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Nanoparticles loading essential oils as alternative solution for the postharvest control of the date moth *Ectomyelois ceratoniae* (Pyralidae) †

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† Presented at the 1st International Electronic Conference on Entomology (IECE 2021), 1–15 July 2021; Available online: https://iece.sciforum.net/.

Abstract: *Ectomyelois ceratoniae* (Pyralidae) is the main insect pest attacking dates in field and storage in Tunisia. Postharvest protection is relied on fumigants. This work investigates fumigant toxicity of 2-hydroxypropyl-beta-cyclodextrin (HP-β-CD) loading *Rosmarinus officinalis* essential oil and its major constituents (1,8-cineole, α-pinene, camphor, borneol) against E. ceratoniae larvae and adults. Results revealed that mortalities reached 56.86; 60.71; 64.28; 50 and 75% respectively for the crude essential oil, α-pinene, camphor, borneol and 1,8-cineole after 30 storage days at the concentration 90 µl/l air. Also, results indicated that 1,8-cineole, camphor and α-pinene showed the strongest inhibitory effects of adults emergence.

Keywords: *Ectomyelois ceratoniae*; *Rosmarinus officinalis*; Cyclodextrin; Dates; Fumigation

Citation: Ben Abada, M.; Tahri, M.; Fourmentin, S; Mediouni-Ben Jemâa, J. Nanoparticles loading essential oils as alternative solution for the postharvest control of the date moth *Ectomyelois ceratoniae* (Pyralidae), in Proceedings of the 1st International Electronic Conference on Entomology, 1–15 July 2021, MDPI: Basel, Switzerland, doi:10.3390/IECE-10394

Published: 30 June 2021

1. Introduction

Essential oils and their compounds exhibited a variety of biological functions including insecticidal activities [1,2]. Indeed, various studies reported the potential of many plant derived essential oils against insect pests mainly those infesting stored products [3,4].

Despite the promising potential of EO, their applications are limited due to their degradation and volatilizations because of their volatile components [5]. So, the usage of controlled-release formulations could be an interesting option for the development of bioinsecticide [6]. In this respect, in last years, cyclodextrin were successfully used as enclusion matrix to encapsulate bioactive substances including essential oils' compounds [7]. Encapsulation prolongs the active compounds viability [8]; increase the compounds solubility in water [9]; and prevent from light and environmental degradation factors [10]. Rosemary (*Rosmarinus officinalis* L.) is native to Mediterranean region [11]. Rosemary essential oil is kwnown by its biological activities like antifungal [12], antibacterial [13], antioxidant properties [14] and insecticidal potential [15].

The present research was designed to explore the fumigant toxicity of the 2-hydroxypropyl-beta-cyclodextrin (HP-β-CD) loading *R. officinalis* essential oil or its major constituents (1,8-cineole, α-pinene, camphor,borneol) nanoparticles against larvae and adults of the date moth *Ectomyelois ceratoniae* for different storage periods.

2. Materials and Methods

2.1. Insect rearing.

E. ceratoniae rearing was conducted under controlled laboratory conditions (Temperature 28 ± 1°C, photoperiod of 12L/12 D, 75 ±5% relative humidity). Rearing was conducted according to the procedure described by [16].

2.2. Plant materials and essential oils extraction

Arial parts of spontaneously grown R. officinalis were collected from Korbous arboretum (36° 48′ 59'' N 10° 34′ 07'' E 401) in March 2020. Samples were air-dried at room temperature for two weeks. Essential oils were obtained by hydrodistillation using a modified Clevenger-type device for 4 h. Essential oils were dried using anhydrous sodium sulphate. Yields were calculated based on plant materials dry weight. Chromatographic analyzes were performed using an Agilent-Technologies 6890 N Network GC system equipped with a flame ionization detector and HP-5MS capillary column (30 mx0.25mm, film thickness 0.25 μm; Agilent-Technologies, Little Falls, CA, USA).

2.3. Standards and chemicals

1,8-cineole, pinene and camphor standards were supplied from Sigma-Aldrich (purity grade).

2.4. Encapsulation in HP-β-CD

Excess amounts from standards (1,8-cineole, pinene, camphor, borneol) were added to 5 ml HP-β-CD solutions (10,25,50,75,100 mM). Mixtures were shaken at 25°C for 24h then filtered using a 0.45 μm membrane filter. HPLC method was used to determine the oil and standards concentrations in the filtrates. Encapsulation efficiency was calculated according to the equation: EE (%) = $(m_{exp}/m_i)*100$ where m_{exp} is the mass of the oil or standards experimentally determined by HPLC in the inclusion complex solution and m_i is the mass of the oil or standards initially used to prepare the inclusion complex.

