneol, and β-caryophylen) are mostly retained by β-CD. While camphor is the only compound that is more retained by HP-β-CD (LC = 21.62$\mu g.mg^{-1}$powder).

Table 3
Encapsulation Efficiency (EE) of *Rosmarinus officinalis* essential oil in HP-β-CD and β-CD (supernatant and residue) inclusion complexes

	HP-β-CD	β-CD (Supernatant)	β-CD (Residue)
EE (%)	25.25	17.73	32.5

Table 4

Loading capacity (LC) (μg.mg^{-1}powder) of *Rosmarinus officinalis* essential oil components in HP-β-CD and β-CD (supernatant and residue) inclusion complexes

	HP-β-CD	β-CD (supernatant)	β-CD (residue)
α-pinene	1.55	5.62	7.02
Camphene	3.47	5.71	8.82
β-pinene	3.69	4.35	8.89
p-cimene	0.82	2.00	7.18
1,8-cineole	10.59	19.63	36.30
Bornéol	3.83	0.28	8.21
Camphor	21.62	11.53	25.89
β-caryophyllene	0.03	1.15	5.63

Release studies

The release studies of *R. officinalis* EO and its components, free and encapsulated in CDs (β-CD and HP-β-CD) were performed using MHE as shown in Table 5 and Fig. 1. The remaining percentage of EO volatile compounds at each extraction corresponding to 45 min interval, was determined based on their individual chromatographic peak area using Eq. (4). The release of the EO and its compounds showed an exponential asymptotic trend whether they were tested in their crude or encapsulated form. The calculated release rate constants were 0.0009 and 0.0007min^{-1} for the essential oil encapsulated in β-CD and HP-β-CD, respectively, compared to 0.002 min^{-1} for the free EO. This observation revealed also that the β-CD released the essential oil faster than the HP-β-CD. The statistical analysis reported in Table 6, showed a significant difference between the release rate of the free and encapsulated EO in the β-CD and the HP-β-CD($F = 1729.41$, $p < 0.001$, ddl = 2). The same observation could be made for EO components. The results indicated that encapsulation reduced the release rate of these volatiles by2.81, 4.14, 7, 5.25, 5, 3.14, 4 and 8.86 fold for HP-β-CD inclusion complex and 3.88, 3.63, 4.67, 3.82, 2.5, 2, 2 and 5.17 fold for α-pinene, camphene, β-pinene, *p*-cymene, 1,8-cineole, borneol, camphor and β-caryophyllene, respectively (Table 5). Additionally, we found that HP-β-CD increased the retention of EO and its major components more than β-CD. In fact, after 6 hours the remaining percentage of α-pinene, camphene, β-pinene, *p*-cymene, 1,8-cineole, borneol and camphor were 75.29, 78.53, 85.58, 72.33, 94.96, 74.43, and 96.78% when encapsulated in HP-β-CD against 68.89, 73.71, 79.71, 65.36, 92.93, 66.48 and 93.25% when encapsulated in β-CD. Except for β-caryophyllene it is more retained by β-CD (86.77%) than by the HP-β-CD (77.55%).

Table 5

Release rate constants k(min^{-1}) of *Rosmarinus officinalis* essential oil compounds free and encapsulated in HP-β-CD and β-CD

	Free	In HP-β-CD	In β-CD
HE	0.0020	0.0007	0.0009
α-pinene	0.0031	0.0008	0.0011
Camphene	0.0029	0.0007	0.0008
β-pinene	0.0028	0.0004	0.0006
p-cymene	0.0042	0.0008	0.0011
1,8-cineole	0.0005	0.0001	0.0002
Borneol	0.0022	0.0007	0.0011
Camphor	0.0004	0.0001	0.0002
β-caryophyllene	0.0062	0.0007	0.0012

Table 6

Effect of matrix and exposure period, release rate, mortality and persistence of *Rosmarinus officinalis* essential oil

Variables		Df	F	P
Matrix	Retention capacity	1	216	< 0.001
	Release rate	2	1729.41	< 0.001
	Mortality rate	2	11376299.64	< 0.001
	Persistence	2	3062450.15	< 0.001
Exposure periods	Mortality rate	3	24880753.11	< 0.001
	Persistence	3	4285603.04	< 0.001
Matrix * Exposure periods	Mortality rate	6	471765.58	< 0.001
	Persistence	6	1094913.61	< 0.001

Encapsulated essential oil fumigant toxicity

The results of fumigation tests with free and encapsulated *R. officinalis* essential oil against *E. ceratoniae* fifth instars larvae are presented in Fig. 2. The results showed that both free and encapsulated EO in HP-β-CD and β-CD were toxic to the date moth. In addition, our results demonstrated that free EO was more effective than the encapsulated ones. In fact, after 30 days of exposure, larval mortalitie was

94.23, 40.38 and 53.85% for the free EO, HP-β-CD/EO and β-CD/ EO inclusion complexes, respectively. Furthermore, the EO encapsulated in β-CD was shown to be more effective against the date moth larvae than that encapsulated in HP-β-CD. After 37 days of exposure, larval mortality reached 62.75 and 78.43% for the EO encapsulated in the HP-β-CD and β-CD, respectively (Fig. 2). Statistical analysis reported in Table 6, indicated that treatments and exposure periods had significant effects on *E. ceratoniae* larval mortalities (Treatments: $F = 11376299.64$, $p < 0.001$, ddl = 2 and exposure periods: $F = 24880753.11$, $p < 0.001$, ddl = 3) (Table 6).

