Scientific Review – Engineering and Environmental Sciences (2020), 29 (4), 544–556
Sci. Rev. Eng. Env. Sci. (2020), 29 (4)
Przegląd Naukowy – Inżynieria i Kształtowanie Środowiska (2020), 29 (4), 544–556
Prz. Nauk. Inż. Kszt. Środ. (2020), 29 (4)
http://iks.pn.sggw.pl
DOI 10.22630/PNIKS.2020.29.4.47

Ayoub AINANE[1], Fatouma MOHAMED ABDOUL-LATIF[2], Talal MOHAMED ABDOUL-LATIF[2], Tarik AINANE[1]

[1]University of Sultan Moulay Slimane, Superior School of Technology of Khenifra
[2]Center for Research and Study of Djibouti, Medicinal Research Institute

Evaluation of biological activities of two essential oils as a safe environmental bioinsecticides: case of *Eucalyptus globulus* and *Rosmarinus officinalis*

Key words: *Eucalyptus globulus*, *Rosmarinus officinalis*, essential oils, insecticidal activity, microbial activity

Introduction

Insects harm crops in the field and may cause extensive damage during storage. The amount of insect damage in stored grain is 10–40% in countries that do not apply modern storage techniques (Banga, Kotwaliwale, Mohapatra & Giri, 2016; Pandey, Tripathi & Singh, 2018). In Africa the rate of insect damage in stored products can potentially reach 100% if control methods and storage conditions are poor. These environmental effects and the need to maintain a sustainable environment have created the need for environmental-safe, degradable and target-specific insecticides (Cheng et al., 2017; Bottrell & Schoenly, 2018; Costa, Freitas, Cruz, Silveira & Morais, 2019). Many extracts and compounds have insecticidal activities, most being repellants, ovipositional deterrents and antifeedants against both agricultural pests and medically important insect species (Singh & Kaur, 2018; Vetal & Pardeshi, 2019; Isman, 2020).

The rather broad use of conventional insecticides as grain protectants remains a dominant pest management tactic against stored product insects particularly in warmer climates (Lamichhane et al., 2016). Such use and recommendations are largely based on acute lethal studies, which although valuable, neglect the likely occurrence and importance of potential sublethal effects of insecticides on these pest species and associated community (Vélez, Barbosa, Quintero, Chediak & Guedes, 2017). Nonetheless, sublethal effects of conventional grain protectants have been recorded in the granary, but compounds of more recent use in stored product protection were not targeted in such studies, as the biopesticide spinosad, in contrast with pyrethroids (Awan, Ahmad, Saleem & Shakoori, 2019).

The sustained levels of pyrethroid use against grain weevils have been leading to problems of resistance to these compounds in *Sitophilus granarius* (Puthur, Anoopkumar, Rebello & Aneesh, 2019). Therefore, new alternatives have been developed and launched in the market as grain protectants. One of them is the biopesticide spinosad, which belongs to a class of naturally derived compounds currently available in many countries (Shah et al., 2019). Therefore, essential oils have emerged as viable alternatives to synthetic pesticides for control of stored-grain insect pests; they are generally non-toxic to mammals, birds, fish, or humans, have limited persistence, are readily biodegradable, and are renewable resources (Owolabi, Ogundajo, Alafia, Ajelara & Setzer, 2020).

In this study, we assessed the insecticidal activities of *Eucalyptus globulus* and *Rosmarinus officinalis* essential oils against *Sitophilus granarius* adults. We also examined the effect of essential oils on the microbial activity against: *Staphylococcus aureus*, *Enterococcus faecium*, *Listeria monocytogenes*, *Bacillus subtilis*, *Escherichia coli*, *Yersinia enterocolitica* and *Candida albicans*. This research provides the first view of the molecular events underlying the response to plant essential oils in *Sitophilus granarius*. In the future, it could provide the foundation for developing plant essential oils as a novel environmentally friendly bioinsecticide against insect pests.

Material and methods

Plant material

The aerial part of plant were randomly collected from *Eucalyptus globulus* and *Rosmarinus officinalis* in planting base (the planting base belongs to EST--Khenifra) in Morocco country (Middle Atlas in Morocco, 32°58’22.9” N 5°39’38.7” W), and the samples were collected once a month from March 2018 to May 2019. The plants were identified by our team from the research laboratory (University Sultan Moulay Slimane), and a voucher specimen for each plant (ESTK/2018/25 and ESTK/2018/29) were deposited in the laboratory collections. The herbs were air-dried in the room and then were milled into 80 mesh powder before hydrodistillation.

50 g dried of samples (50 g) were distilled by Clevenger-apparatus to collect 3 L of distillate (distillation time: 6 h), and the distillate was collected for extracting essential oils of the plants using 100 mL anhydrous diethyl ether under room temperature. The organic phases were dehydrated with anhydrous sodium sulfate and concentrated to obtain essential oils for each biomass. The essential oils were stored at –20°C in dark glass bottles until required for chemical and biological analyzes (maximum four weeks). The process was executed in 10 replicates.