2.5. Fumigant toxicity bioassays

2.5.1. Larval mortality and adult emergence

Larval mortality was assessed for three essential oil concentrations, respectively 30, 60 and 90 μl/l air. For the standards, the respective used concentrations were 30; 60 and 90 μg/l air. For each trial, three replications were performed. 2 cm diameter Whatman filter papers were impregnated with the different oil concentrations. Impregnated filter paper was attached to the covers of 1L glass bottle. Each bottle contained 20 unsexed larvae (placed in dates). Mortality was recorded after 7, 15, 30 and 37 days. Abbott formula [17] was used to assess the mortality.

Moths' emergence was registered daily for each trial. Percentage of emerged adults was determined by formula of Arivoli [18].

2.5.2. Lethal concentration and Lethal time bioassays

Probit analysis [19]) was used to determine the respective lethal concentration (LC*50*) and lethal Time (LT*50*) values for the various treatments after 15 days of exposure.

2.5.3. Formulation insecticidal efficacy

Same procedure was employed to evaluate the larvicidal and adulticidal effect of the formulations. For that, the solid HP-β-CD/R. officinalis and HP-β-CD /standards inclusion complex were placed in 1L glass bottles containing 20 unsexed larvae and 10 adults. Mortality data was assessed by Abbott's formula [17], adult emergence percentages were determined by Arivoli formula [18].

2.6. Statistical analysis

Data were subjected to analysis of variance ANOVA using SPSS software version 20 (IBM Corporation NY, USA). 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.

3. Results

3.1. Essential oil yield and chemical composition

The chemical composition of *R. officinalis* essential oil is reported in Table 1 as percentages of chemical classes.

Table 1. Chemical classes of *R. officinalis* essential oil collected from arboretum of Korbous and extraction yield

Chemical classes	Proportion (%)
Monoterpenes hydrocarbons	34.47
Oxygenated Monoterpenes	61.88
Sesquiterpene Hydrocarbons	2.24
Others	0.53
Total of identified compounds	99.12
Extraction yield	0.6

Results showed that *R. officinalis* essential is dominated by Monoterpenes with respective percentages of 61.88% for oxygenated Monoterpenes and 34.47% for Monoterpenes hydrocarbons. On the other hand, extraction yield was 0.6%.

3.2. Encapsulation efficiency

The Encapsulation Efficiency Encapsulation of the various compounds (α-pinene, 1,8-cineole, Camphor)r and *R. officinalis* essential oil in HP-β-CD inclusion complex are presented in Table 2.

Table 2. Encapsulation Efficiency (EE%)of α-pinene, 1,8-cineole, Camphor and R. officinalis essential oil in HP-β-CD inclusion complex.

α-pinene	1,8-cineole	Camphor	Essential oil
20	63	23	22

Results indicated that the encapsulation efficiency varied among compounds and the essential oil. The highest EE value was obtained for 1,8-cineole with 63%. However, α-pinene, Camphor and the essential oil presented close respective EE values of 20, 23 and 22%.

3.3. Insecticidal toxicity

3.3.1. Insect mortality

The insecticidal activities of free and encapsulated compounds and *R. officinalis* essential oil were illustrated in figure 1.

Results (Figure 1) revealed that fumigant toxicity varied between free and encapsulated compounds. For all compounds and *R. officinalis* essential oil, toxicity was greater in the case of free compared to encapsulation. Moreover, results indicated that toxicity increased for encapsulated compounds over exposure periods.

