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Full Length Article

Chemical Investigations on Algerian *Mentha rotundifolia* and *Myrtus communis* Essential Oils and Assessment of their Insecticidal and Antifungal Activities

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Abstract

This work aimed to assess *in vitro* insecticidal and antifungal activities of *Mentha rotundifolia* and *Myrtus communis* essential oils against the red flour beetle (*Tribolium castaneum*) and three fungal species (*Botrytis cinerea*, *Fusarium solani* and *Colletotrichum acutatum*). Oxygenated monoterpenes presented the dominant group with 72.94 and 58.92% respectively for *M. rotundifolia* and *M. communis* essential oils. *M. rotundifolia* and *M. communis* essential oils composition was dominated by 72.94 and 58.92% of oxygenated monoterpenes, respectively. The determined lethal concentrations of mentha essential oils against *T. castaneum* adults revealed high toxicity respectively for fumigant and contact tests, LC_{50} = 0.113 μL cm^{-2} and LC_{50} = 32.71 μL L^{-1} air. However, common myrtle oil showed a weak fumigant activity (LC_{50} = 357.67 μL L^{-1} air) and no contact toxicity. Furthermore, *M. rotundifolia* essential oil showed a marked antifungal toxicity against all the fungal strains. The mycelial growth of the three fungal strains was completely inhibited at the concentrations of 0.33 μL L^{-1} by contact application and 8, 10 and 12 μL by fumigant application. *M. communis* essential oil displayed only a contact antifungal toxicity against *B. cinerea* at the concentration 21.33 μL L^{-1}. Additionally, *M. rotundifolia* completely inhibited conidial germination of *B. cinerea* and *F. solani*, and significantly affected their morphology, with morphological modifications at the rate of 92.94 and 51.11% respectively. In light of *in vitro* tests results, the mentha essential oil appeared to be an excellent source of antifungal and insecticidal components and will allow the potential development of this species in the biological control of several pests and fungal diseases.

Key words: Biocontrol; Conidia germination; Mycelial growth inhibition; Rot molds; *Tribolium castaneum*

Introduction

Plant pathogens and insect pests pose a serious threat to crops and harvested products, leading to marked yield losses in the field and during storage (Chandrasekaran *et al*. 2016). Pests of stored products are a chronic problem because they contaminate and depreciate the quality of stored food products (Bande-Borujeni *et al*. 2018). In developing countries, there is up to 50% fruit loss during storage and transport and about 35% of crops are lost annually because of fungi and insect pests (Nunes 2012). The insect *Tribolium castaneum* (Coleoptera: Tenebrionidae), and the pathogens *Botrytis cinerea, Colletotrichum acutatum* and *Fusarium solani* are among the best examples of the most widespread and devastating pests of stored products (Pimentel *et al.* 2007; Dean *et al.* 2012). The severity of *T. castaneum* is related to its high multiplication rate coupled with a short life cycle (20 days) under favorable conditions (Kumar *et al*. 2011). In addition to corpses and wastes, adults contaminate and decrease grain quality by secreting a pungent gas from the thoracic and abdominal glands (Salem *et al.* 2018).

B. cinerea, C. acutatum and *F. solani* are associated with diseases in important economical crops. *B. cinerea*, the causal agent of the grey mould is known as a polyphagous and a high-risk pathogen due to its large resistance to anti-botrytis fungicides (Elad *et al.* 2016). Owing to its great genotypic and phenotypic variability and its adaptability to

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various environments (Júnior *et al*. 2014), it is classified as the second most important phytopathogenic fungus in the world (Dean *et al.* 2012). It can even develop successfully over long periods just above the freezing temperatures on cold-stored fruits (Williamson *et al*. 2007). *C. acutatum* have been ranked eighth most important pathogen in the world according to Dean *et al*. (2012). It causes anthracnoses in plants in the form of very damaging black spots, especially when they affect the fruits. This fungus has a wide host range of great economic importance such as strawberry, avocado, citrus, almond, mango and olive. *F. solani* is a soil fungus and parasite of plant species; it is a complex of at least twenty six filamentous fungi associated with numerous diseases on economically important plants. Contamination by fungal diseases decreases the post-harvest storage life and declines the market quality of fruits (Tripathi *et al*. 2007).

Recently, growing public concern regarding the adverse effects of pesticides and possible damage to the environment and human health has led to increasing attention being given to natural products to control pests (Ali *et al.* 2020; Khan *et al.* 2020). Currently, pests control strategies tend to emphasize the non-chemical aspects of pest control (Titouhi *et al.* 2017; Banaras *et al.* 2020, 2021; Javed *et al.* 2021). Essential oils are complex mixtures of volatile compounds, principally monoterpenoids, sesquiterpenoids and phenylpropanoids (Fujita and Kubo 2004), distributed at a quite different concentrations. The bioactivity of essential oils is drastically related to their chemical composition (Zapata and Smagghe 2010), which differs widely within the same species according to the seasonal variations, geographic areas, climatic and edaphic conditions, type of material and methods used for analysis (Salehi *et al*. 2018). Insect and herbivore attack's is among the causes that push aromatic plants to synthesize them (Bakkali *et al*. 2008). In recent years, essential oils have been widely selected for their interesting biological applications as insecticides, bactericides, and fungicides (Rahali *et al.* 2017; Cheraif *et al.* 2020).

Mentha rotundifolia L. (Lamiaceae) and *Myrtus communis* L. (Myrtaceae) are the two aromatic plants widely distributed in the north of Algeria. Decoction and infusion of their leaves are used in traditional Algerian medicines to treat several diseases such as hypertension, diabetes, disorders of the digestive and genitourinary system (Boudjelal *et al*. 2013; Brahmi *et al*. 2016). The biological activities of the round-leaved mint and common myrtle essential oils such as antioxidant (Benabdallah *et al.* 2018), antibacterial (Riahi *et al.* 2013), insecticidal (Aouadi *et al.* 2020; Kharoubi *et al.* 2020) and antifungal (Leblalta *et al.* 2020) have been little described in the literature. In order to develop a new generation of botanical pesticide from natural products, the effectiveness of the fumigant and contact potential of the Algerian *M. rotundifolia* and *M. communis* essential oils was evaluated *in vitro* on virulent strains of *T. castaneum*, *B. cinerea*, *C. acutatum* and *F. solani.*

Materials and Methods

Plant material

Fresh leaves of *M. communis* and *M. rotundifolia* were harvested respectively in October 2017 and August 2018 from two different areas of Annaba region (*M. communis* from Ain Barbar: 36°55'N, 7°36'E and *M. rotundifolia* from Berrahel: 36°50'N, 7°27'E) both situated in Northeastern Algeria. The collected samples were air-dried in shadow at room temperature (20–25°C) for a week and then stored in glass boxes for further use.

Extraction of the essential oils

Essential oils of each species were extracted from dried leaves (100 g) using Clevenger apparatus during 90 min. Essential oils were stored in amber flasks and tightly closed at 4°C. Essential oils' yields were calculated according to dry weight of the plant materials (Afnor 1986).

