J. Crop Prot. 2022, 11 (2): 173-184

Research Article

Rosemary essential oil potential as a bio-insecticide for protecting stored dates against the date moth *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae)

Selma Adouane[1, 2*], Yamina Bouatrous[2], Mohamed Seghir Mehaoua[1, 3], Jose Tudela[4], Sara Mechaala[2] and Virginia Tomas[5]

1. Department of Agricultural Sciences, Mohamed Khider University, BP 145 RP, 07000 Biskra, Algeria.
2. Laboratory of Genetics, Biotechnology, and Valorization of Bio-resources (GBVB), Faculty of Exact Sciences and Sciences of Nature and Life, Mohamed Khider University, Biskra, Algeria.
3. Laboratory of Diversity of Ecosystems and Dynamics of Agricultural Production Systems in Arid Zones (DEDSPAZA), Faculty of Exact Sciences and Sciences of Nature and Life, Mohamed Khider University, Biskra, Algeria.
4. Department of Biochemistry and Molecular Biology, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, E-30100, Espinardo, Murcia, Spain.
5. Department of Analytical Chemistry, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, E-30100, Espinardo, Murcia, Spain.

Abstract: The present work was undertaken to compare the insecticidal activity of two *Rosmarinus officinalis* L. (Lamiaceae) essential oils chemotypes from the Mediterranean region against different life stages of *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae) in constant environmental conditions. The essential oils were extracted by hydrodistillation and analyzed by the gas chromatography-mass spectrometry technique (GC-MS). α-pinene (29.42%), camphene (24.62%), and camphor (20.95%) were obtained as the majority compounds in the essential oil of *R. officinalis* from Algeria, as well as the main essential oil compounds of *R. officinalis* from Spain were α-pinene (25.62%), 1,8-cineole (21.06%) and camphor (18.39%). Ovicidal activity of oils was studied by topical application, while adulticidal and larvicidal activities were assessed by fumigation and ingestion, respectively. *R. officinalis* from Spain presented the highest toxic activity against eggs (78.33% inhibition), young larvae (LC_{50} = 3.40 mg/ml) and adults (LC_{50} = 0.17 mg/ml). On the other hand, *R. officinalis* from Algeria presented lower egg hatching inhibition with 60% and fewer fumigant and antifeedant activities (LC_{50} = 0.30 and 4.97mg/ml for adults and young larvae, respectively). The results of this study indicated the efficacy of rosemary essential oil as an alternative to synthetic insecticides in a postharvest treatment program for the control of *E. ceratoniae*.

Keywords: Insecticidal activity, Essential oils, *Rosmarinus officinalis*, *Ectomyelois ceratoniae*, Bio-insecticide

Introduction

In arid and semi-arid regions, phoéniciculture is of great socio-economic importance (Majourhat *et al*., 2002). Dates and their secondary products are the main agricultural products of the oases of the North African Sahara (Botes and Zaid,1999). They constitute an essential source of nutrients and energy (Boudries *et al*., 2007). Algeria is one of the major date-producing countries; the

Handling Editor: Saeid Moharramipour

* Corresponding author: adouane.selma7@gmail.com
Received: 02 December 2020, Accepted: 08 July 2022
Published online: 25 July 2022

[DOR: 20.1001.1.22519041.2022.11.2.1.4]

