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**Ghozlene Aouadi, Soumaya Haouel, Abir Soltani, Maha Ben Abada, Emna Boushah, Salem Elkahoui, Faiza Taibi, Jouda Mediouni Ben Jemâa, et al**

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# Screening for insecticidal efficacy of two Algerian essential oils with special concern to their impact on biological parameters of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae)

Ghozlene Aouadi<sup>1</sup> · Soumaya Haouel<sup>2</sup> · Abir Soltani<sup>2</sup> · Maha Ben Abada<sup>2</sup> · Emna Boushah<sup>2</sup> · Salem Elkahoui<sup>3</sup> · Faiza Taibi<sup>1,4</sup> · Jouda Mediouni Ben Jemâa<sup>2</sup> · Salima Bennadja<sup>4</sup>

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## Abstract

Chemical composition of Algerian *Mentha rotundifolia* and *Myrtus communis* essential oils, their insecticidal activities and their impact on some biological parameters of the Mediterranean flour moth *Ephestia kuehniella* were assessed. Results showed that *M. rotundifolia* essential oil contained piperitenone oxide (46.06%), D-limonene (9.10%), cis-piperitone oxide (6.81%), and endo-borneol (4.64%) as major compounds, while *M. communis* oil was rich in  $\alpha$ -pinene (29.08%), 1,8-cineole (36.82%),  $\alpha$ -terpineol (6.42%), geranyl acetate (4.38%), and  $\beta$ -linalool (4.04%). The fumigant potential and contact toxicity tests against *E. kuehniella* demonstrated the effectiveness of *M. rotundifolia* essential oil ( $LC_{50}=0.54 \mu\text{L/L}$  air,  $LC_{50}=0.004 \mu\text{L/cm}^2$ ) compared to *M. communis* oil ( $LC_{50}=2.91 \mu\text{L/L}$  air,  $LC_{50}=0.025 \mu\text{L/cm}^2$ ). Moreover, results revealed that all biological parameters were significantly affected (fecundity: 6 eggs/female, oviposition deterrence: 96.62%, log fertility: 0, hatching rate: 0%, copulation rate: 0% for *M. rotundifolia* oil against fecundity: 93 eggs/female, percentage of oviposition deterrence: 47.85%, log fertility: 6.7, hatching rate: 57%, copulation rate: 53.33% for *M. communis* oil). This work supports the use of botanical insecticide as active pest control agents under storage conditions.

**Keywords** *Ephestia kuehniella* · Essential oil · Round-leaved mint · Common myrtle · Copulation rate

## Introduction

Agricultural products are often subject to biotic and abiotic damage during production and conservation. Contamination of food commodities by insect pests is an important quality control issue of concern for the food industries (Rajendran and Sriranjini 2008). About 35% on the field

and 14% in storage (around 50% in total) crops are lost annually because of insect pests, which adversely affected the world food production during crop growth, harvest and storage (Jitendra et al. 2009). Among damaging insects, the Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) is one of the major pests in industrial flour mills (Jacob and Cox 1977), in Algeria (Hami et al. 2005) and Tunisia (Jarraya 2003). It is a worldwide pest that causes major economic losses in the Mediterranean basin and near East regions (Al-Izzi et al. 1985). Its main habitats are flour and grout mills, corn milling plants, bakeries and any other place used for processing grains or preparing flour products (Ben Achour et al. 2008). Larvae of *E. kuehniella* reduce both quantitative and qualitative quality of the products by feeding, by their presence and by the production of feces and webbing (Johnson et al. 1997). Last years, the overuse of synthetic insecticides and fumigants such as phosphine for grain storage has resulted in a number of problems, including the development of insecticide resistance among insect pests of stored grains (Sousa et al. 2009). Currently, stored-product pest control

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strategies tend to emphasize the non-chemical aspects of pest control with the judicious use of pesticides (Titouhi et al. 2017). In this respect, plant extracts are safe, eco-friendly and more compatible with environmental components compared to synthetic pesticides and so ranked under Green pesticides category (Rahman et al. 2016).

Besides, Kim et al. (2003) showed that plant extracts are often active against a limited number of target insects, are biodegradable, and are potentially suitable for use in integrated pest management. These authors reported also that plant extracts could lead to the improvement in new classes of nontoxic insect control. Plant essential oils have been recently qualified as ecological alternatives to chemical pesticides due to their multifunctional efficacy including anti-insect pest activity (Isman and Grieneisen 2014; Mediouni Ben Jemâa 2014). Furthermore, essential oils possess important contact and fumigant toxicity (Liu and Ho 1999; Abdelgaleil et al. 2009; Kasrati et al. 2015; Quan et al. 2018), antifeedant activity (Huang et al. 1997; Ebadollahi 2013), repellent activity (Hori 2003; Prajapati et al. 2005; Nerio et al. 2009; Chang et al. 2017) as well as development and growth inhibitory activity (Waliwitiya et al. 2009; Sangha et al. 2017) against various insect species and are regarded as environmentally compatible pesticides (Cetin et al. 2004).

Their lipophilic nature facilitates their interference with basic metabolic, biochemical, physiological and behavioral functions of insects (Nishimura 2001). Commonly, essential oils with anti-insect pest ability are known to possess insecticidal and repellent effects by neurotoxic mode of action (Kostyukovsky et al. 2002). *Mentha rotundifolia* (L.) huds (Lamiales: Lamiaceae) and *Myrtus communis* (L.) (Myrtales: Myrtaceae) are commonly known under the vernacular names of round leaf and common myrtle. These aromatic plant species, grow wild in northern Algeria, are widely used by the population in traditional phytotherapy for their therapeutic virtues. The essential oils extracted from the leaves of *Myrtus communis* have shown insecticidal activity against *E. kuehniella*, *Plodia interpunctella*, *Acanthoscelides obtectus* (Ayvaz et al. 2010), *Tribulium castaneum* (Senfi et al. 2013), *Callosobruchus maculatus* (Khani and Farzaneh 2012), and *Trogoderma granarium* (Tayoub et al. 2012). Few studies have reported the insecticidal potential of *M. rotundifolia* essential oil on stored-product pests (El Arch et al. 2003; Brahmi et al. 2016). Nevertheless, no study has been reported before on the insecticidal activity of *M. rotundifolia* against *E. kuehniella* adults.