Persistence of the encapsulated EO

The persistence of the insecticidal effect of *R. officinalis* EO free and encapsulated in CDs (β-CD and HP-β-CD) over time is depicted in Fig. 3. The results showed that treatment efficacy decreased with increasing exposure time. Persistence tests indicated that the insecticidal toxicity of *R. officinalis* EO lasted up to 9 days, whereas the insecticidal efficacy of EO encapsulated in β-CD and HP-β-CD was 30 days. Moreover, statistical analyses reveled that treatments and exposure times affected significantly the persistence of the insecticidal effect on *E. ceratoniae* larval (treatments: $F = 3062450.15$, $p < 0.001$, ddl = 2, exposure periods: $F = 4285603.04$, $p < 0.001$, ddl = 3) (Table 6).

Additionally, the estimated LT_{50} values showed that the half-life of the inclusion complexes CDs/EO were significantly longer than that of free EO (Table 7). Indeed, LT_{50} values of the free and the encapsulated EO in HP-β-CD and β-CD were 5.245, 9.620 and 8.045 days, respectively. Furthermore, the inclusion complexes were effective for a longer period of time compared to free EO. The results also showed that *R. officinalis* EO encapsulated in in HP-β-CD had the best persistence compared to EO encapsulated in β-CD.

Table 7
LT_{50} values (days) expressing the persistence of free and encapsulated *Rosmarinus officinalis* essential oil on *Ectomyelois ceratoniae* fifth instar larvae

	Free EO	β-CD/EO inclusion complex	HP-β-CD/EO inclusion complex
LT_{50}[a. b]	5.245 (3.269–9.456)	8.045 (13.040–5.804)	9.620 (6.579–21.121)
Slope± SEM	3.829 ± 1.577	3.333 ± 0.605	3.579 ± 0.755
Degrees of Freedom	4	4	4
χ^2	53.177	13.534	19.719
[a]Units LT_{50} = days			
[b]95% lower and upper confidence limits are shown in parentheses			

Correlation analyses between the matrix proprieties and the efficiently of encapsulated EO

Correlation analyses between the CD properties (retention and release rate) and the efficacy of the encapsulated EO (mortality rate and persistence) after four exposure periods (7, 15, 30 and 37 days) are recorded in Table 8. A highly positive correlation was observed between the retention capacity and the release rate ($r = 0.995^{**}$). In fact, the matrices that retained the greater amounts of EO released more rapidly (β-CD). For this reason, the mortality of *E. ceratoniae* larvae correlated positively with the retention capacity (r = 0.422) and the release rate (r = 0.411). In addition, a highly positive correlation was found between the mortality of *E. ceratoniae* and the exposure time (r = 0.849). The mortality rate increased as the exposure period increased. On the other hand, a strong negative correlation was found between the persistence and retention capacity (r= -0.489) and the persistence and the release rate (r= -0.488). Therefore, HP-β-CD, which was characterized by the lowest retention capacity and release rate exhibited the most significant persistence of the insecticidal effect.

Table 8
Correlation analyses between the matrix proprieties (Retention capacity and Release rate) and the efficacy of encapsulated EO (Mortality rate and persistence) for four exposure periods (7, 15, 30 and 37 days).

	Matrix	Time	Retention capacity	Release rate	Mortality rate	Persistence
Matrix	1					
Time	0.000	1				
Retention capacity	**-0.811****	0.000	1			
Release rate	**-0.783****	0.000	**0.995****	1		
Mortality rate	**-0.472****	**0.849****	**0.422***	**0.411***	1	
Persistence	**0.388***	**-0.671****	**-0.489****	**-0.488****	**-0.729****	1

Discussion

Terpenic components, such as 1,8-cineole, camphor, α-pinene and borneol were detected at high levels in *R. officinalis* EO. The composition *R. officinalis* in this investigation is qualitatively similar to that reported in previously published studies (Andrade et al. 2018; da Silva Bomfim et al. 2015; Elyemni et al. 2022). However, some notable quantitative differences include the relatively high concentrations of α-pinene (26–28%) in relation to the relatively low concentration of 1.8 cineole (11–25%) in the previous reports (Angioni et al. 2004; Melito et al. 2019; Pintore et al. 2002). Such differences may be due to several exogenous and endogenous factors such as geographical location, harvest time, extraction method and genetic makeup (Al-Maharik et al. 2022; Rašković et al. 2014; Teixeira et al. 2013). This EO was a complex mixture of bioactive compounds that have been shown to be toxic against to many pests (Gallardo et al. 2012; Kordali et al. 2008; Matos et al. 2020). Our study demonstrated that *R. officinalis* EO exhibited promising fumigant toxicity against *E. ceratoniae* larvae (LC_{50} = 15.79 µl/l air after 15 days of exposure). Previous reports have investigated the fumigant activity associated with different concentrations of plant EOs against the date moth. The high fumigant activity of *Artemisia herba* and *Citrus sinensis* EOs reported by Chaaban et al. (2018), showed complete (100%) and 64% mortality of *E. ceratoniae* L5 larvae, respectively. Furthermore, the results of Mediouni Ben Jemâa et al. (2012) indicated that *Eucalyptus camaldulensis*, *Eucalyptus astringens*, *Eucalyptus leucoxylon*, *Eucalyptus lehmannii* and *Eucalyptus rudis* had fumigant activity against the date moth. LC_{50} values were 37.26; 41.76; 44.67; 56.34; 36.01 µl/l air within 24 hours of exposure, respectively.