national date production exceeds seven million quintals per year, of which more than half is exported (Dehliz *et al*., 2016). Nevertheless, date production is confronted with several problems, particularly in a phytosanitary order, such as pests attack (Mehaoua *et al*., 2013). The carob moth *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae) is the most important and destructive insect attacking date crops (Mediouni-ben Jemâa*et al*., 2004). It can cause considerable damage, reaching 30% of date production in the Mediterranean basin (F.A.O, 2006). The damage caused to the field at fruit maturity varies from 1 to 4%, but the real damage was in storage with 70% (Sedra, 2003). On the other hand, postharvest control of this pest is exclusively based on synthetic insecticides. Whereas their indiscriminate use resulted in several problems, such as the elimination of natural enemies, pest resistance, and toxic residues in food, water, air, and soil which affect humans and the environment (Balaraju *et al*., 2011). All those reasons make it urgent the orient to other means of control that use natural compounds as an alternative to toxic insecticides (Lamiri *et al*., 2001). Biopesticides have an advantage over chemical pesticides by their highly effective, safe, and ecologically acceptable nature (Leonard *et al*., 2000). The research focused on essential oils, which play an important role in protecting crops against insect infections (Batish *et al*., 2008). They constitute a rich source of bioactive compounds showing contact, fumigant, antifeedant, and repellent activity (Silva *et al*., 2003). According to an ethnobotanical survey, *Rosmarinus officinalis* L. (Lamiaceae) is native to Mediterranean countries and grows under different climatic conditions (Begum *et al*., 2013). Rosemary essential oil is important for its powerful antibacterial (Fu *et al*., 2007), antispasmolytic (Mothana *et al*., 2011), antifungal (Carvalhinho, 2012), antioxidant (Hendel *et al*., 2016), anticancer (Gezici *et al*., 2017) as well as insecticidal properties, and it is the active ingredient in several commercial insecticides (Isman*et al.*, 2008). This study explored for the first time the comparison and the relationship between chemical composition and insecticidal activity of two Mediterranean rosemary oil; *R. officinali*s from Algeria *and R. officinali*s from Spain, against the different developmental stages of the stored-date moth: *E. ceratoniae*, to promote them as biopesticides.

Materials and Methods

Insect rearing

Rearing of the moth *E. ceratoniae* was established in the laboratory of the Regional Station of Plant Protection-Biskra (S. R. P. V) from infested field-collected dates of the palm groves of Biskra. Rearing was performed in plastic boxes (30 × 20 × 15cm) placed in a growth chamber under constant environmental conditions (27 ± 2 °C, 65 ± 10% RH, and L: D 16:8h) (Al-izzi *et al*., 1987) on an artificial diet based on wheat bran (44%), citric acid (1%), vitamin mixture (1%), methylparaben (0.5%), salt mixture (1%), sugar (5%), ascorbic acid (0,5%), yeast (4%), gluten (3%) and distilled water (40%) (Mediouni-ben Jemâaand Dhouibi, 2007). One-day-old eggs, adults, and first instar larvae (L1) (0-24 h), of *E. ceratoniae*, were used for bioassays.

Plant material

Leaves of wild *R. officinalis* were collected from Biskra steppes (35°13′00″N, 5°42′37″E) located in Southern Algeria, where it grows spontaneously during April 2019. Leaves from *R. officinalis* were obtained in March 2019 from the province of Murcia (37°59′00″N, 1°08′00″W) situated in South-East Spain. The harvested material was air-dried at room temperature (20-25 °C) in darkness for further use.

Extraction and chemical analysis of the essential oils

Essential oils were obtained using a modified Clevenger-type apparatus. The weight of 100 g of each dry leaf was hydro-distilled in 1000 ml of distilled water for 4 h. The oils were dried over anhydrous sodium sulfate and then stored at 4 °C for further analysis. For the identification and quantification of volatile components,

[DOR: 20.1001.1.22519041.2022.11.2.1.4]

Essential oils were diluted 1:100 with isooctane in vials of 1.5 ml with mandrel inserts of 250 µl. Then, their compositions were determined by using an Agilent GC7890 gas chromatograph (GC), equipped with an Agilent MS5975 mass spectrometry detector (MSD). The GC system was equipped with a polar and low bleed capillary fused-silica column, SupelcoWax10, with 15 m length× 0.1 mm internal diameter x 0.1 µm film thickness. Hydrogen was used as carrier gas (0.6ml/min), generating a head pressure of 38.17 psi and produced with an electrolytic Parker-Domnik-Hunter generator. Sandwich injections (plunger to needle: 0.2 µl air, 0.2 µl isooctane, 0.2 µl air,0.3 µl sample, and 0.2 µl air) were performed using a Gerstel sampler MPS-2XT. Other injection conditions: injector temperature 260 ºC, septum purge 3 ml/min, and split valve 100:1. The oven temperature was programmed from 60 ºC to 260 ºC at 30 ºC/min and kept constant at 260 ºC for 5 min. The MSD operated with transfer line, ion source, and quadrupole temperatures at 260 ºC, 230 ºC, and 150 ºC, respectively. Other MSD conditions: electron ionization energy 70 eV, electron multiplier voltage 1129, acquisition mass range 30-500 m/z, 21.04 scan/s. Compounds were identified by comparison of their linear retention indexes (Van den Dool and Kratz, 1963), mass spectra of NIST and Wiley spectral libraries, and mass spectra of commercially available pure standards.