Essential oil analysis

Gas chromatography-mass spectrometry (GC/MS) analysis was carried out using a GC/MS apparatus (Hewlett Packard 5971A, Vienna, Austria). The samples of essential oils components were separated on capillary column (30 m × 0.25 mm × 0.25 µm thickness). Helium (99.999%) was employed as carrier gas at a constant linear velocity of 35 cm·s^{-1}. The sample volume of 1 µL was injected using AOC-20i+s autoinjector. The injection port was set at 290°C in splitless mode. The GC oven temperature was programmed as follows: 5 min at 50°C, heated in 1 min from 2°C to 300°C and held for 10 min. Ionization by MS of the sample components was performed in the EI mode (70 eV).

The identification of the essential oil constituents was accomplished by comparing retention indices from the literature data and mass spectra by computer library search.

Insecticidal activity

The insects *Sitophilus granarius* were reared respectively on wheat, tender in plastic boxes of one-liter capacity, transparent and wire mesh (Yattinamani, Bharati & Chimmad, 2019). The whole is placed in enclosures whose temperature is 30°C and the relative humidity is 70%. The insecticide evaluation procedure used in this work is as follows: in Petri dishes (experimental chambers) containing 10 insects, the EOCS were tested at increasing concentrations: C_1 = 0.01 µL·cm^{-3}, C_2 = 0.02 µL·cm^{-3} and C_3 = 0.03 µL·cm^{-3} and at different temperatures: T_1 = 25°C, T_2 = 30°C and T_3 = 35°C. The essential oils were placed in steel cylinders with a constant depth of 0.5 cm and diameters: D_1 = 1 cm, D_2 = 2 cm and D_3 = 3 cm. The assembly was introduced into a fumigation chamber included in the experimental enclosure (semi-ventilated). Repetitions were carried out in triplicate for minimize errors with an oil-free control. The number of dead insects was recorded as a function of time after 24 h (Paventi et al., 2020).

Adjusted mortality in treated insects is expressed according to the equation:

$$M = \left(\frac{M_I - M_C}{100 - M_C}\right) 100\ [\%]$$

where:
M – mortality;
M_I – mortality observed in insects;
M_C – mortality observed in controls.

The determination of the lethal dose of 50% LD_{50} was determined by linear interpolation on curves giving the percentage of mortality as a function of the logarithm of the concentration tested.

Antimicrobial activity

Antimicrobial tests of essential oils were performed against strains Gram(+) bacteria such as: *Staphylococcus aureus* (CECT 976), *Enterococcus faecium* (CECT 4932), *Listeria monocytogenes* (CECT 911) and *Bacillus subtilis* (CECT 4071), strains Gram(–) bacteria such as: *Escherichia coli* (CECT 431), *Yersinia enterocolitica* (CECT 4315) and the yeast *Candida albicans.*

The *MIC* and the *MBC* were determined in 96-well plates (12 columns and 8 rows). For each EO, 10 different concentrations were tested (3.125, 6.25, 12.5, 25.0, 50.0 and 100.0 μl·mL^{-1}). Negative (MH broth and DMSO) and positive (MH broth and microbial inoculum, without essential oils). The plates were incubated overnight at 37°C. The *MIC* values corresponded to the first well of each row where no visible microbial growth was detected. The *MBC* was determined from the first three wells of each row that showed no microbial growth after plate incubation (Man, Santacroce, Iacob, Mare & Man, 2019; Abedini et al., 2020). For that, 10 μL from the corresponding wells were seeded on MacConkey agar plates. After overnight incubation at 37°C, any microbial growth was checked. The *MBC* values represent the concentrations from plates where no bacterial colonies were found.

Statistical analysis

All experiments were performed in triplicate and data were expressed as mean values ±standard deviation (*SD*). The statistical software used for this analysis is SPSS V20.

Results and discussion

The results of chemical analysis carried out by GC/MS of two essential oils of *Eucalyptus globulus* and *Rosmarinus officinalis* were mentioned in Tables 1 and 2, respectively. All spectroscopic data revealed the presence of 82 organic volatile compounds representing 98.63% of the total constituents of *Eucalyptus globulus* and the presence of 45 organic volatile compounds representing 98.53% of the total constituents of *Rosmarinus officinalis*.

Moreover, the major compounds for *Eucalyptus globulus* were estragole (28.14%), terpinolene (7.12%), 1,4-hexadiene-5-methyl-3-(1-methylethylidene) (7.01%), linalool (5.54%) and furfural (4.66%). According to Ferrentino, Morozova, Horn and Scampicchio (2020), 1,8-cineole or/and eucalyptol are the main constituents (70–80%) and the other constituents are mainly terpene with low concentration.