Controlling the high volatility of EOs is the main challenge to be addressed through the development of various encapsulation techniques. Encapsulation can retain EOs by physical or chemical interaction with a matrix that retains the EO for a longer period of time, increasing the retention time and allowing a slow and continuous release of the active ingredients, enabling their application for pest management

(Abdollahdokht et al. 2022; Maes et al. 2019; Melanie et al. 2022). In the present study, *R. officinalis* was encapsulated by the freeze-drying method using HP-β-CD and β-CD in a 1:1 ratio. Our finding showed that rosemary EO was efficiently retained by the CDs (the retention capacities are 98.06% and 97.82% for β-CD and HP-β-CD, respectively). Our results are in agreement with those found by Sebaaly et al. (2018). These authors point out that β-CD and its derivatives showed the highest retention capacity toward nine EOs (69%-78%). The same authors demonstrated that these EOs were more restrained by β-CD than HP-β-CD. Similarly, Hammoud et al. (2022) attribute this high retention capacity to the affinity between the active molecule and the coating matrix. According to the encapsulation efficiency our results pointed out that the EE of rosemary EO in β-CD (EE = 17.73%) is lower than that in HP-β-CD (EE = 25.25%) because of the low solubility of β-CD, the most of the EO is retained by the precipitated fraction. In this regard, Ciobanu et al. (2013) indicated that among the four CDs (α-CD, β-CD, γ-CD and HP-β-CD), the highest encapsulation efficacy was observed for HP-β-CD.

Previous studies have showed that the encapsulation efficacy of 1,8-cineole (63.72%), the main compound of *R. officinalis* EO, in the HP-β-CD was very important compared to whole EO (25.25%) (Abada et al. 2019). This difference in the EE can be attributed to the presence of other oil components (such as α-pinene, camphene, β-pinene, *p*-cymene, borneol, camphor and β-caryophyllene), that also have high affinity for CD (Astray et al. 2010; Haiyee et al. 2009; Jiang et al. 2020; Kfoury et al. 2019; Kfoury et al. 2014; Numanoğlu et al. 2007; Tao et al. 2014).

This research has demonstrated that *R. officinalis* EO microencapsulated in HP-β-CD and β-CD is highly effective against the date moth *E. ceratoniae*. Additionally, the EO encapsulated in β-CD was shown to be more effective against the date moth larvae than that encapsulated in HP-β-CD. This observation is due to high ability of the β-CD to retain the *R. officinalis* EO bioactive components (α-pinene, camphene, β-pinene, *p*-cymene, 1,8-cineole, borneol, and β-caryophyllene). Results of the present study are consistent with those of Abada et al. (2019). These authors highlighted the usefulness of 1,8-cineole (the main component of rosemary EO) encapsulated in the HP-β-CD to overcome limitations in date moth control during storage. Furthermore, Ziaee et al. (2014) reported the toxicity of gelatin- based microencapsulated *R. officinalis* and *Thymus vulgaris* EOs against the Indian meal moth (*Plodia interpunctella*) larvae. It was found that the introduction of these microcapsules in larvae diet caused the death of 17.5 and 20% by *R. officinalis* and T. *vulgaris* Eos, respectively, after 7 days.

On the other hand, the main reason limiting the use of EOs in pest management strategies is the low persistence of their insecticidal toxicity. In the present study, the fumigant persistence of *R. officinalis* EO was improved by the encapsulation in CDs (β-CD and HP-β-CD). The HP-β-CD/EO inclusion complex showed the best persistence compared to the β-CD one. This can be explained by the fact that the β-CD released the EO and its bioactive components faster than HP-β-CD. Numerous studies have shown that encapsulation of EOs provides prolonged protection against insect attack. Further studies showed that the encapsulation *Cuminum cyminum* EO in myristic acid-chitosan nanogels prolonged its fumigant toxicity against the confused flour beetle (*Tribolium confusum* Duv.) and the granary weevil (*Sitophilus granaries* L.) (Ahmed and Al-Moajel 2005). In addition, Negahban et al. (2012) ascertained that the

encapsulation of clove EO improved its stability and allowed a slow and continuous release of the active agent, delaying its evaporation and degradation. These authors showed that encapsulation improved the persistence of the insecticidal toxicity (the half-life of encapsulated essential oil (LT_{50} = 28.73 days) and that of crude oil (LT_{50} = 4.27 days)).

Conclusion

In this study, we demonstrated that encapsulation in CDs is a promising approach for the incorporation of essential oils in the date industry as they are able to overcome the major limitation in the use of EOs. EOs was successfully encapsulate in CDs and allow a delayed and controlled its release. Moreover, our novel larvicide based on rosemary EO encapsulated in CDs was effective against *E. ceratoniae* fifth instars larvae. Encapsulation in CDs maintains or even enhances the insecticidal activity and the functionalities of EOs. This approach may provide a solution to the long-standing problem of controlling the date moth with minimal harm to non-target organisms. Although, to carry out all these processes, a few main concerns are cost reduction and the efficiency of manufacturing.

Declarations

Acknowledgements

We thank the “Agence Universitaire de la Francophonie, Projet de Coopération Scientifique Inter-Universitaire 2018-2020” for supporting the work.

Conflicts of Interest: None

Ethical Approval: Not applicable, because this article does not contain any studies with human or animal subjects.

Consent to Participate: All participants consent to participate of the present study.

Consent to publish: All participants consent to publish the present study.

Authors Contributions: Maha Ben Abada wrote the manuscript with input from all authors. Jouda Mediouni-Ben Jemâa and Sophie Fourmentin devised the project, the main conceptual ideas and proof outline. Soumaya Haoual Hamdi, Abir Soltani, Emna Boushih, and Hélène Greige-Gerges provided critical feedback and helped shape the research, analysis and manuscript. All authors discussed and commented on the manuscript.