Insecticidal activities

Contact toxicity bioassay

The ovicidal activity was carried out by topical application. Preliminary tests were done to choose the right doses. Twenty fresh eggs (24 h) were transferred to a Petri dish (Ø = 90mm) containing a thin layer of food substrate (artificial diet). Then sprayed with 1 ml essential oil solutions in the following concentrations: 0.5, 1, 1.5, 2, and 2.5 mg/ml for each sample. Tests were performed at 30 °C and 80% RH, and three repetitions were performed for each dose with a corresponding control sprayed only with diluted Tween 20. Treated eggs were checked daily under a binocular loupe for hatching.

Fumigant toxicity bioassay

Preliminary tests were conducted to determine the range of concentrations for each essential oil. A solution of each oil dissolved in 1 ml diluted Tween 20 corresponds to the following concentrations: 0.1, 0.2, 0.3, and 0.4 mg/ml was applied to a piece of cotton. Then, the piece of cotton was attached to the cap's under surface of a 500 ml plastic bottle. The bottles were tightly closed and covered with parafilm, and each of them contained separately ten newly emerged adults (< 24 h old). In addition to the control test, each treatment was repeated thrice at 30 °C and 80% RH. The insect mortality was determined after 3, 6, 12, and 24 h of the test completion.

Antifeedant toxicity bioassay

To evaluate both essential oils and antifeedant toxicity, ten newly emerged first instar larvae (L1) were introduced in Petri dishes (Ø = 90 mm), containing a thin layer (20 g) of the artificial diet treated with 1 ml essential oil solutions in the following concentrations: 1, 4, 7, and 10mg/ml for each sample. Mortality was recorded every three days using a binocular loupe. The feeding trial was conducted for 12 days in 3 replicates in addition to the control. A larva was considered dead when it was completely immobile after its excitation.

Statistical analysis

The mortality data were corrected using Abbott's correction formula (Abbott, 1925). The corrected percentage of mortalities was statistically computed according to Finney's (1971) method. The computed percentage of mortality was plotted versus the corresponding concentrations on the logarithmic probability paper to obtain the corresponding Log-concentration probit lines. The LC_{50} was determined for established regression lines. Percentage mortality value for different concentrations was subjected to analysis of variance (ANOVA) using XLSTAT (2007). Differences between means were tested through Fisher's test, and values with $P < 0.05$ were considered significantly different.

[DOR: 20.1001.1.22519041.2022.11.2.1.4]

Results

Essential oil composition

Results from the quantitative and qualitative analysis of the two essential oils constituents investigated using GC–MS techniques are presented in Tables 1 and 2. Both oils were shown to be complex mixtures of many components. Results showed that 26 compounds were identified in the essential oil of *R. officinalis* from Algeria. This oil profile is characterized by α-Pinene (29.42%), camphene (24.62%), and camphor (20.95%). For *R. officinalis* essential oil from Spain, 24 components were identified. The chemical composition of this essential oil is mainly dominated by α-pinene (25.62%), 1,8-cineole (21.06%), and camphor (18.39%). Besides, components such as camphene (9.61%) and β-myrcene (3.16%) were identified in trace amounts.

Table 1 Essential oil composition of *Rosmarinus officinalis* from Algeria.