On the other hand, the major compounds for *Rosmarinus officinalis* were (-)-camphor (31.16%) and β-caryophyllene (18.55%), 3,4-dimethyl-(Z,Z)--2,4-Hexadiene (9.08%), α-fenchene (4.67%), cis-verbenone (4.33%) and Bornyl acetate (3.4%). According to numerous previous studies, the essential oil composition of *Rosmarinus officinalis* strongly related chemotypes as well as the degree of development of the plant. Its main constituents can be 1,8--cineole, α-pinene, camphor, other compounds such as: borneol, bornyl acetate, verbenone, p-cymene or p-myrcene. They can be accompanied by β-caryophyllene, limonene, linalool, sabinene, γ-terpinene, α-terpineol and terpinenol-4 (Conde-Hernández, Espinosa-Victoria, Trejo & Guerrero-Beltrán, 2017; Chung, Lee, Lee, Chung & Lee, 2020).

The results of the insecticidal tests of the essential oils of *Eucalyptus globulus* and *Rosmarinus officinalis* against *Sitophilus granarius* are displayed in Table 3 in the form of lethal doses of 50% (LD_{50}), according to the studied parameters such as: the cylinder diameter (*D*) and the temperature of the incubation (*T*). The study of the insecticidal activity of these essential oils on an organism harmful to stored products *Sitophilus granarius* confirmed that the two essential oils studied have important insecticidal activity.

TABLE 1. Chemical composition of the essential oil of *Eucalyptus globulus*

Peak	R_T	Compound	C [%]
1	4.19	(-)-camphor	31.16
2	4.31	(+)-2-Bornanone	2.17
3	4.64	3.4-dimethyl-(Z.Z)-2.4-Hexadiene	9.08
4	4.82	3-Cyclopentene-1-ethanol. 2.2.4-trimethyl-	4.57
5	4.95	Isoborneol	4.37
6	5.17	α-Fenchene	4.67
7	5.25	cis-Verbenone	4.33
8	5.35	D-Verbenone	2.08
9	5.55	Cyclohexanone. 5-methyl-2-(1-methylethylidene)-	0.24
10	5.61	5-Hepten-1-ol. 2-ethenyl-6-methyl-	0.11
11	5.65	Cyclodecene. 1-methyl-	0.10
12	5.73	Piperitone	0.15
13	6.26	Bornyl acetate	3.44
14	6.47	β-Terpinene	0.58
15	6.69	p-Cymen-7-ol	0.18
16	6.78	4-Hydroxy-3-methylacetophenone	0.17
17	6.89	(Z)-Ocimenone	0.14
18	6.98	4-Hydroxy-3-methylacetophenone	0.24
19	7.11	2H-Inden-2-one. 1.4.5.6.7.7a-hexahydro-7a-methyl-. (S)-	0.33
20	7.45	α-Copaene	0.51
21	8.51	β-Caryophyllene	18.55
22	8.86	1.4.7.-Cycloundecatriene. 1.5.9.9- tetramethyl-. Z.Z.Z-	3.67
23	9.19	γ-Muurolene	0.43
24	9.39	ar-Curcumene	0.48
25	9.52	9-epi-(E)-Caryophyllene	0.15
26	9.62	α-Bergamotene	0.11
27	9.71	α-Amorphene	0.23
28	9.85	β-Bisabolene	0.85
29	9.95	δ-Cadinene	0.24
30	10.01	β-Sesquiphellandrene	0.23
31	10.19	Caryophyllene oxide	0.27
32	10.69	(-)-Globulol	1.85
33	11.02	Humulene epoxide II	0.22
34	11.48	β-Longipinene	0.31
35	11.48	10.10-Dimethyl-2.6-dimethylenebicyclo[7.2.0]undecan-5.beta.-ol	0.31
36	11.87	trans-Z-α-Bisabolene epoxide	0.44

TABLE 1, cont.

Peak	R_T	Compound	C [%]
37	12.06	1-Methyl-6-methylenebicyclo[3.2.0] heptane	0.15
38	13.93	Succinic acid. 3-methylbut-2-yl non-5-yn-3-yl ester	0.12
39	15.05	Isopimara-9(11).15-diene	0.19
40	15.33	(3aS.4R.7R)-1.4.9.9-Tetramethyl-5.6.7.8-tetrahydro-4H-3a.7-methanoaz ulene	0.16
41	16.05	Phosphonous dichloride. (1.7.7-trimethylbicyclo[2.2.1]hept-2-yl)-	0.18
42	16.17	(2.6.6-Trimethylcyclohex-1-enylmethanesulfonyl)benzene	0.24
43	16.62	Cyclohexanemethanol. 3.3-dimethyl- 2-(3-methyl-1-butenyl)-	0.29
44	17.17	Aminosalicylic Acid	0.11
45	17.55	4-Carene. (1S.3R.6R)-(-)-	0.13

R_T – retention time.