Funding: This research was funded by the “PHC Utique” program of the French Ministry of Foreign Affairs and Ministry of higher education, research and innovation and the Tunisian Ministry of higher education and scientific research in the CMCU project number 20G0904".

Competing Interests: The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

Availability of data and materials: The authors confirm that the data supporting the finding of this study are available within the article and its material. Raw data that support the findings of this study are available from the corresponding author, upon reasonable request.

References

1. Abada MB, Hamdi SH, Gharib R, Messaoud C, Fourmentin S, Greige-Gerges H, Jemâa JMB (2019) Post-harvest management control of *Ectomyelois ceratoniae* (Zeller)(Lepidoptera: Pyralidae): new insights through essential oil encapsulation in cyclodextrin. Pest Manag Sci 75: 2000-2008.
2. Abada MB, Hamdi SH, Masseoud C, Jroud H, Bousshih E, Jemâa JMB (2020). Variations in chemotypes patterns of Tunisian *Rosmarinus officinalis* essential oils and applications for controlling the date moth *Ectomyelois ceratoniae* (Pyralidae). S Afr J Bot 128: 18-27.
3. Abbott W (1925) A method of computing the effectiveness of an insecticide. J. econ. Entomol 18: 265-267.
4. Abdollahdokht D, Gao Y, Faramarz S, Poustforoosh A, Abbasi M, Asadikaram G, Nematollahi MH (2022) Conventional agrochemicals towards nano-biopesticides: An overview on recent advances. Chem Biol Technol Agric 9: 1-19.
5. Ahmed S, Al-Moajel NH (2005) Effect of tooth pick (*Ammi visnaga* L.) seed extracts on the rice weevil *Sitophilus oryzae* (Coleoptera: Curculionidae). Arab Univ J Agric Sci 13: 549-557.
6. Al-Maharik N, Jaradat N, Hawash M, Al-Lahham S, Qadi M, Shoman I, Jaber S, Rahem RA, Hussein F, Issa L (2022) Chemical Composition, Antioxidant, Antimicrobial and Anti-Proliferative Activities of Essential Oils of *Rosmarinus officinalis* from five Different Sites in Palestine. Sep 9: 339-347.
7. Andrade JM, Faustino C, Garcia C, Ladeiras D, Reis CP, Rijo P (2018) *Rosmarinus officinalis* L.: an update review of its phytochemistry and biological activity. Future Sci OA 4: 283-289.
8. Angioni A, Barra A, Cereti E, Barile D, Coïsson JD, Arlorio M, Dessi S, Coroneo V, Cabras P (2004) Chemical composition, plant genetic differences, antimicrobial and antifungal activity investigation of the essential oil of *Rosmarinus officinalis* L. J Agric Food chem 52: 3530-3535.
9. Astray G, Mejuto JC, Morales J, Rial-Otero R, Simal-Gándara J (2010) Factors controlling flavors binding constants to cyclodextrins and their applications in foods. Food Res Int 43: 1212-1218.
10. Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils–a review. Food Chem Toxicol 46: 446-475.
11. Campolo O, Giunti G, Russo A, Palmeri V, Zappalà L (2018) Essential oils in stored product insect pest control. J Food Qual 2018: 1-18.
12. Chaaban A, Martins CEN, Bretanha LC, Micke GA, Carrer AR, Rosa NF, Ferreira L, Molento MB (2018) Insecticide activity of *Baccharis dracunculifolia* essential oil against *Cochliomyia macellaria* (Diptera: Calliphoridae). Nat Prod Res 32: 2954-2958.

13. Ciobanu A, Landy D, Fourmentin S (2013) Complexation efficiency of cyclodextrins for volatile flavor compounds. Food Res Int 53: 110-114.
14. Da Silva Bomfim N, Nakassugi LP, Oliveira JFP, Kohiyama CY, Mossini SAG, Grespan R, Nerilo SB, Mallmann CA, Abreu Filho BA, Machinski JM (2015) Antifungal activity and inhibition of fumonisin production by *Rosmarinus officinalis* L. essential oil in *Fusarium verticillioides* (Sacc.) Nirenberg. Food chem 166: 330-336.
15. Dhouibi MH, Hermi N, Soudani D, Thlibi H (2016) Biocontrol of the carob moth *Ectomyelois ceratoniae* (Lepidoptera, Pyralidae) in pomegranate and citrus orchards in Tunisia. Int J Agri Innov Res 4: 849-856.
16. Dodziuk H (2006) Molecules with holes–cyclodextrins. Cyclodextrins and their complexes: Chem Anal Methods Appl 1-30.
17. Elyemni M, El Ouadrhiri F, Lahkimi A, Elkamli T, Bouia A, Eloutassi N (2022) Chemical composition and antimicrobial activity of essential oil of wild and cultivated *Rosmarinus officinalis* from two Moroccan localities. Journal Ecol Eng 23:1-6
18. Estrada-Cano C, Castro MA, Muñoz-Castellanos L, García-Triana N, Hernández-Ochoa L (2017) Antifungal activity of microcapsulated clove (*Eugenia caryophyllata*) and Mexican oregano (*Lippia berlandieri*) essential oils against *Fusarium oxysporum*. J Microb Biochem Technol 9: 567-571.
19. FAO F (2016) Minimum dietary diversity for women: a guide for measurement. Rome: FAO 82
20. Finney D (1971) Statistical logic in the monitoring of reactions to therapeutic drugs. Methods of Inf Med 10: 237-245.
21. Gallardo A, Picollo MI, González-Audino P, Mougabure-Cueto G (2012) Insecticidal activity of individual and mixed monoterpenoids of geranium essential oil against *Pediculus humanus capitis* (Phthiraptera: Pediculidae). J Med Entomol 49: 332-335.
22. Gupta A, Eral HB, Hatton TA, Doyle PS (2016) Nanoemulsions: formation, properties and applications. Soft Matter 12: 2826-2841.
23. Haiyee ZA, Saim N, Said M, Illias RM, Mustapha WAW, Hassan O (2009) Characterization of cyclodextrin complexes with turmeric oleoresin. Food chem 114: 459-465.
24. Hammoud Z, Abada MB, Greige-Gerges H, Elaissari A, Jemâa JMB (2022) Insecticidal effects of natural products in free and encapsulated forms: an overview. J Nat Pestic Res 100007.
25. Hammoud Z, Gharib R, Fourmentin S, Elaissari A, Greige-Gerges H (2020) Drug-in-hydroxypropyl-β-cyclodextrin-in-lipoid S100/cholesterol liposomes: Effect of the characteristics of essential oil components on their encapsulation and release. Int J Pharmac 579: 119-151.
26. Haouel S, Mediouni-Ben Jemâa J, Khouja ML (2010) Postharvest control of the date moth *Ectomyelois ceratoniae* using eucalyptus essential oil fumigation. Tunis J Plant Prot 5: 201-212.
27. Jiang T, Liao W, Charcosset C (2020) Recent advances in encapsulation of curcumin in nanoemulsions: A review of encapsulation technologies, bioaccessibility and applications. Food Res Int 132: 109-135.