Gas chromatography – mass Spectrometry (GC/MS) analysis

Essential oils were analyzed using an Agilent 7890A gas chromatograph coupled 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 μm and 2.5 μm film thicknesses (5% phenyl methyl silicone, 95% dimethylpolysiloxane; Hewlett-Packard, CA, USA). The column temperature was programmed to rise from 50°C to 250°C at a rate of 7°C/min. The flow rate of carrier gas (Helium) was 1 mL/ min. A sample of 2 μL was manually injected with a constant pressure of 7.65 psi using split mode (split ratio 1:50). The identification of essential oils components was established by comparing their retention indices (RI) to n-alkanes with those published in literature or matching them to spectra of authentic compounds recorded in Wiley Registry 9th Edition/NIST 2011 edition mass spectral library.

Insecticidal activities

Insect rearing: *T. castaneum* adults were obtained from rearing colonies kept at darkness on wheat flour and semolina in 2 L plastic storage boxes at 25 ± 1°C and 65 ± 5% relative humidity. Adult's insects 7–14 days old were used for all bioassays.

Fumigant toxicity: To assess fumigant toxicity of *M. communis* and *M. rotundifolia* essential oils and the exposure time required to kill 50% of the insects, ten adults of *T. castaneum* were placed in Plexiglas flasks of 38 mL volume according to Haouel *et al*. (2010). The bottom surface of the screw caps was lined with Whatman no. 1 filter paper discs (2 cm diameter with a 3 cm length fixing

tab). Using a micro-pipette, filter paper discs were imbued with different essential oils doses of 2.5, 5, 7.5 and 10 µL (without any solvent) corresponding to the following concentrations of 65.8, 131.6, 197.36 and 263.15 μL L^{-1} air. Filter papers were hanged up to the screw caps and were quickly screwed tightly onto the bottles. Control and all concentrations were replicated three times and kept in similar conditions. Insects' mortality was recorded each hour by direct observation. When no antenna or leg movements were detected, insects were considered as dead. The Abbott correction formula (Abott 1925) was used to calculate the percentage of mortality. Lethal concentrations LC_{50} and LC_{95} and lethal time LT_{50} values were estimated by using Probit analysis (IBM SPSS v. 22).

Contact toxicity: Filter paper contact method was used in order to evaluate contact toxicity of *M. communis* and *M. rotundifolia* essential oils according to Zhang *et al*. (2018) with slights modifications. A Whatman (No.1) filter paper discs (9 cm ø) were soaked with a series of essential oil dilutions dissolved in acetone to obtain concentration range of 0.07, 0.11 and 0.15 μL cm^{-2}. Acetone was used as negative control. After five minevaporation of acetone at room temperature, each disc was then putted in a glass Petri dish and 10 adults of *T. castaneum* were placed in it. Control and each concentration have been replicated three times. The number of dead insects was registered until total insect's elimination. The mortality percentage was corrected using Abbott's formula. Probit analysis (IBM SPSS v. 22) was used to calculate LC_{50}, LC_{95} and LT_{50}, LT_{95} values.

Anti-fungal activity

Fungal strains, culture and storage: Strains of *Fusarium solani*, *Botrytis cinerea and Colletotrichum acutatum* were provided from the Laboratory of Biotechnology Applied to Agriculture, INRAT, Tunis. Cultures of micro-organisms were maintained on potato dextrose agar (PDA) medium at 24 ± 2°C for 7–14 days.

Toxic medium method: The antifungal toxicity of *M. rotundifolia* and *M. communis* essential oils against *F. solani*, *C. acutatum* and *B. cinerea* was evaluated according to the method of Regnier *et al*. (2008) with slight modifications. It consists in incorporating essential oil into 15 mL of sterile Potato Dextroseagar media (PDA) and homogenizing the mixture before pouring in Petri dishes. Thereafter, mycelial growth of 8 mm fugal discs recovered from seven days old cultures, was evaluated on PDA essential oil mixture during 5 days at 25°C.

The effectiveness of both essential oils was firstly screened at 21.33 μL mL^{-1} and then eight increasing concentrations of the most efficient oil (0.08, 0.16, 0.33, 0.66, 1.33, 2.66, 5.33 and 10.66 μL mL^{-1}) were similarly tested. Minimum inhibitory concentration was determined solely for the oil having the broadest antifungal spectrum. Three repetitions were performed for each essential oil and each concentration. The growth inhibition was calculated according to the formula of Cakir *et al*. (2005), in percentage inhibition of the radial growth of the treated samples compared to the control.

$$\% \text{ inhibition} = (C - T) / C \times 100$$

Where C = average of mycelial growth of controls, T = average of mycelial growth of treated samples.

The lowest concentration that shows no fungal growth observable to the naked eye was considered as minimum inhibitory concentration (MIC).

Volatile activity method: The effect of essential oil vapors against the tested strains was also estimated using the volatile activity technique as described by Neri *et al*. (2006) with slights modifications. The efficiency of essential oils was first evaluated at a fixed dose 12 μL. Thereafter, the minimum inhibitory concentrations were determined solely for fungal strains whose mycelial growth was completely inhibited by essential oils vapors.

For this test, an 8 mm (ø) agar disc recovered from seven day old culture was inoculated into PDA petri dishes (90 mm) and exposed to volatile substances. Essential oil vapors were provided by squares of Whatman filter paper (No. 1) soaked with (6, 8, 10 μL) crude essential oils and glued to the underside of Petri dishes lids. Petri dishes were hermetically sealed with Parafilm, inverted and then incubated for 5 days in the dark at 25 ± 2°C. Three repetitions were performed for each concentration and each oil. Mycelium growth diameters were noted daily and data were expressed as percentage inhibition of the radial mycelial growth (Plaza *et al.* 2004). The minimum inhibitory concentration (MIC) was determined for the oil having the broadest antifungal spectrum and is assigned to the lowest concentration able to completely inhibiting fungal growth.

Minimal fungicidal concentration (MFC): For both of the above methods, minimal fungicidal concentration (MFC), was determined solely for the oil having the broadest antifungal spectrum by transferring and re-inoculating in fresh PDA medium mycelial disks which showed no visual growth. Fungal development was monitored after 7 days incubation in the dark at 24°C.

Spores germination: Spore germination assay was conducted solely for fungi completely inhibited by essential oils. Fungal conidial suspension was prepared by collecting conidia from ten days old culture resuspended in 5% sterile glucose solution and adjusted by hemocytometer (Malassez) to 10^5 spores/mL. *In vitro* assays were performed using concave micro-culture slides by mixing 40 μL of each crude essential oil with 40 μL of conidial suspension (10^5 cells mL^{-1}). Control was prepared by mixing 40 μL of sterile glucose solution (5%) with 40 μL of conidial suspension (10^5 cells mL^{-1}). Slides were incubated in a wet, dark

chamber at 25°C for 48 h and then observed with an optical microscope (Leica) at 1000 magnification. Each treatment was conducted in quadruplicate. The percentage of conidial germination was evaluated using four regions per slide corresponding to at least 300 conidia.