This work aimed to assess the fumigant and contact toxicity of *M. rotundifolia* and *M. communis* essential oils from Algeria as well as their effects on longevity, fecundity, fertility, copulation rate and hatching rate against new emerged adults of a Tunisian strain of the Mediterranean flour moth *E. kuehniella*.

## Materials and methods

### Insect rearing

A rearing colony of *E. kuehniella* was maintained on wheat flour and semolina under laboratory-controlled condition (temperature of  $25\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ , a relative humidity of  $65 \pm 5\%$  and darkness). Insects were maintained in 2-L plastic storage boxes. Unsexed adults aged between 24 and 48 h were used for the bioassays.

### Plant material

*Myrtus communis* aerial parts samples were collected during October 2017 from the Edough Massif (North-East Algeria-Annaba:  $36^{\circ} 55' \text{N}$ ,  $7^{\circ} 36' \text{E}$ ), while *M. rotundifolia* aerial parts were sampled during August 2018 from the locality of Berrahal (North-East of Algeria-Annaba:  $36^{\circ} 50' \text{N}$ ,  $7^{\circ} 27' \text{E}$ ).

### Extraction of the essential oils

100 g of dry leaves of each plant was hydrodistilled during 90 min using a Clevenger apparatus. The crude-extracted essential oils were stored in opaque flasks in a refrigerator at  $4\text{ }^{\circ}\text{C}$ . Essential oils' yields obtained from each plant species were evaluated according to AFNOR (1986) formula: oil% (w/w) = weight of essential oil (g)/weight of plant material (g)  $\times 100$ .

### Gas chromatography–mass spectrometry (GC/MS) analysis

The analysis was carried out using an Agilent 7890A gas chromatograph combined to an Agilent 5972C mass spectrometer with electron impact ionization (70 eV). The mass spectrometer was equipped with a capillary column HP-5 MS (19091S-433), length 30 m, diameter 250  $\mu\text{m}$ , and 2.5  $\mu\text{m}$  film thicknesses (5% phenyl methyl silicone, 95% dimethylpolysiloxane; Hewlett-Packard, CA, USA). The column temperature was automated to rise from 50 to 250  $^{\circ}\text{C}$  at a rate of 7  $^{\circ}\text{C}/\text{min}$ . The transfer line temperature was 250  $^{\circ}\text{C}$ . The flow rate of helium (carrier gas) was 1 mL/min. A sample of 2  $\mu\text{L}$  was manually injected with a constant pressure of 7.65 psi using split mode (split ratio 50:1). Scan time and mass range were 1 s and of 45–400 m/z, respectively. The essential oils components were identified by comparing their retention indices (RIs) relative to *n*-alkanes with those of authentic compounds published in the literature or available in our laboratory. Furthermore, the identification was confirmed by matching

their mass spectra with those recorded in Wiley Registry 9th Edition/NIST 2011 edition mass spectral library. The composition of the essential oils was stated as a relative percentage of total peak area.

## Bioassays

### Fumigant toxicity

The insecticidal activity of *M. rotundifolia* and *M. communis* essential oils was evaluated by the fumigant test according to Titouhi et al. (2017). Ten adults of *E. kuehniella* were placed in Plexiglas bottles of 38 mL volume. The bottom surface of the screw caps was lined with Whatman n° 1 paper disks (2 cm diameter with a 3 cm length fixing tab). Using a micropipette, a series of essential oils doses (without the use of any solvent) were deposited on the filter paper disks. Doses used in the essay were 0.05, 0.125, and 0.5  $\mu\text{L}$  giving equivalent concentrations of 1.31, 3.28, and 13.16  $\mu\text{L/L}$  air. The caps were quickly screwed tightly onto the bottles. Three repetitions were carried out for each concentration of each essential oil including a negative control without essential oil and maintained under the same conditions described for breeding. Mortality was assessed by direct observation of insects every hour until total death. Insects are considered dead when no leg or antenna movements have been observed. Mortality was adjusted via the Abbott correction formula (Abbott 1925). Probit analysis (IBM SPSS V22.0) was used to estimate lethal concentrations  $\text{LC}_{50}$  and  $\text{LC}_{95}$  values and lethal time  $\text{LT}_{50}$ .

### Contact toxicity

The contact bioassay of *M. communis* and *M. rotundifolia* essential oils against *E. kuehniella* adults was done according to Mami Maazoun et al. (2017). A Whatman filter paper disks, having 9 cm of diameter, were treated with three doses 0.05, 0.125, and 0.5  $\mu\text{L}$  dissolved in acetone to give concentrations equivalent to  $9 \times 10^{-4}$ ,  $24 \times 10^{-4}$ , and  $99 \times 10^{-4}$   $\mu\text{L}/\text{cm}^2$ . Acetone was used as a negative control. The solvent was allowed to evaporate completely at room temperature for 5 min. Each filter paper disk was then placed in a glass Petri dish, and 10 adults of *E. kuehniella* were placed into the center of each Petri dish. Each hour, the number of dead insects was recorded. Tests were done in triplicate. The percentage of mortality was calculated using Abbott's correction formula (1925). Insects are considered dead when no leg or antenna movements have been observed. The  $\text{LC}_{50}$ ,  $\text{LC}_{95}$ , and  $\text{LT}_{50}$  values were calculated by using Probit analysis (IBM SPSS V20.0).

## Impact on biological parameters

The insecticidal effects of *M. communis* and *M. rotundifolia* essential oils through fumigation were studied by assessing their impact on longevity, fecundity, fertility, hatching rate, percentage of oviposition deterrence, and copulation rate of *E. kuehniella*. Five pairs of unsexed adults (0–24 h old) were exposed at the lethal concentration  $\text{LC}_{15}$ . The longevity of males and females, the total number of eggs laid per female (fecundity), the percentage of oviposition deterrence according to Pascual Villalobos and Robledo (1998), fertility, and the percentage of egg hatching were determined. Non-exposed adults kept under the same conditions served as a control. Experiments were replicated three times. For the copulation rate study, prior to exposure, virgin females were obtained by rearing last instar larvae of *E. kuehniella* separately in plastic boxes. Female's bursa copulatrix were dissected out under a binocular microscope in a saline solution (10% NaCl) and observed for the presence of spermatophores as a criterion of successful mating.