TABLE 2. Chemical composition of the essential oil of *Rosmarinus officinalis*

Peak	R_T	Compound	C [%]
1	3.17	Terpinolene	7.12
2	3.27	trans-Verbenol	0.47
3	3.36	Linalool	5.54
4	3.35	Spiro[2.4]heptane. 4-methylene-	1.00
5	3.58	4-Acetyl-1-methylcyclohexene	0.51
6	3.68	1.4-Hexadiene. 5-methyl-3-(1-methylethylidene)-	7.01
7	3.83	Tricyclo[4.1.0.0(2.7)]heptanes	2.15
8	3.90	4-Methyl-1.4-heptadiene	0.94
9	4.05	Dicyclopentadiene. tetrahydro. exo	0.83
10	4.11	Isoborneol	0.66
11	4.24	Terpinen-4-ol	2.07
12	4.33	Furfural	4.66
13	4.43	Myrtenal	1.50
14	4.69	Estragole	28.14
15	5.04	2.6-Dimethyl-3.5.7-octatriene-2-ol . Z.Z-	0.75
16	5.19	Propanal. 2-methyl-3-phenyl-	0.27
17	5.30	(-)-Carvone	1.59
18	5.79	Dihydrocarveol	0.14
19	5.88	α-Terpinen-7-al	0.21
20	6.03	Bornyl acetate	0.75
21	6.23	1-Methyltricyclo[2.2.1.0(2.6)]heptane	0.97
22	6.29	Artemisia triene	2.08

TABLE 2, cont.

Peak	R_T	Compound	C [%]
23	6.46	Cyclohexane. 4-methyl-2-methylene-1-(1-methylethylidene)-	0.61
24	6.65	p-Menth-8-ene. 3-methylene-	1.39
25	6.78	2H-Inden-2-one. 1.4.5.6.7.7a-hexahydro-7a-methyl-. (S)-	0.91
26	6.90	α-Longipinene	2.64
27	7.07	4-(2.4.4-Trimethyl-bicyclo[4.1.0]hept-2-en-3-yl)-butan-2-one	0.83
28	7.14	cis-Carvyl Acetate	1.91
29	7.42	5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol	1.10
30	7.58	α-Bulnesene	0.78
31	7.69	Cycloheptane. 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-viny l	0.66
32	7.82	α-Santalol	0.94
33	8.02	(2S.4R)-p-Mentha-[1(7).8]-diene 2- hydroperoxide	0.89
34	8.23	5-Isopropenyl-2-methyl-7-oxabicyclo[4.1.0]heptan-2-ol	0.56
35	8.33	cis-Sabinene hydrate	0.49
36	8.43	α-Himachalene	0.50
37	8.63	8-Oxabicyclo[5.1.0]oct-5-en-2-ol.1.4.4-trimethyl-	0.52
38	8.93	β-Humulene	0.44
39	9.26	Eremophilene	0.59
40	9.36	β-Himachalene	0.65
41	9.79	α-Sinensal	0.23
42	9.85	β-Longipinene	0.25
43	9.98	Z-Ocimene	0.22
44	10.09	Methane. tribromo-	0.36
45	10.29	Ethanone. 1-(3-methylene cyclopentyl)-	0.15
46	10.44	β-Ocimene	0.38
47	10.53	Spiro[cyclopropane-1.6’-[3]oxatricyclo[3.2.1.0(2.4)]octane]	0.33
48	10.65	p-Menth-8-ene. 3-methylene-	0.19
49	10.96	Cyclohexane. (2-ethyl-1-methyl-1-butenyl)-	0.29
50	11.08	Limonene dioxide	0.12
51	12.60	cis.α-Santalol	0.13
52	12.77	Ocimene	0.10
53	13.28	(E)2.3-Dimethylcyclohex-2-en.oxime	0.37
54	13.38	1.2-Dipropylcyclopropene-3-carboxylic acid	0.36
55	14.13	2(5H)-Furanone. 4-methyl-3-(2-methyl-2-propenyl)-	0.16
56	14.78	(3S.6R)-3-Hydroperoxy-3-methyl-6-(prop-1-en-2-yl)cyclohex-1-ene	0.17
57	15.00	Camphene	0.16

TABLE 2, cont.