Data analysis

Results were analyzed by one-way ANOVA followed by Duncan test to perceive significant differences at the $P \leq 0.05$. All data were expressed as the mean of three replication ± standard deviation ($\bar{x}$ ± SD). All statistical analyses were accomplished using IBM SPSS v. 22.

Results

Chemical composition

The essential oil yields for *M. communis* and *M. rotundifolia* were 0.64 and 1.29% respectively (Table 1). The chemical analyses enabled the identification of twenty volatile compounds amounting 95.13% in *M. communis* oil and thirty constituents in *M. rotundifolia* oil corresponding to 95.51%. Table 1 depicted the identified components ordered into several chemical classes, their percentages and their retention index (RI). Results showed that *M. communis* was dominated by 1, 8 cineole (36.82%) and α-pinene (29.08%). Nevertheless, the major compounds recognized in *M. rotundifolia* were rotundifolone (46.06%) and D-limonene (9.10%). As can be seen, oxygenated monoterpenes class represented the major fraction of both essential oils: *M. rotundifolia* (72.94%) and *M. communis* (58.92%) followed by monoterpene hydrocarbons class which represents 35.25%. for *M. communis* and 17.74% for *M. rotundifolia*.

Fumigant toxicity

As showed in Fig. 1, *M. rotundifolia* exhibited high fumigant toxicity against *T. castaneum* adults comparatively to *M. communis* oil ($F_{1,96}$= 2180.06, $P \leq 0.001$). Results of adult's mortality showed a dose - response relationship with oils concentrations. In fact, mortality increased significantly with increasing essential oil concentrations ($F_{3,96}$ = 86.72, $P \leq 0.001$) and exposure time ($F_{5,96}$ = 269.32, $P \leq 0.001$). For *M. rotundifolia*, the lowest concentration (65.8 μL L^{-1} air) induced complete mortality after 30 hours of exposure time whereas no mortality was registered in the same conditions with *M. communis* oil. After exposition of 24 h at the concentration of 131.6 µL L^{-1} air, *M. communis* oil caused only 3.33% mortality compared to 100% mortality with *M. rotundifolia.* Moreover, at the highest concentration (263.15 μL L^{-1} air), mortality of *T. castaneum* adults attained 20% and 100% for *M. communis*, and *M. rotundifolia* respectively after 18 h of exposure. Additionally, Probit analyses demonstrated that *T. castaneum* was more sensitive to the round leaf mint essential oil. LC_{50} and LC_{95} values were correspondingly to 32.71 μL L^{-1} air and 218.14 μL L^{-1} air at 18 h comparatively to 357.67 μL L^{-1} air and 530.69 μL L^{-1} air for common myrtle oil (Table 2). Likewise, LT_{50} and LT_{95}values confirmed that round leaf mint oil was more toxic than oil of common myrtle (Table 3). LT_{50} and LT_{95} values went from 13.2 h to 17.98 h and 15.6 h to 23.78 h for round leaf mint and from 37.82 h to 97.94 h and 84.17 h to 161.6 h for common myrtle. In the current study, data indicated that *M. rotundifolia* and *M. communis* essential oils expressed fumigant activity against *T. castaneum*, however *M. rotundifolia* was the most effective. *T. castaneum* adults were about six times more susceptible to the fumigant toxicity of *M. rotundifolia* than *M. communis* essential oils.

Contact toxicity

Results of contact test against *T. castaneum* were reported in Table 4 as percentage mortality (± S.E). Statistical analysis showed very high significant differences in mortality as function as plant species ($F_{1,72}$ = 8949.16, $P \leq 0.001$). Indeed, *M. communis* oil did not lead to any mortality with any tested concentrations contrary to *M. rotundifolia* which caused complete elimination of *T. castaneum* adults after 48 h of exposure to 0.15 μL cm^{-2} concentration (Table 4). Furthermore, the toxicity of *M. rotundifolia* oil varied significantly according to concentration ($F_{2,72}$ = 55.96, $P \leq 0.001$), exposure time ($F_{5,72}$ = 40.36, $P \leq 0.001$) and their interaction ($F_{10,72}$ = 7.76, $P \leq 0.001$). Probit analysis revealed the high potential of contact toxicity of *M. rotundifolia* against *T. castaneum*. Table 5 displays LC_{50} and LC_{95} values of *M. rotundifolia* essential oils against *T. castaneum* adults. The concentration for the essential oil to cause 50 and 95% mortality (LC_{50}) and (LC_{95}) in *T. castaneum* was 0.113 μL $cm^{-2}$and 0.164 μL cm^{-2}. Table 6 revealed that LT_{50} values ranged from 12.93 h and 23.18 h for the highest concentration (0.15 μL cm^{-2}) to 37.14 h and 63.29 h for the lowest concentration (0.07 μL cm^{-2}).

Fungicidal activity by toxic medium method

Statistical analyses revealed that growth inhibition of *F. solani*, *B. cinerea* and *C. acutatum* induced by 21.33 μL mL^{-1} of *M. rotundifolia* and *M. communis* essential oils varied significantly according to the essential oil ($F_{1,12}$ = 541.12, $P < 0.001$) and the fungus ($F_{2,12}$ = 139.15, $P \leq 0.001$). Screening of antifungal activity by contact with essential oils revealed the efficiency of *M. rotundifolia* essential oil compared to *M. communis* (Fig. 2). In fact, mycelial growth of all fungal strains was 100% inhibited by *M. rotundifolia* oil while, *M. communis* essential oil did not inhibit all fungus equally as it inhibited 100% *B. cinerea*, 49.96% *F. solani* and 39.13% *C. acutatum* (Fig. 2).

Statistical analyses indicated that the effect of fungus is not significant when studying the activity of different

Table 1: Major compounds of *M. communis* and *M. rotundifolia* essential oils obtained from leaves sampled from Annaba (Algeria)

Compounds		RI	*M. communis*	*M. rotundifolia*
Monoterpene hydrocarbons			35.25	17.74
1	*α*-Pinene	939	29.08	2.61
2	*β*-Pinene	980	0.77	2.04
3	D-Limonene	1028	-	9.10
Oxygenated monoterpenes			58.92	72.94
4	1.8-Cineole	1033	36.82	0.45
5	*β*-Linalool	1098	4.04	-
6	Endo-borneol	1165	-	4.64
7	*α*-Terpineol	1189	6.42	0.82
8	*cis*-piperitone oxide	1261	-	6,81
9	Rotundifolone	1376	-	46.06
10	Geranyl acetate	1383	4.38	-
11	*cis*-jasmone	1394	-	2.47
12	Methyl eugenol	1401	2.59	-
Sesquiterpene hydrocarbons			0.42	9.35
13	Caryophyllene	1420	0.42	3.18
14	GermacreneD	1485	-	3,58
Oxygenated sesquiterpenes			0.96	0.87
Other				3.96
Total identified (%)			95.13	95.51
Extraction yield (%)			0.64	1.29