## Data analysis

For each biological parameter (longevity, percentage of oviposition deterrence, fecundity, fertility, hatching rate, and copulation rate of *E. kuehniella*), data were subjected to two-way ANOVA, with essential oils and exposure time as main fixed factors plus with essential oils \* exposure time interaction term. Differences in values of each essential oil and exposure time were tested by one-way ANOVA followed by Duncan test to detect significant differences in means at 0.05 percent level. In some cases, certain data have been transformed into a common logarithm or square root in order to obtain a normal distribution of the variables. All data values represented the mean of three replications and were expressed as the mean  $\pm$  standard deviation. All statistical analyses were achieved using SPSS statistical software version 22.0.

## Results

### Essential oil composition

Oil yields based on leaf dry matter weight were, respectively, 1.29% and 0.64% for *M. rotundifolia* and *M. communis*. GC and GC/MS analysis of *M. rotundifolia* and *M. communis* essential oils are reported in Table 1. Results indicated that *M. rotundifolia* essential oil contained 30 identified compounds constituting approximately 95.51% of the total content. The essential oil profile was characterized by piperitenone oxide (46.06%), d-limonene (9.10%), *cis*-piperitone

**Table 1** Essential oil composition (%) of *Mentha rotundifolia* and *Myrtus communis* leaves collected from Algeria

No.	Compounds	RI	<i>M. rotundifolia</i>	<i>M. communis</i>
1	$\alpha$ -Pinene	939	2.61	29.08
2	Camphene	954	1.69	1.91
3	Sabinene	976	0.91	–
4	Vinyl amyl carbinol	978	1.44	–
5	$\beta$ -Pinene	980	2.04	0.77
6	$\beta$ -Myrcene	991	1.39	–
7	$\beta$ -Ocimene	995	0.27	–
8	$\Delta$ -3-Carene	1011	–	0.64
9	<i>p</i> -Cimene	1023	–	1.52
10	<i>D</i> -Limonene	1028	9.10	–
11	1,8-Cineole	1033	0.45	36.82
12	$\gamma$ -Terpinene	1053	–	0.53
13	$\alpha$ -Terpinolene	1089	–	0.47
14	$\beta$ -Linalool	1098	–	4.04
15	Amyl vinyl carbinyol acetate	1110	0.67	–
16	<i>trans</i> -Pinocarveol	1138	–	1.04
17	Camphor	1143	0.57	0.46
18	( <i>R</i> )-Lavandulol	1148	0.26	–
19	Endo-borneol	1165	4.64	–
20	Terpinen-4-ol	1178	0.35	1.09
21	$\alpha$ -Terpineol	1189	0.82	6.42
22	5-acetyl-2-hydrazino-4-methylpyrimidine	1211	1.85	–
23	<i>trans</i> -Carveol	1230	–	0.33
24	Geraniol	1255	–	1.15
25	Linalyl acetate	1257	–	0.51
26	<i>cis</i> -Piperitone oxide	1261	6.81	–
27	Bornyl acetate	1285	0.52	–
28	<i>m</i> -Thymol	1290	0.37	–
29	Piperitenone oxide	1376	46.06	–
30	Geranyl acetate	1383	–	4.38
31	<i>cis</i> -Jasmone	1394	2.47	–
32	Methyl eugenol	1401	–	2.59
33	Caryophyllene	1420	3.18	0.42
34	$\alpha$ -Humulene	1454	0.42	–
35	<i>trans</i> - $\beta$ -farnesene	1458	0.87	–
36	$\alpha$ -Amorphene	1475	0.34	–
37	Germacrene D	1485	3.58	–
38	$\gamma$ -Cadinene	1513	0.46	–
39	1 <i>S</i> . <i>cis</i> -calamenene	1519	0.50	–
40	Caryophyllene oxide	1581	–	0.96
41	Veridiflorol	1592	0.47	–
42	$\alpha$ -Cadinol	1643	0.40	–
Total			95.51	95.13

–, compound not detected; RI, retention index calculated on a HP-5MS capillary column (30 m  $\times$  0.25 mm  $\times$  0.25 mm)

oxide (6.81%), and endo-borneol (4.64%). Concerning the essential oil of *M. communis*, 20 compounds representing 95.13% of the total oil composition were identified. The major components were  $\alpha$ -pinene (29.08%), 1,8-cineole (36.82%),  $\alpha$ -terpineol (6.42%), geranyl acetate (4.38%), and  $\beta$ -linalool (4.04%).