Peak	R_T	Compound	C [%]
58	15.08	Triselenothane	0.22
59	15.26	α-Farnesene	0.46
60	15.34	Acetic acid. 2.2-selenobis-	0.10
61	15.39	(E.E.E)-3.7.11.15-Tetramethylhexadeca-1.3.6.10.14-pentaene	0.24
62	15.69	1.6.10.14.18.22-Tetracosahexaen-3- ol. 2.6.10.15.19.23-hexamethyl-. (all-E)-(.+/-.)-	0.43
63	15.84	(Z)-β-Santalol	0.32
64	16.02	p-Menth-8-ene. 3-methylene-	0.28
65	16.13	p-Camphorene	0.91
66	16.2	β-Terpinene	0.21
67	16.35	Hexane. 1-chloro-5-methyl-	0.45
68	16.55	β-Bisabolene	0.57
69	16.71	Acetic acid. 2.2'-selenobis-	0.39
70	17.02	Succinic acid. 2-methylpent-3-yl 2.2-dichloroethyl ester	0.73
71	17.27	(3R.6R)-3-Hydroperoxy-3-methyl-6-(prop-1-en-2-yl)cyclohex-1-ene	0.31
72	17.43	Formic acid. 3.7.11-trimethyl-1.6.10-dodecatrien-3-yl ester	0.47
73	17.57	Acetic acid. 2.2'-selenobis-	0.75
74	17.83	7-Methylene-bicyclo[3.3.1]nonan-3-one oxime	0.33
75	18.41	Formic acid. 3.7.11-trimethyl-1.6. 10-dodecatrien-3-yl ester	0.55
76	20.21	1.5.6.7-Tetrahydro-4-indolone	0.23
77	20.61	β-Elemenone	0.26
78	20.84	(Z)-epi-β-Santalol	0.12
79	21.00	2-Ethyl-2-[(E)-(4-methoxyphenyl)diazenyl]malononitrile	0.16
80	21.30	Epi-β-Santalol	0.12
81	22.18	(3R.6R)-3-Hydroperoxy-3-methyl-6-(prop-1-en-2-yl)cyclohex-1-ene	0.10
82	28.12	Phenol. 2-(2-aminoethyl)-	0.18

R_T – retention time.

According to the lethal dose of 50% (LD_{50}), the activity of the essential oils tested varies widely depending on the nature of the essential oil and the factors used (Diniz do Nascimento et al., 2020). In addition, the insecticidal activities of these essential oils studied are probably due to the major constituents of each essential oil, thus and their synergetic. All these tests carried out can confirm that the treatment of foodstuffs with essential oil from aromatic and medicinal plants can be very effective in controlling pests of stored foodstuffs. All these tests carried out can confirm that the treatment of foodstuffs with these two essential oils can be very effective in controlling pests of stored foodstuffs (Ainane et al., 2019).

TABLE 3. LD_{50} insecticide activities of different essential oils

Diameter	Temperature	*Eucalyptus globules*	*Rosmarinus officinalis*
D_1 = 1 cm	T_1 = 25°C	0.041 ±0.005	0.016 ±0.005
	T_2 = 30°C	0.007 ±0.005	0.003 ±0.005
	T_3 =35°C	0.005 ±0.005	0.002 ±0.005
D_2 = 2 cm	T_1 = 25°C	0.020 ±0.005	0.011 ±0.005
	T_2 = 30°C	0.009 ±0.005	0.002 ±0.005
	T_3 = 35°C	0.004 ±0.005	0.001 ±0.005
D_3 = 3 cm	$T1$ = 25°C	0.020 ±0.005	0.007 ±0.005
	T_2 = 30°C	0.007 ±0.005	0.002 ±0.005
	T_3 = 35°C	0.002 ±0.005	*

*LD_{50} is less than 0.001 $\mu L \cdot cm^{-3}$.

TABLE 4. Parameters of the antibacterial activity of two essential oils

Strains	Parameter	*Eucalyptus globules*	*Rosmarinus officinalis*
Staphylococcus aureus	*MIC* [$\mu l \cdot mL^{-1}$]	655.13	47.31
	MBC [$\mu l \cdot mL^{-1}$]	815.13	58.21
	MBC / *MIC*	1.24	1.23
Enterococcus faecium	*MIC* [$\mu l \cdot mL^{-1}$]	713.11	54.23
	MBC [$\mu l \cdot mL^{-1}$]	992.11	84.32
	MBC / *MIC*	1.39	1.55
Listeria monocytogenes	*MIC* [$\mu l \cdot mL^{-1}$]	791.31	68.33
	MBC [$\mu l \cdot mL^{-1}$]	871.31	89.53
	MBC / *MIC*	1.10	1.31
Bacillus subtilis	*MIC* [$\mu l \cdot mL^{-1}$]	803.35	64.23
	MBC [$\mu l \cdot mL^{-1}$]	996.35	74.33
	MBC / *MIC*	1.24	1.16
Escherichia coli	*MIC* [$\mu l \cdot mL^{-1}$]	941.21	69.21
	MBC [$\mu l \cdot mL^{-1}$]	1 471.21	88.21
	MBC / *MIC*	1.56	1.27
Yersinia enterocolitica	*MIC* [$\mu l \cdot mL^{-1}$]	804.61	71.53
	MBC [$\mu l \cdot mL^{-1}$]	994.41	91.13
	MBC / *MIC*	1.24	1.27
Pseudomonas aeruginosa	*MIC* [$\mu l \cdot ml^{-1}$]	not detected	56.23
	MBC [$\mu l \cdot mL^{-1}$]	not detected	78.13
	MBC / *MIC*	not detected	1.39
Candida albicans	*MIC* [$\mu l \cdot mL^{-1}$]	867.75	89.15
	MBC [$\mu l \cdot mL^{-1}$]	1 234.75	101.05
	MBC / *MIC*	1.42	1.13