-: compound not detected; RI: Retention Index calculated on a HP-5MS capillary column (30 m x 0.25 mm x 0.25 mm)

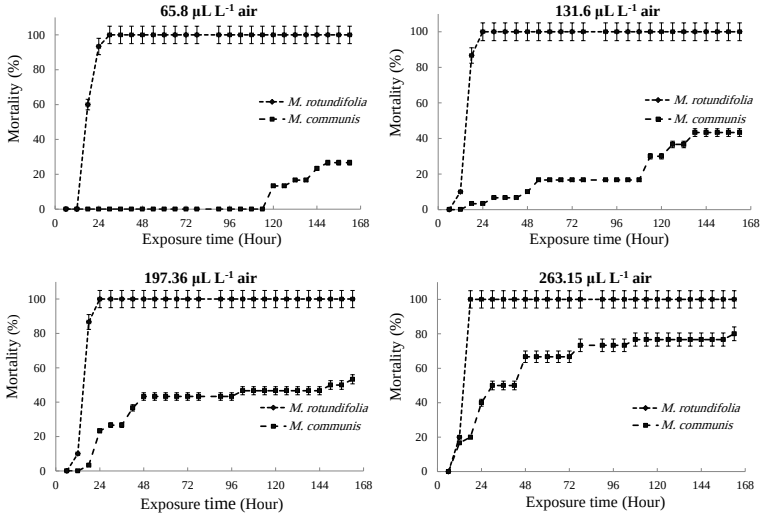

Fig. 1: Mortality (%) of *Tribolium castaneum* adults exposed for various periods of time and various concentrations to *Mentha rotundifolia* and *Myrtus communis*. essential oils

concentration of *M. rotundifolia* oil on mycelial growth of *B. cinerea, F. solani* and *C. acutatum*. Indeed, there was no significant difference in the inhibition percentage of mycelial growth between the fungal strains treated with *M. rotundifolia* oil ($F_{2,48}$ = 3.27, $P > 0.05$) (Fig. 3). At the concentration 0.08 μL mL^{-1}, inhibition percentage had reached 43.33, 52.77 and 69.26% for *F. solani, B. cinerea* and *C. acutatum* respectively. Nevertheless, increasing concentrations of *M. rotundifolia* oil resulted in a significant increase in the percentage of inhibition of the tested strains ($F_{7,48}$ = 56.41, $P < 0.001$). Starting from 0.33 μL mL^{-1} of *M. rotundifolia* essential oil, growth of all fungal strain is completely inhibited (Fig. 3 and 4). Consequently, the concentration 0.33 μL mL^{-1} represented the minimum inhibitory concentration (MIC) of the round leaf mint essential oil against fungal strains (Table 7).

Fungicidal activity by volatile activity method

Statistical analysis showed significant differences in mycelial growth between essential oil treatments ($F_{1,12}$ = 9560.27, $P \leq 0.001$) and between different fungal strains ($F_{2,12}$= 656.79, $P \leq 0.001$). Data showed that *M. rotundifolia* oil inhibited 100% mycelial growth of all tested fungi at 12 μL. However, the fumigation of fugal strains with 12 μL of *M. communis* oil was totally inefficient towards *B. cinerea* and inhibited 47.4 and 55.19% the growth of *C. acutatum* and *F. solani* respectively (Fig. 5). According to these

Table 2: LC_{50} and LC_{95} of *Mentha rotundifolia* and *Myrtus communis* essential oils applied by fumigation against *Tribolium castaneum*

Essential oils	LC_{50} (a, b) (μL L^{-1} air)	LC_{95} (a, b) (μL L^{-1} air)	$\chi 2$	Slope ± S.E.	Sig	df
M. rotundifolia	32.71 (-83.11 - 75.58)	218.14 (176.40-329.33)	2.97	0.009 ± 0.002	0.226	2
M. communis	357.67 (291.15–789.02)	530.69 (394–1495.89)	1.18	0.010 ± 0.004	0.552	2

a: Units LC_{50} and LC_{95} = μL.L^{-1} air, applied for 18 h at 25 °C
b: 95% lower and upper confidence limits are shown in parenthesis

Table 3: LT_{50} values of *Mentha rotundifolia* and *Myrtus communis* essential oils applied by fumigation against *Tribolium castaneum*

Essential oils	Concentrations (μL.L^{-1} air)	LT_{50} (a, b)	LT_{95} (a, b)	$\chi 2$	Slope ± S.E.	Sig	df
M. rotundifolia	65.8	17.98 (5.43 – 25.81)	23.78 (22.13 - 26.77)	10.94	0.306 ± 0.029	0.004	2
	131.6	15.21 (14.63 - 15.78)	19.32 (18.49 - 20.45)	0.023	0.4 ± 0.038	0.989	2
	197.36	15.21 (14.63 - 15.78)	19.32 (18.49 - 20.45)	0.023	0.4 ± 0.038	0.989	2
	263.15	13.2 (12.65 – 15.19)	15.6 (14.2 – 21.53)	0.068	0.685 ± 0.219	0.967	2
M. communis	65.8	-	-	-	-	-	-
	131.6	97.94 (74.16–186.77)	161.6 (141.7 - 340.12)	2.53	0.026 ± 0.008	0.469	3
	197.36	49.36 (37.06 - 278.77)	84.22 (58.73- 849,07)	20.26	0.047 ± 0.005	0.00	3
	263.15	37.82 (30.06- 52.81)	84.17 (63.68 - 155.37)	6,342	0,035 ± 0,004	0.096	3

a: Units LT_{50} = h, applied at 25°C
b: 95% lower and upper confidence limits are shown in parenthesis

Table 4: Mortality (%) of *Tribolium castaneum* adults exposed to various concentrations for different periods of time to *Mentha rotundifolia* and *Myrtus communis* essential oils applied by direct contact

Concentration (μL cm^{-2})	24 h	48 h	72 h	96 h	120 h	144 h
0.07	36.66 ± 0.33a	70 ± 1a	80 ± 0.57a	80 ± 0.57a	83.33 ± 0.33a	100 ± 0
0.11	50 ± 0.57a	90 ± 0.57b	100 ± 0b	100 ± 0b	100 ± 0b	100 ± 0
0.15	93.33 ± 0.66b	100 ± 0b	100 ± 0b	100 ± 0b	100 ± 0b	100 ± 0
F- value	F = 29.62	F = 5.25	F = 12	F =12	F = 25	
P	*P ≤ 0.01*	*P ≤ 0.05*	*P ≤ 0.001*	*P ≤ 0.001*	*P ≤ 0.001*	

For each column, values followed by different letters are significantly different according to Duncan test at *P ≤ 0.05*)

Table 5: LC_{50} and LC_{95} of *Mentha rotundifolia* essential oil applied by contact test against *Tribolium castaneum*

Essential oils	LC_{50} (a, b) (μL cm^{-2})	LC_{95} (a, b) (μL cm^{-2})	$\chi 2$	Slope ± S.E.	Sig	df
M. rotundifolia	0.113 (0.108 - 0.118)	0.164 (0.155 - 0.177)	1,223	32.26 ± 3.04	0,269	1

a: Units LC_{50} and LC_{95} = μL cm^{-2}, applied for 18 h at 25 °C
b: 95% lower and upper confidence limits are shown in parenthesis

results, the vapors of *M. rotundifolia* oil exhibited the highest fumigant toxicity against the tested fungi.