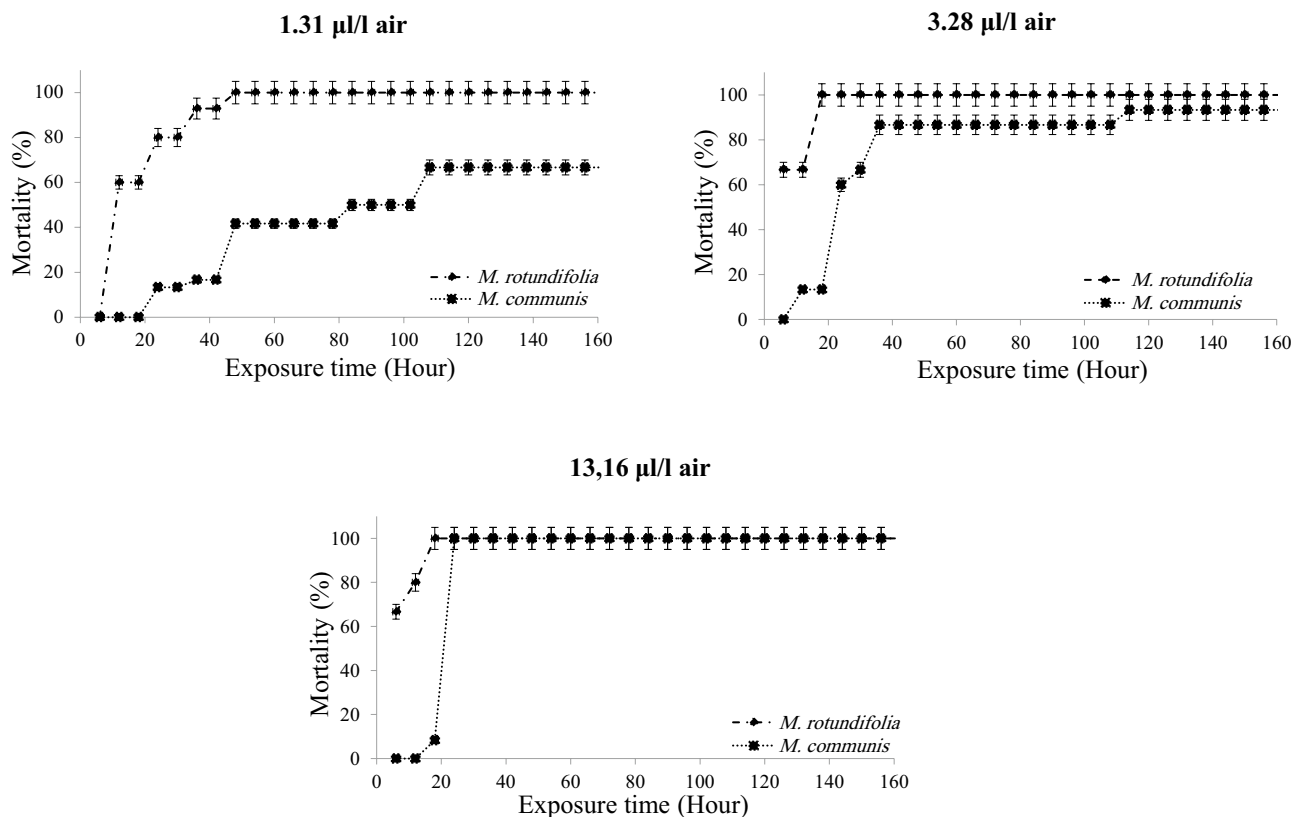
### Essential oil fumigant toxicity

Fumigant toxicity results are presented in Fig. 1 as a percentage of mortality of *E. kuehniella* adult's strains from Tunisia exposed to *M. rotundifolia* and *M. communis* essential oils collected from Algeria. Data revealed that fumigant toxicity depended significantly on plant species ( $F_{1,36} = 33.02$ ,  $p < 0.01$ ), concentrations ( $F_{2,36} = 20.01$ ,  $p < 0.01$ ), and exposure time ( $F_{1,36} = 4.82$ ,  $p < 0.01$ ). The mortality values increased depending on the increasing essential oil concentrations. Furthermore, *M. rotundifolia* essential oil was more toxic than *M. communis*. In fact, for the lowest concentration (1.31  $\mu$ L/L air), percentage mortality of *E. kuehniella* after 48 h attained, respectively, 100% and 41.66% with *M. rotundifolia* and *M. communis* essential oils. For the highest concentration (13.16  $\mu$ L/L air), *M. rotundifolia* and *M. communis* essential oils caused 100% mortality after, respectively, 18 and 24 h of exposure. Moreover, essential oil of *M. rotundifolia* caused significantly the highest adulticidal effects at all tested concentrations. Moreover, results indicated that *M. rotundifolia* essential oil showed 100% effectiveness at 3.28  $\mu$ L/L air after only 18 h of treatment.

Probit analyses have also confirmed that *E. kuehniella* was more susceptible to *M. rotundifolia* oil (Table 2). Additionally, the  $LC_{50}$  and  $LC_{95}$  values proved that *M. rotundifolia* essential oil was more toxic than *M. communis*. The  $LC_{50}$  values reached 0.54  $\mu$ L/L air for *M. rotundifolia* and 2.91  $\mu$ L/L air for *M. communis*. The  $LC_{95}$  was evaluated to be, respectively, 2.11  $\mu$ L/L air and 5.29  $\mu$ L/L air for *M. rotundifolia* and *M. communis* essential oils. Furthermore, the  $LT_{50}$  values ranged between 1.03 and 20.76 h for *M. rotundifolia* and from 20.19 h to 105.09 h for *M. communis* (Table 3).

### Essential oil contact toxicity

Fumigant toxicity data are illustrated in Fig. 2 as percentage of mortality of *E. kuehniella* adults. Statistical analysis revealed significant differences in percentage of mortality as a function of essential oils treatments ( $F_{1,12} = 10.08$ ,  $p < 0.01$ ) and concentrations ( $F_{2,12} = 7.58$ ,  $p < 0.01$ ) after 72 h of exposure. But no significant differences were detected in percentage of mortality after 120 h of exposure time [essential oils treatments ( $F_{1,12} = 4.00$ ,  $p > 0.05$ ) and concentrations ( $F_{2,12} = 1.75$ ,  $p > 0.05$ )]. Results indicated the sensitivity of *E. kuehniella* adults to both essential oils



**Fig. 1** Mortality (%) of *Ephestia kuehniella* adults exposed for various periods of time and various concentrations to *Mentha rotundifolia* and *Myrtus communis* essential oils

**Table 2** Fumigant toxicity of *Mentha rotundifolia* and *Myrtus communis* essential oils against *Ephestia kuehniella*

Essential oils	LC <sub>50</sub> <sup>(a,b)</sup> (µL/L air)	LC <sub>95</sub> <sup>(a,b)</sup> (µL/L air)	χ <sup>2</sup>	Slope ± SE	Sig	Degrees of freedom
<i>M. rotundifolia</i>	0.54 (0.05–0.94)	2.11 (1.74–3.95)	0.26	1.05 ± 0.38	0.56	1
<i>M. communis</i>	2.91 (2.63–4.84)	5.29 (4.61–6.46)	0.01	0.67 ± 0.10	1.00	1

<sup>a</sup>Units LC<sub>50</sub> and LC<sub>95</sub> = ml/l air. Applied for 24 h at 25 °C