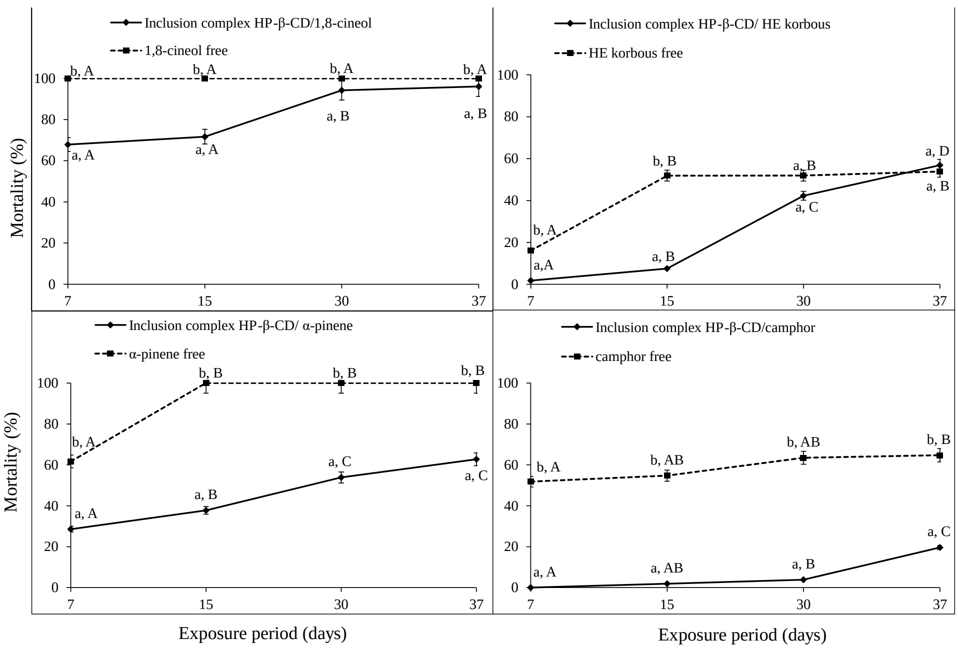

Figure 1. Mortalities (%) of fifth instar larvae of Ectomyelois ceratoniae exposed to free and encapsulated (HP-β-CD inclusion complex) *R. officinalis* essential oil and the compounds (Letters denotes significant differences at P<0.05 in percent mortality for each exposure period (uppercase) and each concentration (lowercase); Duncan's test).

3.3.2. Lethal concentrations

Table 3 cited the lethal concentrations values of *R. officinalis* essential oil and the three standards determined by Probit method.

Table 3. Median Lethal Concentrations values (µl/l air) of 1,8-cineole, α-pinene Camphor and *R. officinalis* against fifth instar larvae of *Ectomyelois ceratoniae*

Compounds	**1,8-cineole**	**α-pinene**	**Camphor**	***R. officinalis***
CL_{50} [a,b]	65.13	97.415	223.764	160.008
(µl/l air)	(46.45-122.61)	(63.71- 146.12)	(47.94- 312,22)	(136.46-229)

a Units LC_{50} = µm/l air
b 95% lower and upper confidence limits are shown in parentheses.

Results pointed out that 1,8-cineole and α-pinene were more toxic than *R. officinalis* and Camphor.

3.3.3. Lethal Time

Table 4 reported the lethal time values of *R. officinalis* essential oil and the three standards determined by Probit method.

Table 4. Median Lethal Time values (days) of 1,8-cineole, α-pinene Camphor and R. officinalis against fifth instar larvae of *Ectomyelois ceratoniae*

Compounds	**1,8-cineole**	**α-pinene**	**Camphor**	***R. officinalis***
LT_{50} [a,b] (days)	23.918	29	22.958	35.806
	(18.12-33.48)	(17.86-48.69)	(15.41-68.74)	(27.233-64.88)

a Units LT_{50} = days
b 95% lower and upper confidence limits are shown in parentheses.

Results revealed that lethal time values varied among compounds and essential oil. 1,8-cineole and Camphor were more toxic compared to α-pinene and *R. officinalis* essential oil.

4. Discussion

The postharvest control of *E. ceratoniae* is mainly based on the use of synthetic fumigants [20]. Recently, the use of essential oils has gained attention as pest management agents, due to their insecticidal proprieties [21].

This work indicated the insecticidal potential of R. officinalis essential oil in controlling the date moth E. ceratoniae. Our results are consistent with those found by [6]. Moreover, [22] pointed out that LC_{50} values varied between 14.02 and 171.11 µl/ l air. Besides, [23] reported that for *E. ceratoniae*, TL*50* values ranged from 39.50 to 18.27 h. On the other hand, this work showed the ability of HP-β-CD to encapsulate and solubilize guests (*R. officinalis* essential oil, 1,8-cineole, α-pinene and camphor) [24,25].

This section is not mandatory but may be added if there are patents resulting from the work reported in this manuscript.

This study pointed out that encapsulated oils could be combined with existing techniques in an integrated pest management strategy for the management of stored dates' pests.

Author Contributions: MAHA BEN ABADA Conceptualization, methodology, writing—original draft preparation, software, data curation, writing—review and editing; MAROUA TAHRI resources, data curation; formal analysis; resources; SOPHIE FOURMENTIN Conceptualization, validation, supervision, project administration, review and editing; JOUDA MEDIOUNI-BEN JEMÂA Conceptualization, validation, supervision, project administration, review and editing. All authors have read and agreed to the published version of 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".

Informed Consent Statement: Not applicable

Data Availability Statement: Data available in a publicly accessible repository.

Acknowledgments: Authors thank Ms. Emna BOUSHIH for her technical support in the laboratory experiments.

Conflicts of Interest: "The authors declare no conflict of interest".

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