The study of different doses of *M. rotundifolia* essential oil effect on fungal growth showed that applied doses ($F_{2,18}$ = 5.06, *P ≤ 0.05*) and the fungal strain are significant ($F_{2,18}$ = 12.55, *P ≤ 0.001*). Indeed, *F. solani* was 100% inhibited with 8 μL of oil vapor whereas; *B. cinerea and C. acutatum* were inhibited by 98.6 and 92.38% respectively (Fig. 6 and 7). At 10 μL of *M. rotundifolia* oil, *B. cinerea* growth was 100% stopped while *C. acutatum* growth was inhibited by 97.25% (Fig. 6 and 7). These results suggest that 8, 10 and 12 μL are the corresponding MIC of *F. solani*, *B. cinerea* and *C. acutatum* respectively (Table 7).

Minimal fungicidal concentration

The values of the minimal fungicidal concentrations of essential oils have been reported in Table 8. After ten days of incubation of the transferred mycelial discs, it has been noted that essential oil vapors presented fungistatic effects contrary to the direct contact application which possessed fungicidal activity. It was also observed that the minimal fungicidal concentrations values were higher than the minimal inhibitory concentrations. Minimal fungicidal concentration of *M. rotundifolia* for *B. cinerea*, *C. acutatum*

Table 6: LT_{50} values of *Mentha rotundifolia* essential oil applied by direct contact against *Tribolium castaneum*

Essential oils	Concentrations (μL cm^{-2})	LT_{50} (a, b)	LT_{95} (a, b)	$\chi 2$	Slope ± S.E.	Sig	df
M. rotundifolia	0.07	37.14. (26.61- 68.80)	63.29 (46.17 – 158.61)	23.30	0.063 ± 0.005	0.000	3
	0.11	20.38 (17.06 - 23.35)	57.71 (50.23 - 69.88)	2.49	0.044 ± 0.006	0.287	2
	0.15	12.93 (-50.18–17.25)	23.18 (18.44 - 161.50)	6.32	0.16 ± 0.02	0.042	2

a: Units LT_{50} = h, applied at 25 °C
b: 95% lower and upper confidence limits are shown in parenthesis

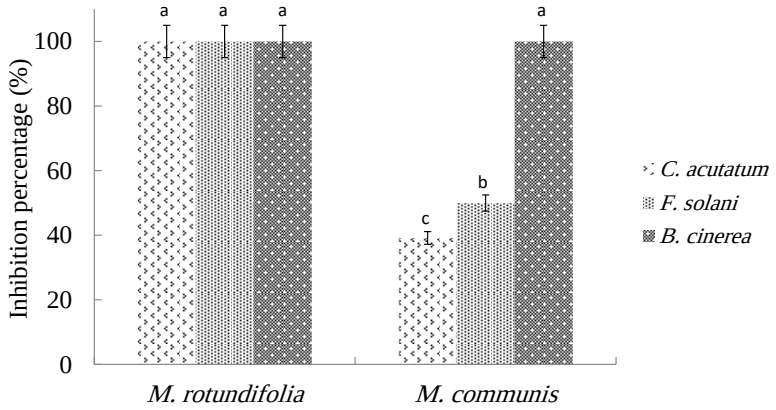

Fig. 2: Screening of contact antifungal activity of *Mentha rotundifolia* and *Myrtus communis* essential oils against *Fusarium solani*, *Colletotrichum acutatum* and *Botrytis cinerea* at 21.33 μL mL^{-1} concentration (Different letters are significantly different according to Duncan test at $P \leq 0.01$)

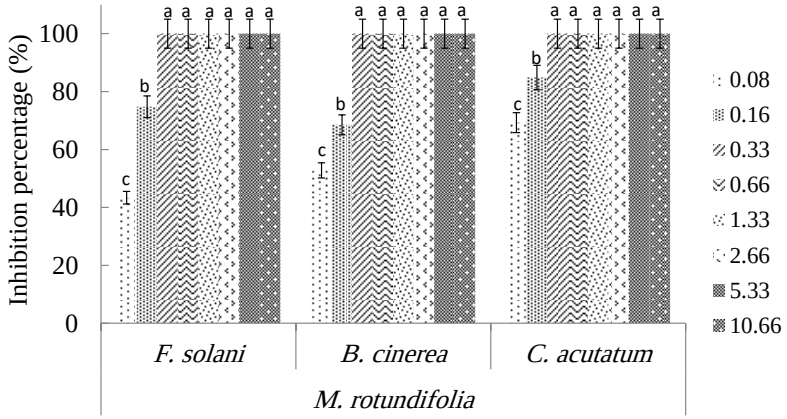

Fig. 3: Inhibition percentage induced by various concentrations of *Mentha rotundifolia* essential oil (μL mL^{-1}) on the growth of *Fusarium solani*, *Colletotrichum acutatum* and *Botrytis cinerea*. Poisonous medium method (Different letters are significantly different according to Duncan test at $P \leq 0.01$)

and *F. solani* were 0.66, 1.33 and 2.66 µL mL^{-1}, respectively.

Spore germination

According to statistical analyses, *M. rotundifolia* crude essential oil inhibited 100% the germination of *B. cinerea* ($F_{1,6}$ = 19.57, $P \leq 0.01$) and *F. solani* spores ($F_{1,6}$ = 19422, $P \leq 0.001$) comparing to controls and induced 51.11% of morphological modifications for *B. cinerea* and 99.94% for *F. solani* conidia (Table 9 and Fig. 8).

Discussion

Biological potential of *M. communis* and *M. rotundifolia* essential oils have been little reported worldwide and especially in Algeria. However, these two aromatic plants widely distributed in the north of Africa, used to be largely recommended in traditional medicine to treat different health disorders. Based on these assumptions, Algerian

Table 7: Minimum inhibitory concentrations (MIC) of *Mentha rotundifolia* essential

	Poisonous medium method (μL mL^{-1})	Volatile activity method (μL)
F. solani	0.33	8
B. cinerea	0.33	10
C. acutatum	0.33	12

Oil against *Fusarium solani*, *Botrytis cinerea* and *Colletotrichum acutatum*

Table 8: Minimum fungicidal concentration (MFC) (μL mL^{-1}) of *Mentha rotundifolia* essential oil against *Fusarium solani*, *Botrytis cinerea* and *Colletotrichum acutatum* with poisonous medium method

	Minimum fungicidal concentration (μL mL^{-1})
F. solani	2.66
B. cinerea	0.66
C. acutatum	1.33