28. Kalemba D, Kunicka A (2003) Antibacterial and antifungal properties of essential oils. Current Med Chem 10: 813-829.
29. Kfoury M, Auezova L, Greige-Gerges H, Fourmentin S (2019) Encapsulation in cyclodextrins to widen the applications of essential oils. Environ Chem Lett 17: 129-143.
30. Kfoury M, Landy D, Auezova L, Greige-Gerges H, Fourmentin S (2014) Effect of cyclodextrin complexation on phenylpropanoids' solubility and antioxidant activity. Beilstein J Org Chem 10: 2322.
31. Khemira S, Mediouni-Ben Jemâa J, Haouel S, Khouja M (2012) Repellent activity of essential oil of *Eucalyptus astringens* against *Rhyzopertha dominica* and *Oryzaephilus surinamensis*. International symposium on Medicinal and Aromatic Plants-SIPAM 2012 997, pp 207-213.
32. Kolb B, Ettre LS (2006) Static headspace-gas chromatography: theory and practice. John Wiley & Sons, New Jersey, pp 191-204.
33. Kordali S, Cakir A, Ozer H, Cakmakci R, Kesdek M, Mete E (2008) Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum acutiden*s and its three components, carvacrol, thymol and p-cymene. Bioresour Technol 99: 8788-8795.
34. Maes C, Bouquillon S, Fauconnier ML (2019) Encapsulation of essential oils for the development of biosourced pesticides with controlled release: A review. Mol 24: 25-39.
35. Matos LF, Da Cruz Lima E, De Andrade Dutra K, Navarro DM, Alves JLR, Silva GN (2020) Chemical composition and insecticidal effect of essential oils from *Illicium verum* and *Eugenia caryophyllus* on *Callosobruchus maculatus* in cowpea. Ind Crops Prod 145: 112-118.
36. Mediouni Ben Jemâa J, Haouel S, Khouja ML (2013) Efficacy of Eucalyptus essential oils fumigant control against *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae) under various space occupation conditions. J Stored Prod Res 53: 67-71.
37. Mediouni Ben Jemâa J, Tersim N, Toudert KT, Khouja ML (2012) Insecticidal activities of essential oils from leaves of *Laurus nobilis* L. from Tunisia, Algeria and Morocco, and comparative chemical composition. J Stored Prod Res 48: 97-104.
38. Mediouni J, Dhouibi M (2007) Mass-rearing and field performance of irradiated carob moth *Ectomyelois ceratoniae* in Tunisia. Area-wide control of insect pests, Springer, Dordrecht, pp 265-273.
39. Melanie M, Miranti M, Kasmara H, Malini DM, Husodo T, Panatarani C, Joni IM, Hermawan W (2022) Nanotechnology-based bioactive antifeedant for plant protection. Nanomater 12: 630-637.
40. Melito S, Petretto GL, Chahine S, Pintore G, Chessa M (2019) Seasonal variation of essential oil in *Rosmarinus officinalis* leaves in Sardinia. Nat Prod Commun 14: 1-7.
41. Mirzabegi Kesbi O, Rajabipour A, Omid M, Goldansaz SH (2023) Effect of postharvest microwave heat treatment of pomegranate on carob moth, *Ectomyelois ceratoniae*, control and quality parameters. J Hortic Postharvest Res 6: 105-114.
42. Moretti Mario DL , Demontis S, Bazzoni E (2002) Essential Oil Formulations Useful as a New Tool for Insect Pest Control. AAPS Pharm Sci Tech 3: 2-11.