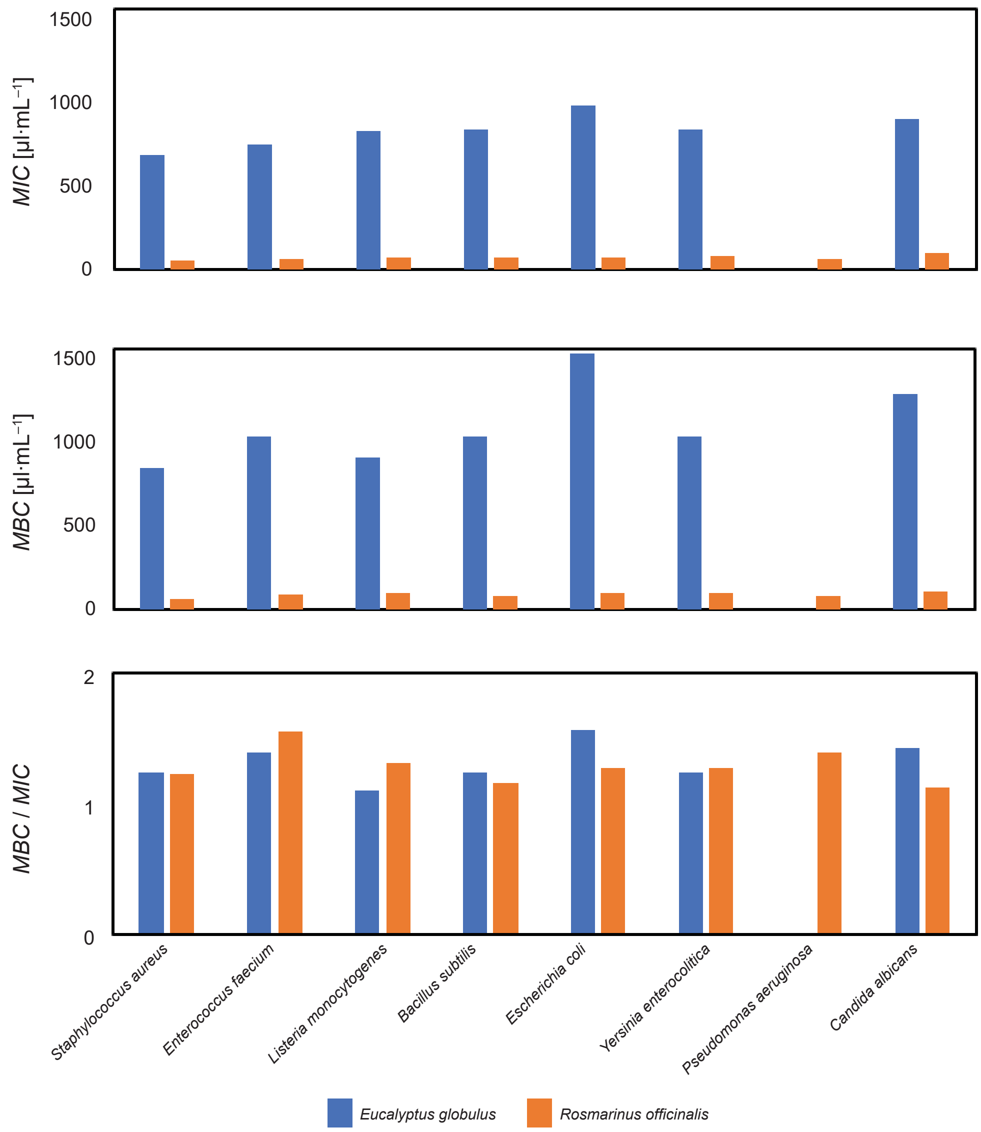

FIGURE. Graphical representation of parameter data: *MIC*, *MBC* and *MBC* / *MIC*

The results of the antibacterial activities of the microdilution broth method applied to the essential oils of *Eucalyptus globulus* and of *Rosmarinus officinalis* are expressed in terms of the minimum inhibitory concentration (*MIC*), the minimum bactericidal concentration (*MBC*) and the fraction (*MBC* / *MIC*). All the values obtained are shown in Table 4 and the figure. It can be concluded after results obtained, that the two essential oils have interesting antimicrobial activities against *Staphylococcus aureus*, *Enterococcus faecium*, *Listeria monocytogenes*, *Bacillus subtilis*, *Escherichia coli*, *Yersinia enterocolitica* and *Candida albicans*. The minimum inhibitory concentrations MIC vary from 47.31 to 941.21 $\mu l \cdot mL^{-1}$ and the minimum bactericide concentrations *MBC* vary in the range from 58.21 to 1,234.75 $\mu l \cdot mL^{-1}$. The essential oil of *Eucalyptus globulus* has weak antimicrobial activities while the essential oil of *Rosmarinus officinalis* has moderate and acceptable antimicrobial activities (El Abboubi et al., 2019).

Finally, all the results obtained during the insecticidal activities and the complementary antibacterial activities, we can target the two essential oils in agricultural uses as bioinsecticides. They can be used as natural insecticides while respecting environmental standards.