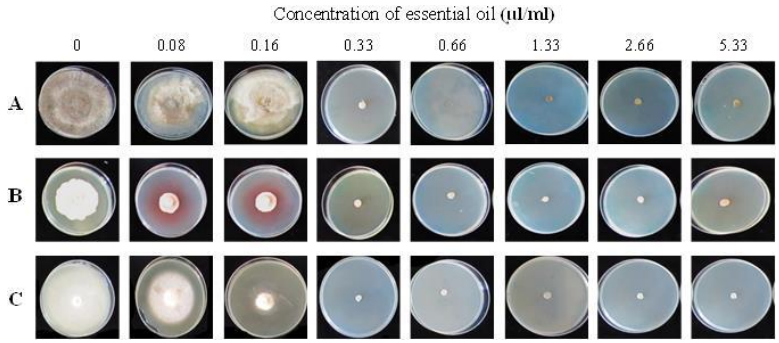

Fig. 4: Effect of various concentrations of *Mentha rotundifolia* essential oil on mycelial growth of (**A**) *Botrytis cinerea*; (**B**) *Fusarium solani* and (**C**) *Colletotrichum acutatum* on PDA

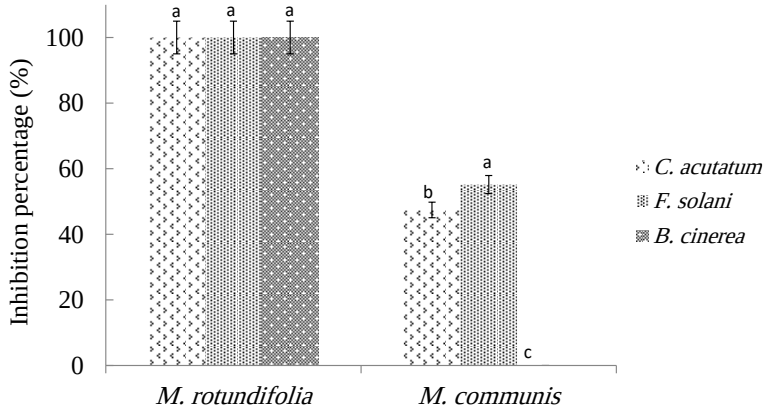

Fig. 5: Screening of volatile activity of *Mentha rotundifolia* and *Myrtus communis* essential oils vapor against *Fusarium solani*, *Colletotrichum acutatum* and *Botrytis cinerea* at 12 µL dose. Different letters are significantly different according to Duncan test at $P \leq 0.01$)

M. communis and *M. rotundifolia* essential oils were screened for their chemical, insecticidal and antifungal properties in this study.

reported in some areas of Algeria and Tunisia (Bouzabata *et al*. 2010; Aidi-Wannes *et al*. 2010; Barhouchi *et al*. 2016) but greater to those stated by Jamoussi *et al*. (2005) in Tunisia, Farah *et al*. (2006) and Satrani *et al*. (2006) in Morocco, and Gardeli *et al*. (2008) in Greece. On the other hand, *M. rotundifolia* essential oil yielded 1.29% which is in agreement with the findings of Riahi *et al*. (2013) in Tunisia.

The extraction of essential oil from *M. communis* dry leaves allowed to obtain a yield 0.64% which is in accordance with the results reported in some areas of Algeria (Barhouchi *et al*. 2016) but greater than those observed in Tunisia (Jamoussi *et al*. 2005), in Morocco (Farah *et al*. 2006; Satrani *et al*. 2006), and in Greece (Gardeli *et al*. 2008). On the other hand, *M. rotundifolia* essential oil yielded 1.29% which is in agreement with the findings of Riahi *et al*. (2013) and different to those

Table 9: Germination and morphological modifications (%) of *Fusarium solani* and *Botrytis cinerea* spores treated by *Mentha rotundifolia* essential oil

	Fungi	*M. rotundifolia*	Control
Germination (%)	*F. solani*	0^{a}	81.32^{b}
	B. cinerea	0^{a}	66.91^{b}
Spores modification (%)	*F. solani*	99.94^{b}	0^{a}
	B. cinerea	51.11^{b}	0^{a}

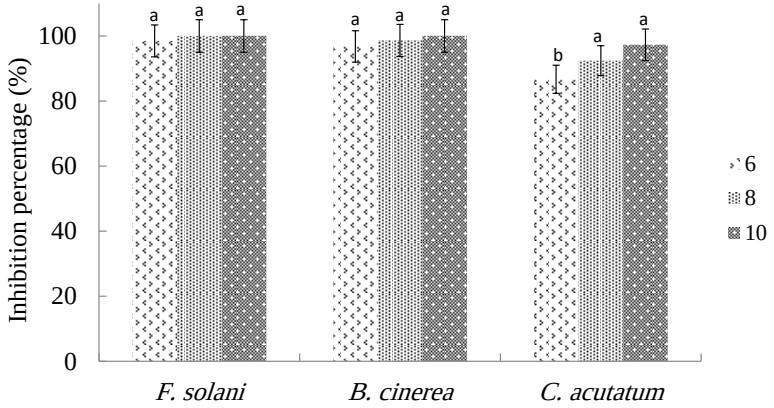

Fig. 6: Inhibition percentage induced by various concentrations of *Mentha rotundifolia* essential oil (µL) on the growth of *Fusarium solani, Colletotrichum acutatum* and *Botrytiscinerea.* Volatile activity method. Different letters are significantly different according to Duncan test at $P \leq 0.01$

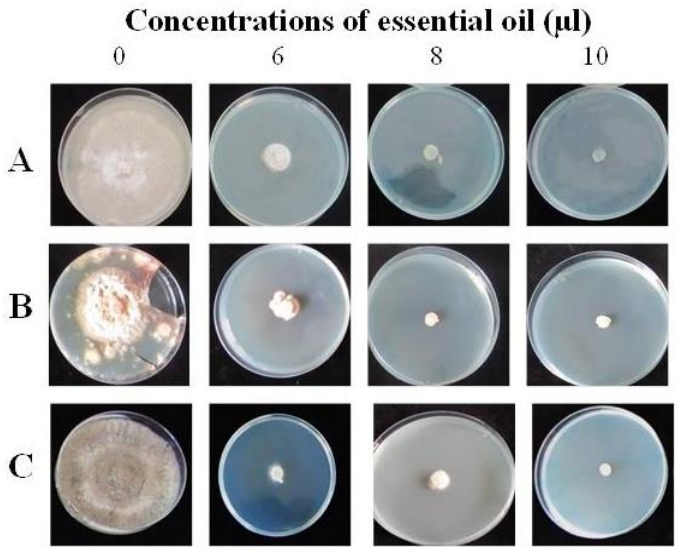

Fig. 7: Effect of various concentrations of *Mentha rotundifolia* essential oil on mycelial growth of (**A**) *Colletotrichum acutatum;* (**B**) *Fusarium solani* and (**C**) *Botrytis cinerea* on PDA

reported by other authors (Brahmi *et al*. 2016; Benabdallah *et al*. 2018).