43. Negahban SM, Mojgan Z , Seyed AH (2012) Fumigant properties of nano-encapsulated essential oil from *Artemisia sieberi* on *Tribolium castaneum*. Proc 9th Int Conf on Controlled Atmosphere and Fumigation in Stored Products, Antalya, Turkey. 15 – 19 October 2012, ARBER Professional Congress Services, Turkey 101-105.
44. Numanoğlu U, Şen T, Tarimci N, Kartal M, Koo OM, Önyüksel H (2007) Use of cyclodextrins as a cosmetic delivery system for fragrance materials: linalool and benzyl acetate. Aaps Pharmscitech 8: 34-42.
45. Pintore G, Usai M, Bradesi P, Juliano C, Boatto G, Tomi F, Chessa M, Cerri R, Casanova J (2002) Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. oils from Sardinia and Corsica. flavour fragr J 17: 15-19.
46. Rakmai J, Cheirsilp B, Torrado-Agrasar A, Simal-Gándara J, Mejuto JC (2017) Encapsulation of yarrow essential oil in hydroxypropyl-beta-cyclodextrin: physiochemical characterization and evaluation of bio-efficacies. CyTA-J Food 15: 409-417.
47. Rašković A, Milanović I, Pavlović N, Ćebović T, Vukmirović S, Mikov M (2014) Antioxidant activity of rosemary (*Rosmarinus officinalis* L.) essential oil and its hepatoprotective potential. BMC complement Altern Med 14: 1-9.
48. Sebaaly C, Charcosset C, Fourmentin S, Greige-Gerges H (2018) Potential applications of cyclodextrin inclusion complexes, liposomes, and drug-in-cyclodextrin-in-liposome in food industry and packaging. Role of materials science in food bioengineering. Elsevier, pp 187-234.
49. Shakeri M, Sadatakhavi Y (2004) Diseases and pests of pomegranate. Yazd: Tasbih Publications, Iran.
50. Sherje AP, Dravyakar BR, Kadam D, Jadhav M (2017) Cyclodextrin-based nanosponges: A critical review. Carbohydr Polym 173: 37-49.
51. Soltani A, Ncibi S, Djebbi T, Sadraoui IA, Abada MB, Chargui H, Hassine K, Laabidi A, Mahmoudi H, Majdoub H (2022) Microencapsulation of rosemary *Rosmarinus officinalis* essential oil by Chitosan-gum Arabic and its application for the control of two secondary pests of stored cereals.
52. Szejtli J (2004) Past, present and futute of cyclodextrin research. Pure Appl Chem 76: 1825-1845.
53. Tao S, Yue W, Zhong M, Chen Z, Ren Y (2014) Fabrication of graphene-encapsulated porous carbon–metal oxide composites as anode materials for lithium-ion batteries. ACS Appl Mater Interfaces 6: 6332-6339.
54. Teixeira EI, Fischer G, Van Velthuizen H, Walter C, Ewert F (2013) Global hot-spots of heat stress on agricultural crops due to climate change. Agric For Meteorol 170: 206-215.
55. Tudi M, Daniel Ruan H, Wang L, Lyu J, Sadler R, Connell D, Chu C, Phung DT (2021) Agriculture development, pesticide application and its impact on the environment. Int J Environ Res Public Health 18: 1112-1118.
56. Waleczek K, Marques HC, Hempel B, Schmidt P (2003) Phase solubility studies of pure (−)-α-bisabolol and camomile essential oil with β-cyclodextrin. Eur J Pharm Biopharm 55: 247-251.

57. Xu C, Lei C, Meng L, Wang C, Song Y (2012) Chitosan as a barrier membrane material in periodontal tissue regeneration. J Biomed Mater Res Part B Appl Biomater 100: 1435-1443.
58. Zhu Y, Li C, Cui H, Lin L (2021) Encapsulation strategies to enhance the antibacterial properties of essential oils in food system. Food Control 123: 107856-107862.
59. Ziaee M, Moharramipour S, Mohsenifar A (2014) Toxicity of C arum copticum essential oil-loaded nanogel against *Sitophilus granarius* and *Tribolium confusum*. J Appl Entomol 138: 763-771.
60. Zielińska A, Ferreira NR, Feliczak-Guzik A, Nowak I, Souto EB (2020) Loading, release profile and accelerated stability assessment of monoterpenes-loaded solid lipid nanoparticles (SLN). Pharm Dev Technol 25: 832-844.