N	Retention Index (min)	LRI	Compound	Concentration (%)	IM
1	1.360	1025	α-Pinene	29.42	L, M, S
2	1.585	1069	Camphene	24.62	L, M, S
3	1.808	1110	β-Pinene	3.76	L, M, S
4	2.139	1161	β-Myrcene	1.01	L, M
5	2.232	1178	α-Terpinene	0.52	L, M, S
6	2.342	1198	D-Limonene	4.04	L, M, S
7	2.398	1211	1,8-Cineole	6.32	L, M, S
8	2.605	1245	γ-Terpinene	0.93	L, M, S
9	2.748	1270	*p*-Cymene	1.03	L, M, S
10	2.809	1282	α-Terpinolene	0.35	L, M, S
11	3.565	1423	α-Thujone	0.74	L, M, S
12	3.656	1440	β-Thujone	0.17	L, M
13	3.904	1491	α-Copaene	0.23	L, M
14	3.966	1508	Chrysanthenone	0.24	L, M
15	4.019	1515	Camphor	20.95	L, M, S
16	4.270	1579	Bornyl Acetate	0.24	L, M, S
17	4.349	1601	Terpinen-4-ol	0.32	L, M, S
18	4.367	1599	trans-Caryophyllene	0.95	L, M, S
19	4.548	1685	Sabinyl acetate	0.30	L, M
20	4.664	1693	α-Amorphene	0.12	L, M
21	4.725	1694	α-Terpineol	0.36	L, M, S
22	4.746	1700	Borneol	0.76	L, M, S
23	4.822	1708	Germacrene-D	0.31	L, M
24	4.856	1723	α-Muurolene	0.11	L, M
25	4.911	1735	Bicyclogermacrene	0.25	L, M
26	4.978	1756	δ-Cadinene	0.20	L, M

LRI: Linear retention index (Van den Dool and Kratz, 1963), agree with NIST or Wiley library.
IM: Identification Method. L: LRI. M: Mass spectra library NIST or Wiley. S: Commercial standard.

[DOR: 20.1001.1.22519041.2022.11.2.1.4]

Table 2 Essential oil composition of *Rosmarinus officinalis* from Spain.

N	Retention Index (min)	LRI	Compound	Concentration (%)	IM
1	1.360	1025	α-Pinene	25.62	L, M, S
2	1.581	1069	Camphene	9.61	L, M, S
3	1.808	1110	β-Pinene	2.97	L, M, S
4	1.901	1122	Thuja-2,4-(10)-diene	0.31	L, M
5	2.046	1147	δ-3-Carene	0.43	L, M, S
6	2.138	1161	β-Myrcene	3.16	L, M, S
7	2.233	1178	α-Terpinene	0.43	L, M, S
8	2.343	1198	D-Limonene	3.98	L, M, S
9	2.403	1211	1,8-Cineole	21.06	L, M, S
10	2.547	1235	cis-Ocimene	0.18	L, M, S
11	2.606	1245	gamma-Terpinene	0.82	L, M, S
12	2.672	1255	3-Octanone	0.07	L, M, S
13	2.749	1270	*p*-Cymene	1.91	L, M, S
14	2.811	1282	α-Terpinolene	0.45	L, M, S
15	3.862	1484	α-Ylangene	0.11	L, M
16	4.020	1515	Camphor	18.39	L, M, S
17	4.079	1545	cis-Pinocamphone	0.67	L, M
18	4.272	1579	Bornyl acetate	0.94	L, M, S
19	4.350	1601	Terpinen-4-ol	0.35	L, M, S
20	4.369	1599	trans-Caryophyllene	1.42	L, M, S
21	4.666	1667	α-Humulene	0.40	L, M, S
22	4.726	1694	α-Terpineol	0.91	L, M, S
23	4.748	1700	Borneol	2.22	L, M, S
24	4.834	1723	L-Verbenone	0.96	L, M, S

LRI: Linear retention index (Van den Dool and Kratz, 1963), agree with NIST or Wiley library.
IM: Identification Method. L: LRI. M: Mass spectra library NIST or Wiley. S: Commercial standard.