Chemical analysis of the two essential oils showed that oxygenated monoterpenes class represented the major fraction of both essential oils with 72.94% in *M. rotundifolia* and 58.92% in *M. communis* followed by monoterpene hydrocarbons class which represents 35.25% for *M. communis* and 17.74% for *M. rotundifolia*. *M. communis* was dominated by 1,8 cineole (36.82%) and α-pinene (29.08%) while *M. rotundifolia* major compounds were rotundifolone (46.06%) and D-limonene (9.10%).

These findings are in accordance with those of Bouzouita *et al.* (2003) and Viuda-Martos *et al*. (2011). On the contrary, in precedent studies carried out by Bouzabata *et al.* (2010) and Barhouchi *et al*. (2016), the common myrtle of the same region was characterized by an α-pinene essential oil chemotype. According to literature, the α-pinene chemotype of the common myrtle essential oil is the

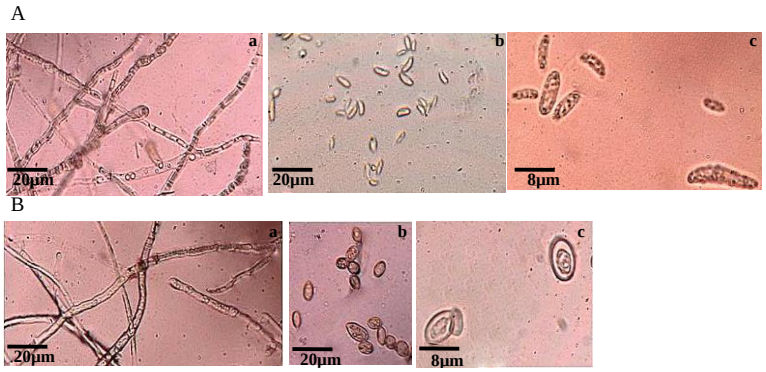

Fig. 8: Micrography displaying the effect of *Mentha rotundifolia* essential oil treatment on (**A**) *Fusarium solani* and (**B**) *Botrytis cinerea* conidia
a: untreated germinated conidia (positive control); **b**: Absence of germination of treated conidia; **c**: Treated conidia showing structure modifications

most widespread around the world; it is the typical chemotype of Tunisian *M. communis* wild populations (Ghnaya *et al*. 2013), Albanian ones (Asllani 2000), Iranian (Bajalan and Pirbalouti 2014) French (Curini *et al*. 2003), Iraqi (Kiralan *et al*. 2012) and Italian (Mulas and Melis 2011). However, other chemotypes of *M. communis* essential oil have been identified in other regions of Algeria such as 1, 8-cineole/*cis*-geraniol in TiziOuzou and myrtenyl acetate/1,8-Cineole in Algiers (Djenane *et al.* 2011). On another side, Myrtenyl acetate chemotype characterized Grecian Spanish and Croatian myrtle essential oil (Jerkovic *et al*. 2002; Gardeli *et al*. 2008). Otherwise, a 1,8-cineole/linalool chemotype has been reported in Turkish myrtle essential oil (Özek *et al*. 2000) while the Moroccan *M. communis* essential oil was dominated by the pair 1,8 cineole/myrtenyle acetate (Farah *et al*. 2006).

In our study, oxygenated monoterpenes chemical class exceeded the level of 50% of the chemical composition of *M. rotundifolia* essential oil (72.94%). *M. rotundifolia* essential oil belonged to piperitenone oxide chemotype. In accordance with our results, piperitenone oxide chemotype was recorded to be the main constituent of *M. rotundifolia* species in different geographic regions around the world (Bounihi 2016; Benabdallah *et al*. 2018). Nevertheless, Brahmi *et al*. (2016) stated trans-piperitone epooxide as main constituent of *M. rotundifolia* growing in Bejaia-Algeria. Moreover, *M. rotundifolia* essential oil with the germacrene chemotype was identified in Constantine-Algeria (Bouhabila *et al*. 2018). Pulegone was identified as the main chemical component of Tunisian and Moroccan species (Riahi *et al*. 2013. Menthol chemotype was also reported in Morocco (Derwich *et al*. 2009). Furthermore, Lawrence (2007) reported a carvone chemotype of *M. rotundifolia* oil. Additionally, Piperitone oxide and menthyl acetate were also found to be two chemotypes of the Grecian specie (Kokkini and Papageorgiou 1988). Whereas, 2, 4 (8), 6-p-menthatrien-2, 3-diol and germacrene D chemotypes characterized Cuban *M. rotundifolia* populations (Pino *et al*. 1999).

Subsequently to chemical composition determination, data of the current study indicated that *M. rotundifolia* and *M. communis* essential oils expressed fumigant activity against *T. castaneum*, with a better activity of *M. rotundifolia*. Indeed, *T. castaneum* adults were about six times more susceptible to the fumigant toxicity of *M. rotundifolia* than *M. communis* essential oils.

In contrast with our finding, Karabörklü *et al*. (2010) reported that Turkish *M. communis* essential oil possessed a strong fumigant activity against *T. castaneum* with a low LC_{50} value (56.98 μL L^{-1} air). Opposing to *M. rotundifolia* which exhibited an interesting contact activity, *M. communis* essential oil was completely ineffective against *T. castaneum* adults. To the best of our knowledge, no published data has previously been reported on the insecticidal activity of Algerian *M. rotundifolia* essential oil on *T. castaneum*. However, *M. rotundifolia* essential oil was assessed for its insecticidal effect on other insects. Thus, Brahmi *et al*. (2016) investigated the insecticidal potential of piperitone epoxide chemotype of Algerian *M. rotundifolia* (Bejaia, Algeria) against *Rhyzopertha dominica* and reported the moderate contact and fumigant toxicity of the essential oil. Arch *et al*. (2003) stated that Moroccan pulegone chemotype of *M. rotundifolia* essential oil presented an interesting fumigant activity. 100% mortality was reached after 24 h of exposure to 35 μL L^{-1} air and 65 μL L^{-1} air for *Sitophilus oryzae* and *R. dominica*, respectively.

According to our results, insecticidal activity of the tested oils varied conferring to the mode of application. *M. rotundifolia* oil displayed more strength in contact toxicity than fumigant activity. Contrary, essential oil of *M. communis* showed moderate fumigant toxicity while it has no toxic effect in contact assay. This is in agreement with the findings of Zapata and Smagghe (2010). The same conclusion was made by Mohamed and Abdelgaleil (2008) when they screened the fumigant and contact effect of essential oils extracted from eight Egyptian aromatic plants against *T. castaneum* adults. They found that all the tested essential oil possessed a better contact toxicity than fumigant toxicity apart *Mentha microphylla* which was the