Figures

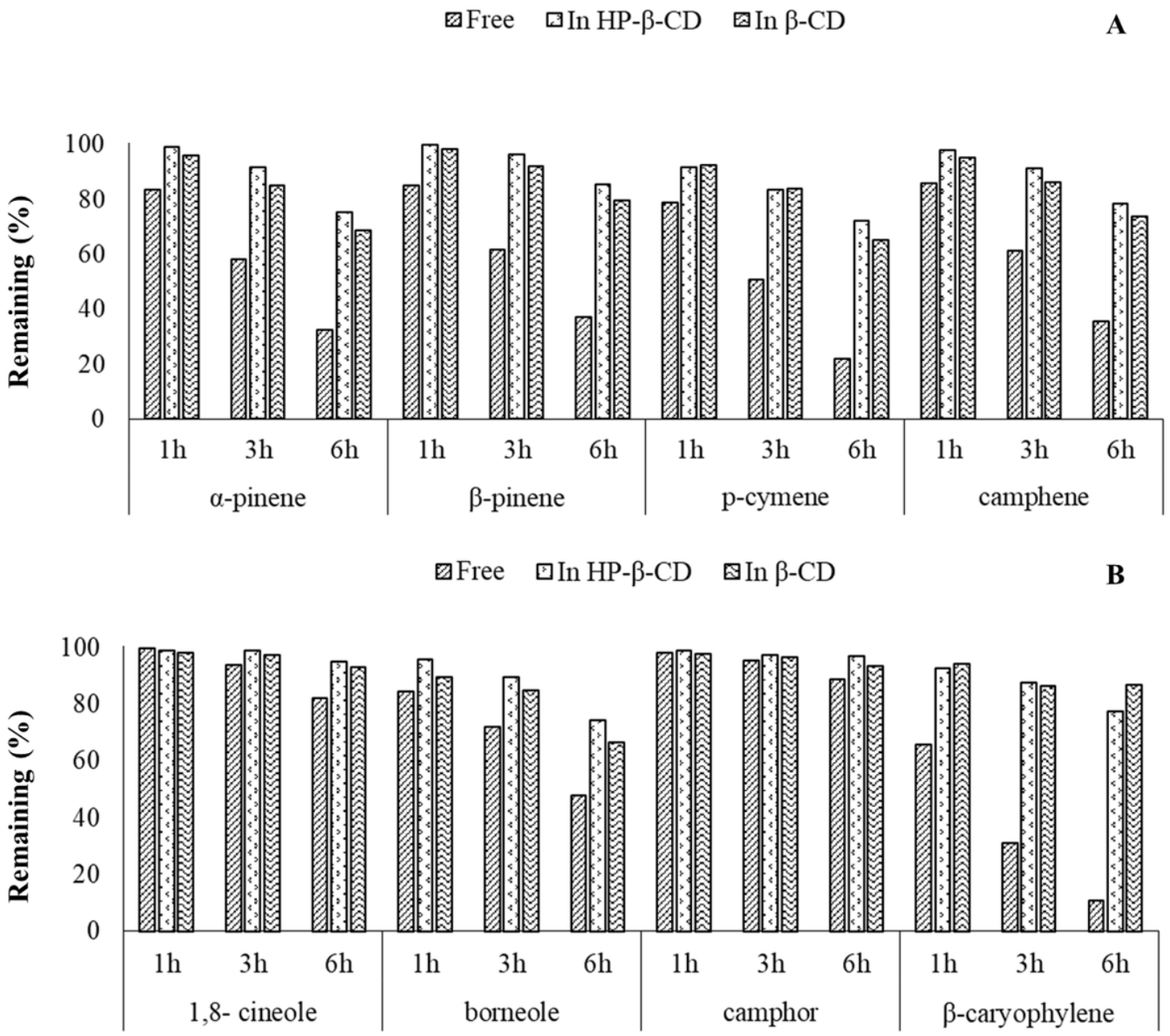

Figure 1

Remaining of *Rosmarinus officinalis*essential oil volatile compounds free and encapsulated in HP-β-CD and β-CD (A: Hydrocarbons Monoterpenes and B: Oxygenated monoterpenes)