<sup>b</sup>95% lower and upper confidence limits are shown in parentheses

**Table 3** LT<sub>50</sub> values of *Mentha rotundifolia* and *Myrtus communis* essential oils against *Ephestia kuehniella*

Essential oils	Concentration (µL/L air)	LT <sub>50</sub> <sup>(a,b)</sup>	χ <sup>2</sup>	Slope ± SE	Sig	Degrees of freedom
<i>M. rotundifolia</i>	1.31	20.76 (9.80–29.16)	5.87	0.10 ± 0.01	0.31	5
	3.28	5.30 (1.6–17.6)	3.52	0.37 ± 0.05	0.10	5
	13.16	1.03 (0.14–3.94)	4.04	0.53 ± 0.08	0.20	5
<i>M. communis</i>	1.31	105.09 (76.42–173.91)	5.66	0.013 ± 0.001	0.34	5
	3.28	22.41 (10.32–36.42)	25.12	0.02 ± 0.002	0.00	5
	13.16	20.19 (17.35–22.77)	3.90	0.06 ± 0.005	1.00	5

<sup>a</sup>Units LT<sub>50</sub> = h. Applied at 25 °C

<sup>b</sup>95% lower and upper confidence limits are shown in parentheses

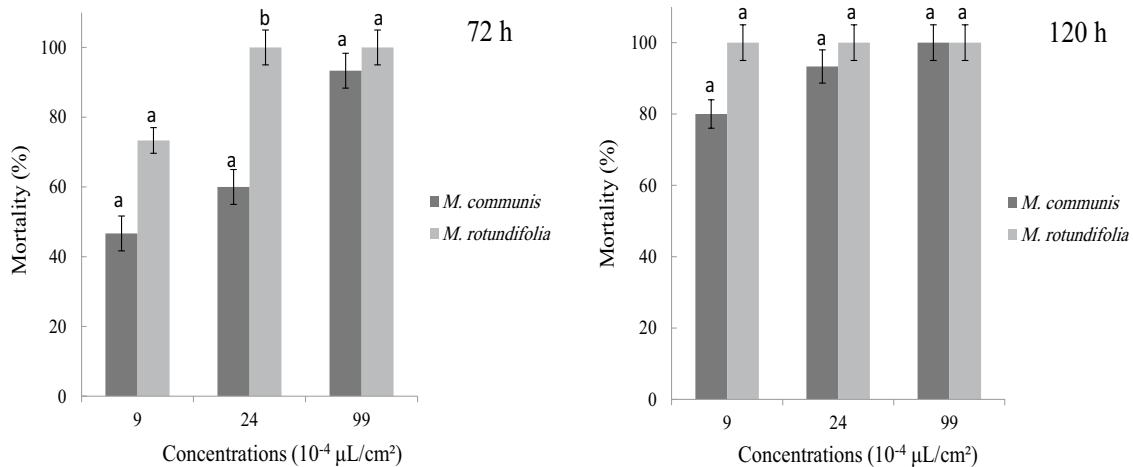


concentrations with a high striking effect of *M. rotundifolia* essential oil. Indeed, the lowest concentration ( $9 \times 10^{-4} \mu\text{L}/\text{cm}^2$ ) of *M. rotundifolia* oil induced 100% mortality after 72 h of exposure, while 80% mortality was achieved after 120 h of exposure to *M. communis* oil.

Table 4 reports  $\text{LC}_{50}$  and  $\text{LC}_{95}$  values calculated for *M. rotundifolia* and *M. communis* essential oils against *E. kuehniella* adults. The median lethal concentration was

highly dependent upon oils. Probit analysis established that *E. kuehniella* adults were more susceptible to *M. rotundifolia* oil.

Table 5 demonstrates that  $\text{LT}_{50}$  values for *M. rotundifolia* ranged from 62 h for the lowest concentration ( $9 \times 10^{-4} \mu\text{L}/\text{cm}^2$ ) to 26 h for the highest concentration ( $99 \times 10^{-4} \mu\text{L}/\text{cm}^2$ ), while for *M. communis* oil  $\text{LT}_{50}$  values



**Fig. 2** Mortality (%) of *Ephestia kuehniella* adults exposed for various concentrations of *Mentha rotundifolia* and *Myrtus communis* essential oils after 72 and 120 h. (For each concentration, compari-

sons were made among essential oils. Different letters are significantly different according to Duncan test at  $p \leq 0.05$ )

**Table 4** Contact toxicity of *Mentha rotundifolia* and *Myrtus communis* essential oils against *Ephestia kuehniella*

Essential oils	$\text{LC}_{50}^{(a,b)}$ ( $\mu\text{L}/\text{cm}^2$ )	$\text{LC}_{95}^{(a,b)}$ ( $\mu\text{L}/\text{cm}^2$ )	$\chi^2$	Slope $\pm$ SE	Sig	Degrees of freedom
<i>Mentha rotundifolia</i>	0.004 (0.0028–0.0063)	0.017 (0.012–0.027)	6.95	$128.74 \pm 51.16$	0.008	1
<i>Myrtus communis</i>	0.025 (0.016–0.041)	0.053 (0.034–0.084)	1.12	$58.03 \pm 57.65$	0.289	1

<sup>a</sup>Units  $\text{LC}_{50}$  and  $\text{LC}_{95}$  = mL/L air. Applied for 24 h at 25 °C

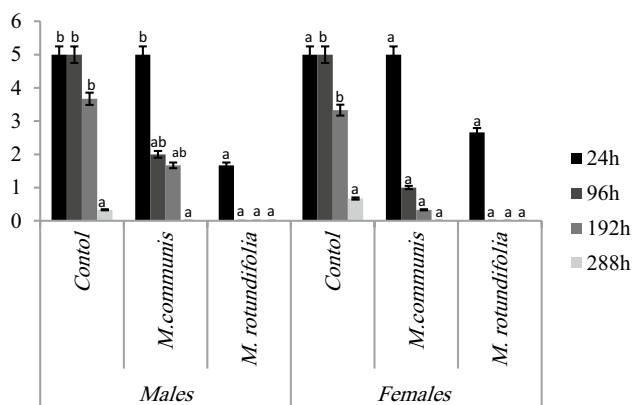
<sup>b</sup>95% lower and upper confidence limits are shown in parentheses