Contact toxicity

Results showed that ovicidal activity depends on oil concentrations. The hatching rate of eggs decreased significantly with increases in the concentration of the two oils ($P < 0.05$). The exposure to vapors of essential oils from Spanish *R. officinalis* caused 78.33% inhibition of the hatching rate at a concentration of 2.5 mg/ml. With the same concentration, the inhibition of hatching rates was 60% when eggs were exposed to Algerien R. officinalis oil (Fig. 1). Most unhatched eggs possessed a dead embryo.

Fumigant toxicity

Both *R. officinalis* oils were toxic to *E. ceratoniae* adults (Fig. 2). At the highest concentration (0.4 mg/ml), *R. officinalis* from Spain achieved more than 82.22% of adult mortality after 24 h of exposure, followed by 66.67% with *R. officinalis* from Algeria. The corresponding LC_{50} values were respectively 0.17 and 0.30 mg/ml after 24 h (Table 3). There was a significant correlation between adult mortality and doses. *Rosmarinus officinalis* from Spain was more toxic ($F = 115.82$; $df = 8$; $P < 0.05$).

Antifeedant toxicity

Results of toxicity of both *R. officinalis* essential oils were presented as a percentage of mortality of *E. ceratoniae* first instar larvae L1 at different doses and different times of exposure (Fig. 3). The mortality values significantly increased depending on the increasing essential oil concentration ($p < 0.05$). The two essential oil showed medium ingestion action. Results indicated that at the concentration of 10 mg/ml

[DOR: 20.1001.1.22519041.2022.11.2.1.4]

air of *R. officinalis* oil from Algeria, only 43.33% of larvae died. However, 70 % of young larvae L1 mortality was obtained at this same concentration with *R. officinalis* from Spain. The concentration of 1 and 4 mg/ml showed little larvicidal activity (< 50% mortality). The results prove that young larvae L1 were significantly more sensitive to *R. officinalis* oil from Spain, with an LC_{50} value equal to 3.40 mg/ml. Whereas young larvae L1 were more resistant to *R. officinalis* oil from Algeria with LC_{50} equal to 4.97mg/ml (Table 4).

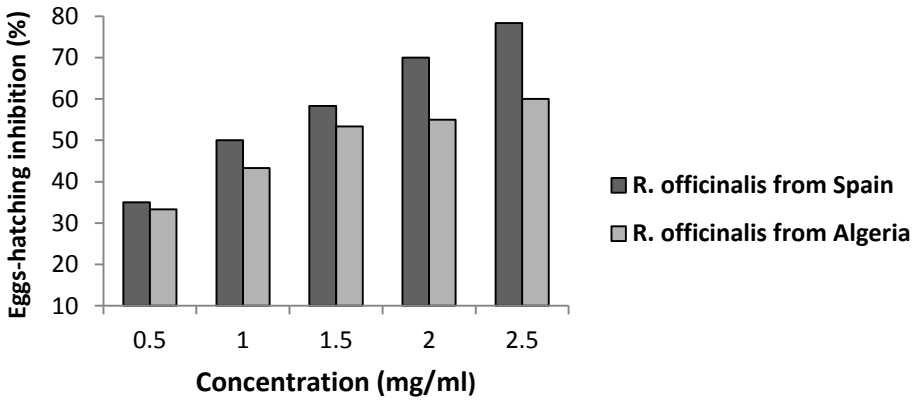

Figure 1 Eggs-hatching inhibition of *Ectomyelois ceratoniae* exposed to various essential oil concentrations from Spanish *Rosmarinus officinalis* and Algerien *Rosmarinus officinalis*.