Conclusions

During this study, the research carried out gave us a general work of view on the development of these two essential oils of *Eucalyptus globulus* and *Rosmarinus officinalis*, from where, we carried out a chemical composition with other biological studies for agricultural applications. The results obtained have shown that the two essential oils have interesting prospects for the conservation of seeds against biotic aggressions (insect pests and infection microbials). These results can help reduce the amount of insecticides applied, and subsequently decrease the negative impact of synthetic agents, such as residues, resistance and environmental pollution.

References

Abedini, A., Colin, M., Hubert, J., Charpentier, E., Angelis, A., Bounasri, H. & Renault, J.H. (2020). Abundant extractable metabolites from temperate tree barks: the specific antimicrobial activity of *Prunus avium* extracts. *Antibiotics*, *9*(3), 111. https://doi.org/10.3390/antibiotics9030111

Ainane, A., Khammour, F., El Kouali, M., Talbi, M., Oussaid, A., Lemhidi, A. & Ainane, T. (2019). Evaluation of the toxicity of the essential oils of certain mints grown in the region of Settat (Morocco): *Mentha piperita*, *Mentha pulegium* and *Mentha spicata* against, *Sitophilus Granarius*, *Sitophilus Oryzae* and *Sitophilus Zeamais*. *Journal of Analytical Sciences and Applied Biotechnology*, *1*(1), 1-10.

Awan, D.A., Ahmad, F., Saleem, M.A., Shakoori, A.R. (2019). Synergistic effect of piperonyl butoxide and emamectin benzoate on enzymatic activities in resistant populations of red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: *Tenebrionidae*). *Environmental Science and Pollution Research*, *26*(14), 14200-14213.

Banga, K.S., Kotwaliwale, N., Mohapatra, D. & Giri, S.K. (2018). Techniques for insect detection in stored food grains: an overview. *Food Control*, *94*, 167-176.

Bottrell, D.G. & Schoenly, K.G. (2018). Integrated pest management for resource-limited farmers: challenges for achieving ecological, social and economic sustainability. *The Journal of Agricultural Science*, *156*(3), 408-426.

Cheng, W., Yang, J., Nie, Q., Huang, D., Yu, C., Zheng, L. & Zhang, J. (2017). Volatile organic compounds from *Paenibacillus polymyxa* KM2501-1 control *Meloidogyne incognita* by multiple strategies. *Scientific reports*, *7*(1), 1-11.

Chung, M.S., Lee, G.W., Lee, S.S., Chung, B.Y. & Lee, S. (2020). Comparative Analysis of Volatile Terpenoids Composition in Rosemary Leaves in Response to Ionizing Radiation. *Journal of Essential Oil Bearing Plants*, *23*(3), 594-600.

Conde-Hernández, L.A., Espinosa-Victoria, J.R., Trejo, A. & Guerrero-Beltrán, J.Á. (2017). CO_2-supercritical extraction, hydrodistillation and steam distillation of essential oil of rosemary (*Rosmarinus officinalis*). *Journal of Food Engineering*, *200*, 81-86.

Costa, J.A.V., Freitas, B.C.B., Cruz, C.G., Silveira, J. & Morais, M.G. (2019). Potential of microalgae as biopesticides to contribute to sustainable agriculture and environmental development. *Journal of Environmental Science and Health, Part B*, *54*(5), 366-375.

Diniz do Nascimento, L., de Moraes, A.A.B., da Costa, K.S.., Pereira Galúcio, J.M., Taube, P.S., Costa, C.M.L. & Faria, L.J.G.D. (2020). Bioactive natural compounds and antioxidant activity of essential oils from spice plants: new findings and potential applications. *Biomolecules*, *10*(7), 988. https://doi.org/10.3390/biom10070988

El Abboubi, M., Bennani, L., Ainane, A., Charaf, S., Bouhadi, M., Elkouali, M. H., Ainane, T. (2019). Treatment of mycoses by essential oils: Mini Review. *Journal of Analytical Sciences and Applied Biotechnology*, *1*(2), 35-40.

Ferrentino, G., Morozova, K., Horn, C. & Scampicchio, M. (2020). Extraction of essential oils from medicinal plants and their utilization as food antioxidants. *Current Pharmaceutical Design*, *26*(5), 519-541.

Isman, M.B. (2020). Botanical insecticides in the twenty-first century – fulfilling their promise? *Annual Review of Entomology*, *65*, 233-249.

Lamichhane, J.R., Aubertot, J.N., Begg, G., Birch, A.N.E., Boonekamp, P., Dachbrodt--Saaydeh, S. & Kiss, J. (2016). Networking of integrated pest management: a powerful approach to address common challenges in agriculture. *Crop protection*, *89*, 139-151.