**Table 5**  $\text{LT}_{50}$  values of *Mentha rotundifolia* and *Myrtus communis* essential oils against *Ephestia kuehniella*

Essential oils	Concentrations ( $10^{-4} \mu\text{L}/\text{cm}^2$ )	$\text{LT}_{50}^{(a,b)}$	$\chi^2$	Slope $\pm$ SE	Sig	Degrees of freedom
<i>Mentha rotundifolia</i>	9	61.73 (55.62–69.10)	0.67	$0.06 \pm 0.014$	0.99	6
	24	28.76 (20.13–35.67)	7.55	$0.05 \pm 0.01$	0.27	6
	99	26.58 (14.54–35.44)	2.7	$0.03 \pm 0.008$	0.84	6
<i>Myrtus communis</i>	9	85.84 (72.35–102.16)	8.87	$0.02 \pm 0.003$	0.18	6
	24	73.32 (64.02–86.05)	5.43	$0.03 \pm 0.007$	0.48	6
	99	48.45 (42.74–53.90)	2.62	$0.08 \pm 0.01$	0.85	6

<sup>a</sup>Units  $\text{LT}_{50}$  = h. Applied at 25 °C

<sup>b</sup>95% lower and upper confidence limits are shown in parentheses



**Fig. 3** Effects of *Mentha rotundifolia* and *Myrtus communis* essential oils on the longevity of *Ephestia kuehniella* adults. (For each sex, comparisons were made among essential oils. Different letters are significantly different according to Duncan test at  $p \leq 0.05$ )

varied between 86 and 48 h for the lowest and highest concentration, respectively.

**Biological parameters**

**Longevity**

Essential oils significantly reduced adults' longevity at LC<sub>15</sub> concentration (Fig. 3). Statistical analysis showed highly significant differences in longevity as a function of essential oils treatments for both sex [males ( $F_{2,24} = 21.08, p < 0.01$ ), females ( $F_{2,24} = 22.57, p < 0.01$ )], time (males:  $F_{3,24} = 16.07, p < 0.01$ ; females:  $F_{3,24} = 23.42, p < 0.01$ ), and their interaction [males ( $F_{6,24} = 2.85, p < 0.01$ ), females ( $F_{6,24} = 3.57, p < 0.05$ )]. Moreover, significant differences were observed in the longevity of males

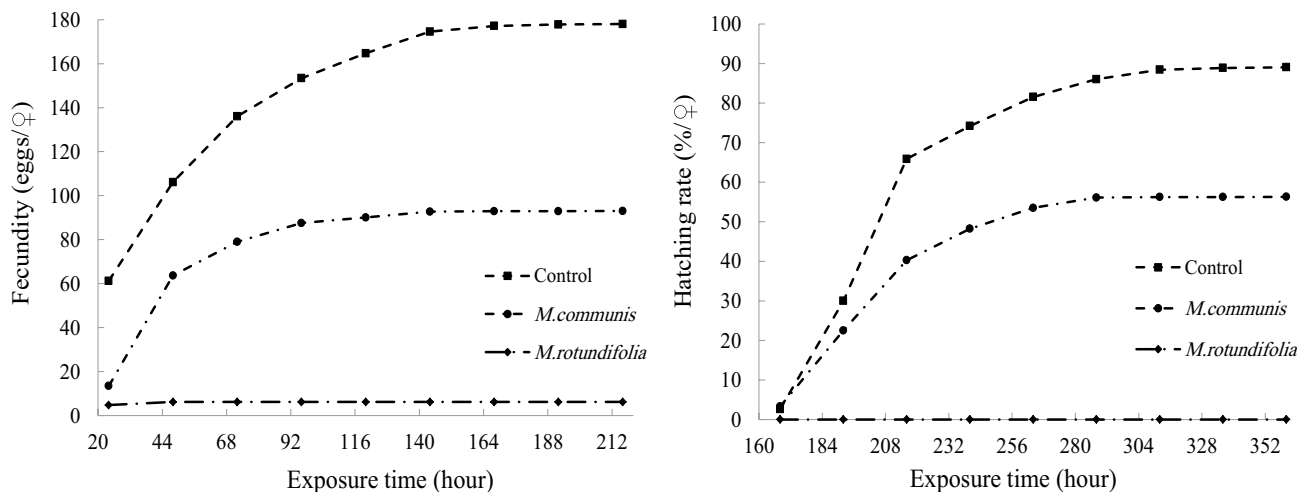
adults treated by *M. rotundifolia* and *M. communis* essential oils ( $F_{1,22} = 5.56, p < 0.05$ ), whereas no significant differences were detected in the longevity of females ( $F_{1,22} = 1.32, p > 0.05$ ).

**Fecundity, oviposition deterrence, and hatching rate**

According to the data presented in Fig. 4, the fecundity of females was highly dependent on essential oil and exposure time. In fact, the number of eggs laid increased over time, whether in the control or in *M. communis* essential oil treatment. Based on statistical analysis, there is significant differences in fecundity between control and treated females ( $F_{2,6} = 412.81, p < 0.01$ ). The mean number of eggs bequeathed by the untreated females was 178 eggs compared to 93 eggs for the essential oil of *M. communis* and 6 eggs for *M. rotundifolia*. As it can be perceived in Fig. 4, the essential oils adversely affected the hatching rate (control: 89%, common myrtle: 57%, round-leaved mint: 0%) which decreased over time. Data showed that hatching rate declined significantly with *M. rotundifolia* essential oil treatment ( $F_{1,4} = 148.23, p < 0.01$ ) contrary to *M. communis* treatment ( $F_{1,4} = 3.73, p > 0.05$ ). Furthermore, results showed significant differences in percentage of oviposition deterrence as a function of essential oils treatment ( $F_{2,6} = 21.02, p < 0.01$ ). *Mentha rotundifolia* showed the high percentage of oviposition deterrence  $96.62\% \pm 1.87$  against  $47.85\% \pm 18.13$  for *M. communis*.