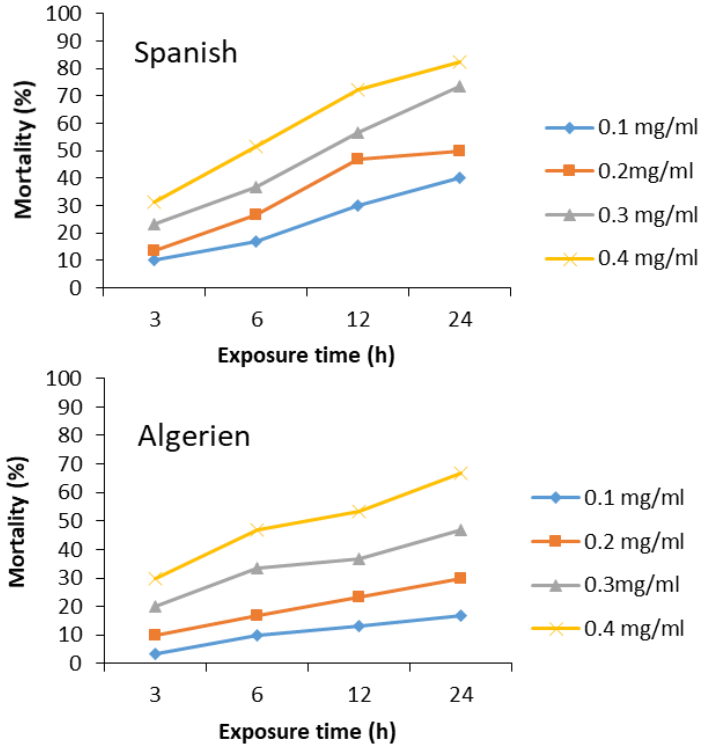

Figure 2 Mortality of *Ectomyelois ceratoniae* adults exposed to various periods and concentrations of essential oil from Spanish and Algerien *Rosmarinus officinalis*.

[DOR: 20.1001.1.22519041.2022.11.2.1.4]

Table 3 LC_{50} value of Spanish and Algerien *Rosmarinus officinalis* essential oils against adults of *Ectomyelois ceratoniae* by fumigant toxicity.

Exposure time (h)	*R. officinalis*essential oil	LC_{50} (CI_{95}) (mg/ml)	Slope	Probability
3	Spanish	0.61(0.44-0.86)	3.81	0.018
	Algerien	0.73(0.56-0.95)	2.87	0.004
6	Spanish	0.39(0.28-0.55)	3.82	0.023
	Algerien	0.49(0.37-0.66)	3.17	0.032
12	Spanish	0.24(0.18-0.31)	2.84	0.029
	Algerien	0.39(0.30-0.53)	3.12	0.034
24	Spanish	0.17(0.13-0.22)	2.60	0.009
	Algerien	0.30(0.23-0.39)	2.79	0.028

LC_{50} lethal concentration causes 50% mortality in the exposed insect population.
CI_{95} 95% confidence interval.