strongest one ever tested as well in fumigant test (LC_{50} = 4.51 μL L^{-1} air) as in contact test (LC_{50} = 0.01 $mgcm^{-2}$). Several investigations testified the interesting insecticidal potential of many species of the genus *Mentha* against *T. castaneum* (Eliopoulos *et al.* 2015; Kasrati *et al.* 2015). On the bases of the low LC_{50} values in contact (0.11 μL cm^{-2}) and fumigant (32.71 μL L^{-1} air) activity of our study, *M. rotundifolia* oil revealed a strong insecticidal potential against stored product pests. This effective activity could be attributed to its major components: piperitenone oxide D-Limonene and *Cis* piperitone oxide. Oumzil *et al.* (2002), reported an antibacterial activity of piperitenone oxide and piperitone oxide. Additionally, Tripathi *et al.* (2004) studied the insecticidal effect of piperitenone oxide against various stage of *Anopheles stephensi* and indicated a high level of toxicity, repellency and decreasing of reproduction parameters. Many reports related the fumigant, contact and antifeedant toxicity of 1,8 cineol, which is the major component of *M. communis* essential oil (Lee *et al*. 2004; Rozman *et al*. 2007; Palacios *et al.* 2009). Moreover, the insecticidal activity of several essential oils major components against *T. castaneum* has been reported in several researches (Mondal and Khalequzzaman 2010; Eljazi *et al*. 2018). Generally, essential oils and their main components act on the nervous system of the insect either by inhibiting the activity of the enzyme acetylcholinesterase or by increasing the concentrations of cAMP and Ca^{2+} in nervous cells or as an antagonist to octopamine receptors (exclusive to invertebrates including insects) (Jankowska *et al*. 2017). According to the same authors, the multitude potential target sites in the nervous system of insects make essential oils components interesting candidates for bio-insecticides. Numerous papers have reported the antifungal activity of *M. communis* and *M. rotundifolia* essential oil against human pathogenic fungi, but few studies have been carried out on phytopathogenic strains. To the best of our knowledge, no previous study has reported the antifungal toxicity of Algerian *M. rotundifolia* and *M. communis* essential oils.

Results obtained from our study revealed that essential oils extracted from *M. rotundifolia* exhibited a powerful antifungal activity. *In vitro* tests have shown that *M. rotundifolia* was very effective against all fungal strains in comparison with *M. communis* essential oil, which was effective only by contact application on *B. cinerea*. Our results corroborate those of Curini *et al*. (2003) showing that the essential oil of the Italian species of *Myrtus communis* had also exerted a weak inhibitory power on the mycelial growth of *F. solani* (15, 59% inhibition at 1600 ppm). The same observations were reported for the Tunisian species for which the essential oil with chemotype α - pinene/Limonene had slightly reduced the mycelial growth of *F. solani* to 32% at the concentration of 10 μL mL^{-1} (Slim *et al*. 2017). Besides, according to Mirzabagheri *et al*. (2014), Iranian common myrtle essential oil has shown the weakest antifungal activity against *Penicellium digitatum* compared to other essential oils.

It should be noted that the sensitivity of micro-organisms to the action of essential oils varied considerably depending on the method of application. Indeed, *M. rotundifolia* essential oil possessed a fungitoxic potential by contact unlike the vapors which exerted a fungistatic effect by fumigation. Likewise, the essential oil of *M. communis* was effective against *B. cinerea* by contact and completely ineffective by fumigation. Our findings corroborate the results of Regnier *et al.* (2014) which indicated the fungitoxic and the fungistatic effects of essential oils by contact and fumigation application respectively. According to Cox *et al*. (2001), the variability in essential oil efficacy related to the mode of application (contact or fumigation) can be explained by the differences in the polarities and volatilities of the individual essential oil components. Hydrophilic polar constituents mix and diffuse easily in aqueous media and consequently exhibit higher effects in direct contact method. Referring to the minimum inhibitory and fungicidal concentrations, *M. rotundifolia* expressed a strong antifungal toxicity; *C. acutatum, F. solani* and *B. cinerea* colonies were completely inhibited at the low concentration of 0.33 µL mL^{-1}. Moreover, *M. rotundifolia* essential oil vapors even entirely stopped the mycelial growth of *F. solani, B. cinerea* and *C. acutatum* at the low concentrations of 8, 10 and 12 μL respectively. Previous studies attested the toxicity of round leaf mint essential oil and its main components against several micro-organisms strains (Ladjel *et al*. 2011). This powerful antifungal ability of *M. rotundifolia* essential oil can be attributed to its main chemical components and their synergistic action with minor components (Mahboubi and Haghi 2008). Essential oils with a high level of oxygenated monoterpenes components are biologically more active compared to oils rich in hydrocarbon monoterpenes (Carson and Riley 1995), which is the case with our findings. Other species of *Mentha* genus had also displayed an effective antifungal activity such as *M. spicata*, *M. pulegium* (Yadav *et al*. 2006; Mohammadi *et al.* 2013), *M. arvensis* (Kumar *et al*. 2009) and *M. piperita* (Plavšić *et al*. 2017) against *Alternaria alterna* (700 ppm), *Pyricularia oryzae*, *Penicillium digitatum* (1000 ppm), *Aspergillus ochraceus* (1100 ppm), *F. oxysporum*, f. spp. *ciceris*, *Macrophominaphaseolina*, *Dreshlera spicifera* and *Eurotium herbariorum*. These essential oils act on the fungus by altering the mycelium but also by inhibiting spores germination. *M. rotundifolia* inhibited completely the spore germination of *F. solani* and *B. cinerea*. The essential oil has also induced morphological changes in the spores causing up to the exuviation of cellular content. The inhibitory action of essential oils on the germination of fungal spores has been underlined in several works (Vitoratos *et al*. 2013; Farzaneh *et al*. 2015). The mechanism of antifungal action of essential oils remains ambiguous and misunderstood. Nevertheless, previous studies have shown that the antifungal activity of essential oils is due to their ability to disrupt the structure of cell

membranes in fungi (Pei *et al*. 2020). According to Shao *et al*. (2013), tea tree essential oil altered mycelial morphology and ultrastructure. The low ratio of unsaturated/saturated fatty acids increases the permeability and electrical conductivity of the membrane and causes the exuviation of cytoplasm. Based on the results of our study, the strong insecticidal and antifungal potential expressed by the essential oil of round-leaved mint can be exploited in biological control as part of pest control strategies within the framework of sustainable development.

Conclusion

In conclusion, our research pointed out the potent antifungal and insecticidal activity of *Mentha rotundifolia* essential oil. Indeed, on the one hand, it acted effectively on the three tested fungal strains by inhibiting completely their mycelial growth at low concentrations and by stopping totally the spore's germination by inducing deep alterations in their morphologies leading even to their explosion. On the other hand, it caused the complete death of *Ephestia kuehniella* adults by contact and fumigation application. Therefore, our results support the use of *M. rotundifolia* oil in the biological control of stored foods pests and diseases. Nevertheless, additional tests on the impact of essential oil on food quality as well as in vivo tests on artificially inoculated fruits are needed.

Author Contributions

GA and LKG did data curation, formal analysis, writing original draft editing. AS, SH and MBA wrote methodology, and involved in writing-revision. TF was Project administration. SEK, EB and MC involved in resource management. MRH did supervision; validation. JMBJ was involved in conceptualization, supervision and validation.

Conflicts of Interest

All authors declare no conflicts of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable in this paper

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