**Fertility and copulation rate of E. kuehniella females**

Results of fertility and copulation rate of *E. kuehniella* females are illustrated in Table 6. Data showed that fertility and copulation rate significantly depended on essential



**Fig. 4** Effect of *Mentha rotundifolia* and *Myrtus communis* essential oils on fecundity and hatching rate of *Ephestia kuehniella*

**Table 6** Effect of *Mentha rotundifolia* and *Myrtus communis* on the fertility and copulation rate of *Ephesthia kuehniella*

	Copulation rate (%)	Log fertility
<i>Mentha rotundifolia</i>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>
<i>Myrtus communis</i>	53.33 ± 6.66 <sup>b</sup>	4.04 ± 1.39 <sup>b</sup>
Control	100.00 ± 0.00 <sup>c</sup>	6.69 ± 0.14 <sup>c</sup>

For each column, values followed by different letters are significantly different according to Duncan test at  $p \leq 0.01$

oil. Statistical analyses indicated a significant reduction in fertility between control and treated insects ( $F_{2,6} = 14.90$ ,  $p < 0.01$ ). Significant differences were detected in the copulation rate among control and treatments ( $F_{2,6} = 169$ ,  $p < 0.01$ ). In control, 100% of females had been fertilized by the males and had filled spermathecae. Almost 50% of the females who were treated with *M. communis* oil have been fertilized. Thus, the mating rate had been halved by the action of this oil. Mating rate in females treated with essential oil of *M. rotundifolia* was null (0%).

## Discussion

The present study showed that the essential oils of *M. rotundifolia* and *M. communis* collected from the northeast of Algeria are a promising source of phytochemicals. *Mentha rotundifolia* yielded 1.29% which is in accordance with the results reported for the same species in Tunisia 1.26%, (Riahi et al. 2013). Contrary, *M. rotundifolia* from other locations of Algeria have higher essential oil yields 1.6–1.8% (Brada et al. 2007) and 1.65% (Benabdallah et al. 2018) but lower than those reported in Morocco 1.54% (Derwich et al. 2009) and 4.33% (Derwich et al. 2010).

In our case, the yield of *M. communis* essential oil was 0.64%. This result was in agreement with the studies of Barhouchi et al. (2016) in Algeria (0.6%) and Aidi Wannes et al. (2007) in Tunisia (0.61%) but seemed to be higher than those found in Greece 0.28–0.3% (Gardeli et al. 2008) and Morocco 0.3–0.4% (Satrani et al. 2006).

In this research, GC/MS analysis revealed 30 compounds in essential oil of *M. rotundifolia*, the major one being piperitenone oxide or rotundifolone (46.06%). Preceding works pointed out that piperitenone oxide was reported as main constituent of some chemotype of *M. rotundifolia* in diverse geographic areas (Brada et al. 2006, 2007; Benabdallah et al. 2018; Lorenzo et al. 2002; Gende et al. 2014; Bounihi 2016; Sumio 1956). However, Kokkini and Papageorgiou (1988) confirmed that piperitone oxide and menthyl acetate were also two chemotypes of Grecian populations. Moreover, Reitsema (1958) and Lawrence (2007) reported carvone as a principal constituent in *M. rotundifolia* oils. Similarly, El

Arch et al. (2003) and Riahi et al. (2013) stated pulegone as a major compound, respectively, in Morocco and Tunisia. Other previous studies investigated that the chemical composition of *M. rotundifolia* revealed the presence of specific chemotypes such as trans-piperitone epoxide in Algeria (Brahmi et al. 2016), 2,4(8),6-*p*-menthatrien-2,3-diol and germacrene D in Cuba (Pino et al. 1999), and menthol in Morocco (Derwich et al. 2009). Many studies reported that piperitenone oxide was also the main constituent of *Mentha suaveolens* (Oumzil et al. 2002; Amzouar et al. 2016; Maffei 1988; Baser et al. 1999), *Mentha longifolia* (Venskutonis 1996; Ghoulami 2001), and *Mentha villosa* (Sousa et al. 2009). In the present study, *M. communis* essential oil was characterized by 1,8-cineole (36.82%) and  $\alpha$ -pinene (29.08%) as major compounds. Likewise, data showed a high level of 1,8-cineole which corroborates the findings of Bouzouita et al. (2003) and Viuda Martos et al. (2011). However, Ben Ghnaya et al. (2013) and Aidi Wannes et al. (2007) stated that two populations of Tunisian *M. communis* essential oils presented the pair  $\alpha$ -pinene/1,8-cineole as major compounds with a prevalence for  $\alpha$ -pinene. Moreover, Barhouchi et al. (2016) reported that  $\alpha$ -pinene and 1,8-cineole represent together 88% of common myrtle essential oil from the Northeast of Algeria. Also, Messaoud et al. (2005) investigated twelve Tunisian natural populations of *M. communis* and detected that  $\alpha$ -pinene and 1,8-cineole were the main volatile components. In the same way, it can be observed that Iranian, French, and Italian myrtle oil revealed the same main mixture:  $\alpha$ -pinene, 1,8-cineole (Rasooli et al. 2002; Curini et al. 2003; Tuberoso et al. 2006). Chalchat et al. (1998) compared *M. communis* essential oils from different Mediterranean locations and reported that oils from Corsica and Tunisia presented a level of  $\alpha$ -pinene above 50% and Morocco, Lebanon, Yugoslavia oils' presented a level under 35%. In contrast, Spanish, Croatian, and Grecian common myrtle was characterized by the myrtenyl acetate chemotype (Boelens and Jimenez 1992; Jerkovic et al. 2002; Gardeli et al. 2008), whereas in the present study there was no trace of this compound. Otherwise, the ratio 1,8-cineole, myrtenyl acetate characterized the Moroccan oil (Satrani et al. 2006). Another study from Tizi Ouzou Algeria had shown that 1,8-cineole (47%) and *cis*-geraniol (25%) were the main volatile compounds (Djenane et al. 2011).