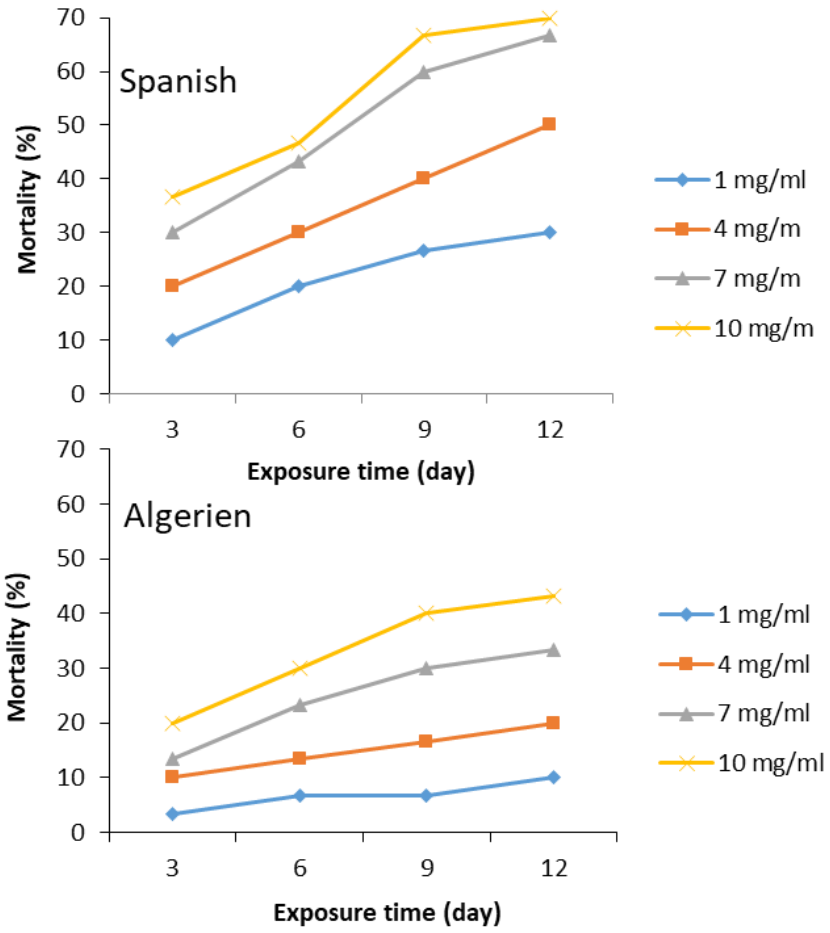

Figure 3 Mortality of *Ectomyelois ceratoniae* first instar larvae (L1) exposed to various periods and concentrations of essential oil from Spanish and Algerien *Rosmarinus officinalis.*

[DOR: 20.1001.1.22519041.2022.11.2.1.4]

Table 4 LC_{50} value of Spanish and Algerien *Rosmarinus officinalis* essential oils against first instar larvae (L1) of *Ectomyelois ceratoniae* by antifeedant toxicity.

Exposure time (days)	*R. officinalis* essential oil	LC_{50} (CI_{95}) (mg/ml)	Slope	Probability
3	Spanish	25.95 (13.94-48.30)	11.67	0.010
	Algerien	88.80 (48.27-163.35)	11.44	0.008
6	Spanish	13.67 (6.46-28.92)	19.34	0.023
	Algerien	24.46 (13.71-43.65)	9.87	0.024
9	Spanish	4.55 (2.63-7.90)	8.84	0.037
	Algerien	18.99 (11.80-30.56)	6.56	0.017
12	Spanish	3.40 (1.99-5.81)	8.33	0.025
	Algerien	4.97 (03.80-06.50)	2.90	0.080

LC_{50} lethal concentration causes 50% mortality in the exposed insect population.
CI_{95} 95% confidence interval.

Discussion

Concerning our GC-MS results of the Algerien *R. officinalis* oil, they are similar to those reported by Boutekedjiret *et al*. (2005) showed that *R. officinalis* oil from North Algeria was formed mainly by α-pinene (15.50%) followed by β-caryophyllene (10.60%) and camphor (9.00%). However, our results are in agreement with those of Chalchat *et al*.(2011) found that *R. officinalis* essential oil extracted from the region of Murcia (Spain) was characterized by a majority composition ofα-pinene (19.00 %), 1,8-cineole (17.00%) and camphor (12.00%). The chemical composition of essential oil is strongly influenced by geographical origin, genetic characteristics, climatic conditions (Da Silva *et al*., 2014), harvest period, and extraction technique (Isman *et al.*, 2008). The effect of plant maturity at the time of oil production can also drastically affect the chemical composition (Lahlou and Berrada, 2003). These variations are of particular importance in the study of the insecticidal activities of these products, as the value of essential oils in aromatherapy must be related to their chemical compositions (Lawrence, 2000). Monoterpenoids such as α-pinene, 1,8-cineole, and camphor as the majority components have been considered to be toxic to stored insect pests and possess repellent, fumigant (Yari *et al*., 2014), and antifeedant properties (Hough-Goldstein, 1990).