Man, A., Santacroce, L., Iacob, R., Mare, A. & Man, L. (2019). Antimicrobial activity of six essential oils against a group of human pathogens: a comparative study. *Pathogens*, *8*(1), 15. https://doi.org/10.3390/pathogens8010015

Owolabi, M.S., Ogundajo, A.L., Alafia, A.O., Ajelara, K.O. & Setzer, W.N. (2020). Composition of the essential Ooil and insecticidal activity of *Launaea taraxacifolia* (Willd.) Amin ex C. Jeffrey growing in Nigeria. *Foods*, *9*(7), 914. https://doi.org/10.3390/foods9070914

Pandey, A.K., Tripathi, S. & Singh, P. (2018). Plant essential oils: a substitute for conventional insecticides against Tribolium species (Coleoptera: Tenebrionidae) – achievements and challenges. *Archives of Phytopathology and Plant Protection*, *51*(13-14), 696-728.

Paventi, G., Acutis, L., de, De Cristofaro, A., Pistillo, M., Germinara, G.S. & Rotundo, G. (2020). Biological Activity of *Humulus lupulus* (L.) Essential Oil and Its Main Components against *Sitophilus granarius* (L.). *Biomolecules*, *10*(8), 1108. https://doi.org/10.3390/biom10081108

Puthur, S., Anoopkumar, A.N., Rebello, S., Aneesh & E.M. (2019). Synergistic control of storage pest rice weevil using *Hypericum japonicum* and deltamethrin combinations: a key to combat pesticide resistance. *Environmental Sustainability*, *2*(4), 411-417.

Shah, F.M., Razaq, M., Ali, Q., Shad, S.A., Aslam, M. & Hardy, I.C. (2019). Field evaluation of synthetic and neem-derived alternative insecticides in developing action thresholds against cauliflower pests. *Scientific Reports*, 9(1), 1-13.

Singh, B. & Kaur, A. (2018). Control of insect pests in crop plants and stored food grains using plant saponins: a review. *LWT*, *87*, 93-101.

Vélez, M., Barbosa, W. F., Quintero, J., Chediak, M. & Guedes, R.N.C. (2017). Deltamethrin- and spinosad-mediated survival, activity and avoidance of the grain weevils *Sitophilus granarius* and *S. zeamais*. *Journal of Stored Products Research*, *74*, 56-65.

Vetal, D.S. & Pardeshi, A.B. (2019). Larvicidal potential of *Argemone mexicana* L. Plant extracts against *Spodoptera litura* fab. *The Pharma Innovation Journal*, *8*(6), 698-702.

Yattinamani, N.M., Bharati, P. & Chimmad, B.V. (2017). Breakfast behaviour of adolescents of karnataka and its contribution to nutrient intake. *Life Science Bulletin*, *14*(2), 193-197.

Summary

Evaluation of biological activities of two essential oils as a safe environmental bioinsecticides: case of *Eucalyptus globulus* and *Rosmarinus officinalis*. All works of this article were conducted to investigate chemical composition and insecticidal and antimicrobial properties of *Eucalyptus globulus* and *Rosmarinus officinalis* essential oils isolated by hydro-distillation of its aerial parts. Analysis of the essential oils on the basis of gas chromatography and mass spectrometry (GC/FID and GC/MS) revealed the presence of 82 organic volatiles representing 98.63% of the total constituents of *Eucalyptus globulus* and the presence of 45 organic volatiles representing 98.53% of the total constituents of *Rosmarinus officinalis*. The major compounds for *Eucalyptus globulus* were estragole (28.14%), terpinolene (7.12%), 1,4-hexadiene-5-methyl-3-(1--methylethylidene) (7.01%), linalool (5.54%) and furfural (4.66%) and for *Rosmarinus officinalis* were (-)-camphor (31.16%) and β-caryophyllene (18.55%), 3,4-dimethyl--(Z,Z)-2,4-Hexadiene (9.08%), α-fenchene (4.67%), cis-verbenone (4.33%) and Bornyl acetate (3.4%). The efficacy of the two essential oils was evaluated on the insect pests *Sitophilus granarius* of wheat and was remarkable with lethal doses of 50% tending towards 1 $\mu L \cdot cm^{-3}$. The broth microdilution method as a complementary test was conducted to test the antimicrobial activity of the essential oil against: *Staphylococcus aureus*, *Enterococcus faecium*, *Listeria monocytogenes*, *Bacillus subtilis*, *Escherichia coli*, *Yersinia enterocolitica*, *Pseudomonas aeruginosa* and *Candida albicans*, further for the two oils of shows promising activity against all strains.

Authors' address:
Ayoub Ainane
(https://orcid.org/0000-0002-5004-6416)
Tarik Ainane – corresponding author
(https://orcid.org/0000-0001-6743-2666)
University of Sultan Moulay Slimane
Superior School of Technology of Khenifra
(EST-Khenifra)
PB 170, Khenifra 54000
Morocco
e-mail: t.ainane@usms.ma

Fatouma Mohamed Abdoul-Latif
(https://orcid.org/0000-0001-7213-4700)
Talal Mohamed Abdoul-Latif
Medicinal Research Institute
Center for Research and Study of Djibouti
BP 486, Djibouti