As we can observe, there are several chemotypes of *Myrtus communis* and *M. rotundifolia* essential oils over the world; this difference in the chemical composition is closely linked to several factors like adaptive metabolism of plant (Nikšić et al. 2014), geographical position, temperature, day length, nutrients, maturation stage, polyploidy (Scora 1973), air movement, rainfall (Boukhebt et al. 2011), harvest period, distillation method, and time of extraction (Rajendran and Sriranjini 2008).

Plant extracts have been traditionally used around the world to protect stored products, but the recent increased interest in essential oil was observed with the emergence of scientific evidences on their fumigant and contact insecticidal toxicity to a large range of pests (Isman 2000). In fact, essential oils represent excellent alternatives to chemicals because of their low mammalian toxicity, fast degradation, and local obtainability (Rajendran and Sriranjini 2008). Essential oils could be regarded among the new generation of chemicals that interrupt insects' life cycle and thus seen as more target-oriented softer technique of control with less impact on the environment. The essential oils used in the current work showed a potent toxicity to *E. kuehniella* adults, especially *M. rotundifolia*. Our results demonstrated a high fumigant and contact toxicity and exceptional disruptive effects on the biological parameters of *E. kuehniella*. Brahmi et al. (2016) and El Arch et al. (2003) have reported the insecticidal activity of *M. rotundifolia* against pest beetles. Furthermore, context of KarabÖrkülü et al. (2011) evaluated the fumigant action of eight essential oils from Turkish aromatic plants including *M. communis* and demonstrated that this oil showed a moderate toxicity with  $LC_{50}$  value of 15.15  $\mu\text{L/L}$  air. In another study, Ayvaz et al. (2010) testified the insecticidal activity (fumigant test) of *M. communis* ( $LC_{50} = 12.75 \mu\text{L/L}$  air). This is in contradiction with our results, since we demonstrate that myrtle oil was more efficient with a low lethal concentration ( $LC_{50} = 2.21 \mu\text{L/L}$  air). Mediouni Ben Jemâa et al. (2012) studied the impact of seasonal variations on the chemical composition and the fumigant bioassay of five *Eucalyptus* species essential oils against *E. kuehniella* and confirmed that all species were characterized by the same major compounds: 1, 8-cineole and  $\alpha$ -pinene, and *Eucalyptus camaldulensis* was the toxic one with  $LC_{50} = 26.73 \mu\text{L/L}$  air and  $LT_{50} = 30.46$  h. Also, 100% mortality of *E. kuehniella* larvae was reached by *Satureja hortensis* essential oil at 228.5  $\mu\text{L/L}$  air after 12 h of exposure (Maedeh et al. 2011). Similarly, in the study of Emamjomeh et al. (2014), *Zataria multiflora* exhibited high fumigant toxicity against *E. kuehniella* adults and larvae at 0.98  $\mu\text{L/L}$  air and 20.67  $\mu\text{L/L}$  air  $LC_{50}$ , respectively. Also, fumigant toxicity screening on different development stages of *E. kuehniella* showed the effectiveness of *Mentha spicata* essential oil with  $LC_{50}$  value of 0.5  $\mu\text{L/L}$  air for adults and eggs mortality reaching 50–60% (Eliopoulos et al. 2015). In addition, Kheirkhah et al. (2015) noted that *E. kuehniella* adults were more sensitive to the fumigant effect of *Ziziphora clinopodioides* oil than larvae ( $LC_{50} = 1.39 \mu\text{L}$ , 42.17  $\mu\text{L/L}$  air). Mediouni Ben Jemâa et al. (2013) conducted fumigant assays using *Laurus nobilis* essential oil from Algeria and Tunisia against *E. kuehniella* adults and recorded the superiority of the Algerian oil ( $LC_{50} = 20.77$ , 33.75  $\mu\text{L/L}$  air). Likewise, Ecran et al. (2013) observed that *Prangos ferulacea* oil was more toxic to *E. kuehniella*

adults ( $LC_{50} = 1$ ) than eggs ( $LC_{50} = 320.37 \mu\text{L/L}$  air). Pandir and Baş. (2016) recorded that paprika, basil, papermint, and rosemary essential oils were toxic to eggs, larvae, and adults *E. kuehniella*. Ben Chaaban et al. (2019) proved the fumigant efficacy of *Mentha pulegium* against *E. kuehniella* adults ( $LC_{50} = 0.3 \mu\text{L/L}$  air).

Inhibition of oviposition using essential oils represents a pertinent criterion to control pest infestation and manage stored products (Singh and Pandey 2018). In this respect, Ulukanli et al. (2014) pointed out that essential oils extracted from cortex of *Pinus pinea* and *Pinus brutia* presented ovicidal activity on *E. kuehniella* eggs ( $LC_{50} = 343.57$ , 299.9  $\mu\text{L/L}$  air). According to Tunç et al. (2000), cumin and anise induced 100% mortality of *E. kuehniella* eggs. Besides, Bachrouch et al. (2010) cited that *Pistacia lentiscus* was toxic against *E. kuehniella* ( $LC_{50} = 1.84 \mu\text{L/L}$  air) and reduced adults' longevity, fecundity, hatching rate, and copulation rate. Generally, as recorded by Delimi et al. (2013) and Taibi et al. (2018), topical bioassay of *Artemisia herba-alba* and *Origanum vulgare* oils on *E. kuehniella* chrysalis causes disorders of nymphal duration, disrupts adult reproduction, reduces the longevity of females, and therefore decreases their fecundity.

As reported in this work, strong contact and fumigant toxicities were exhibited by both Algerian essential oils. The round-leaved mint *M. rotundifolia* showed most promising results in terms of adult mortality and impact on biological parameters of the target pest *E. kuehniella*. Indeed, a complete inhibition of egg hatching and copulation rate was accomplished. Consequently, our results clearly support the application of this oil as a potential biocontrol agent that may be an appropriate alternative for achieving a safer and more sustainable management of stored commodities in Algeria. Therefore, further research including the efficacy of this oil on other stored-product pests (coleopteran species) and its impact on food quality are warranted.

## Compliance with ethical standards

**Conflict of interest** The author declares that they have no conflict of interest.

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