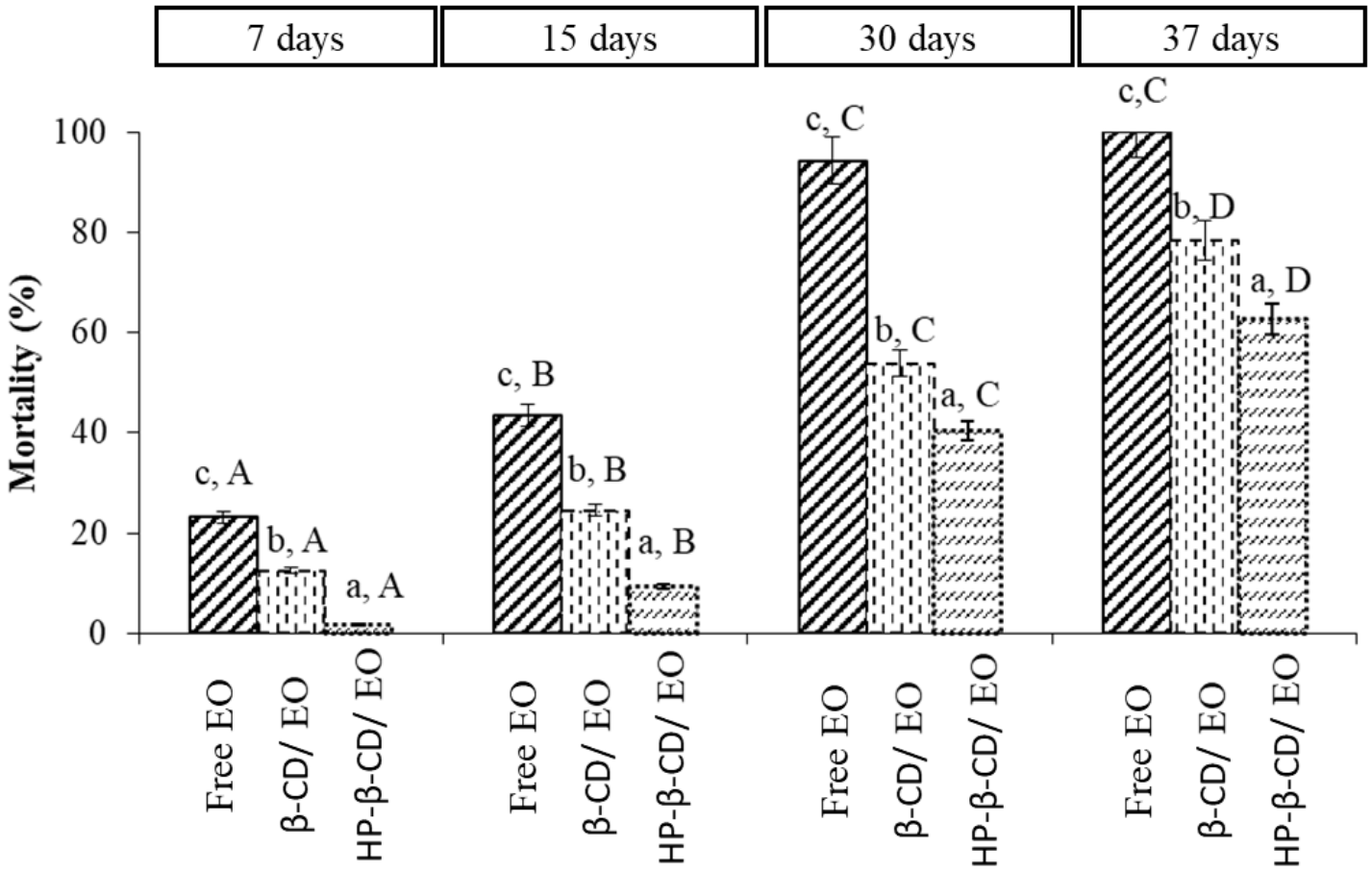

Figure 2

Mortality of *Ectomyelois ceratoniae* fifth instars larvae exposed to free and encapsulated *Rosmarinus officinalis* essential oil within (β-CD and HP-β-CD) (Letters denotes significant differences at $P<0.05$ in percent mortality for each exposure period (uppercase) and each treatment (lowercase); Duncan's test)

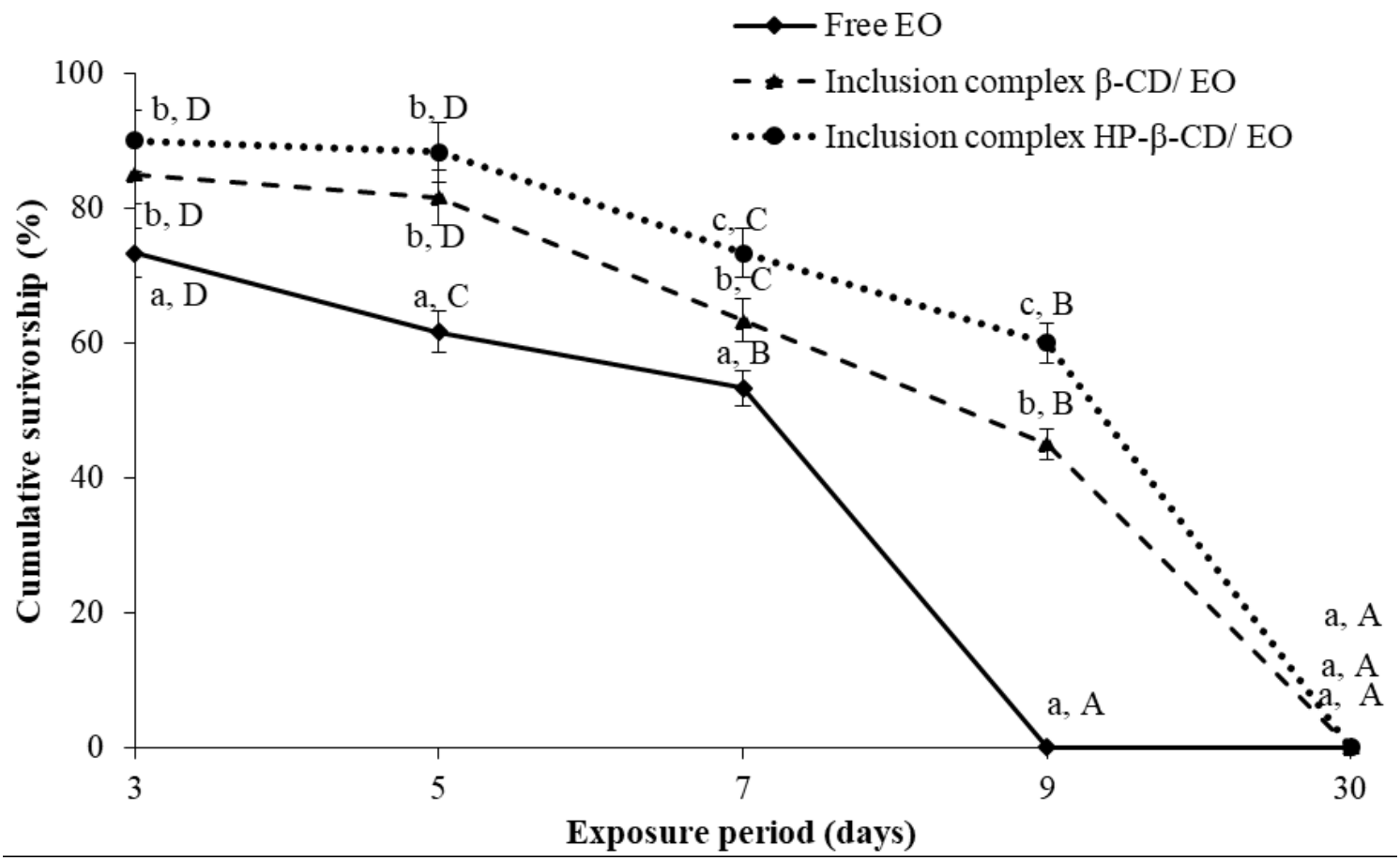

Figure 3

Persistence of the insecticidal effect of the free and encapsulated *Rosmarinus officinalis* essential oil (different letters indicate significant differences in percent mortality for each treatment (lowercase) and exposure period (uppercase); Duncan's test at P <0.05)