The findings of ovicidal potential accorded with results reported by Amri *et al*. (2014), which showed that *R. officinalis* essential oil from Tunisia achieved 100% mortality for *E. ceratoniae* eggs at 20 µl ml^{-1}. While at the same dose, 100% and 84% mortality were obtained respectively with *Thymus capitatus* and *Pinus halepensis* essential oils. Results demonstrated that the egg stage of *E.ceratonia* was the most resistant to the action of oils, followed by larvae and adults. Stamopoulos *et al*. (2007) explained that the tolerance of the eggs was related to their lower surface permeability at the beginning of embryogenesis. Another possible explanation is due to the neurotoxic action of essential oils compounds, which act only after the nervous system of the embryo begins to grow (Credland, 1992).

Regarding adulticidal activity, our results seem to be moderate compared to those obtained by Ben Chaaban *et al*. (2018) have shown that essential oil from *Mentha pulegium* showed 100% effectiveness at 14.54 µl/l air after 7 h on adults of *E. ceratoniae*. While at the same concentrations and at the same time of exposure, *Ocimum basilicum* showed only 14%. Pare and Tumlinson (1999) reported that volatiles penetrates the respiratory system in the insect body and results in abnormal breathing, leading to asphyxiation and final death. Changes in the physiology and behavior of insects are also affected by essential oils (El-wakeil, 2013).

[DOR: 20.1001.1.22519041.2022.11.2.1.4]

To the best of our knowledge, this is the first study of the antifeedant effect of essential oils on *E. ceratoniae*. Our results agree with those reported by Arasu et al. (2013) indicated that essential oils' aromatic properties make insects disgusted by food and reduce or stop feeding. Stability in the mortality rate is noted after the 9th day in almost all concentrations. This can be explained by the fact that the quantity of bioactive substances is low in the doses or the persistence of the active principle does not last beyond the 9th day. Moreover, our results noticed a decrease in the size of the survived larvae compared to those of the control. Sahayaraj (1998) showed notable antifeedant activity in *Vitex negundo* L. essential oil, which touched the growth of *Spodoptera litura* F. larvae, and was indicated by very low food consumption and digestibility, fecal pellets production, and reduced body weight.

Conclusion

In this study, we performed the insecticidal potential of *R. officinalis* essential oils against *E. ceratoniae* as a major date pest. The strongest contact/fumigant/antifeedant potential of these volatile oils seems to be related to the richness of monoterpene compounds. Results of the present work recommended Mediterranean rosemary essential oils as a plant-based insecticide alternative to improve date safety during storage or for managing populations of *E. ceratoniae* in the field. Nevertheless, it is important to identify the bioactivity of individual compounds in both plant's essential oils and their mode of action against the date moth or other stored-product pests and investigate the effect of *R. officinalis* essential oil on date quality.

Conflict of interests

The authors have no conflict of interest

Acknowledgments

We thank -Pr. Oudjehih Bachir for botanic identification, Laboratory of the Regional Station of Plant Protection-Biskra (S. R. P. V) for *E. ceratoniae* samples, Mohamed Khider, and Murcia Universities for providing laboratory facilities.

References

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18: 265-267.

Al-izzi, M. A. J., Al-Maliky, S. K. and Jabbo, N. F. 1987. Culturing the carobmoth, *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae), on an artificial diet. Journal of Economic Entomology, 80(1): 277-280.

Amri, I., Hamrouni, L., Hanana, M., Jamoussi, B. and Lebdi, K. 2014. Essential oils as biological alternatives to protect date palm (*Phoenix dactylifera* L.) against *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae). Chilean Journal of Agricultural Research, 74: 273-279.

Arasu, M. V., Al-dhabi, N. A., Saritha, V., Duraipandiyan, V., Muthukumar, C. and Kim, S. J. 2013. Antifeedant, larvicidal, and growth inhibitory bioactivities of novel polyketide metabolite isolated from Streptomyces sp. AP-123 against *Helicoverpa armigera* and *Spodoptera litura*. BMC Microbiology, 13(105): 1-6.

Balaraju, K., Vendan, S. E., Ignacimuthu, S. and Park, K. 2011. Antifeedant and larvicidal activities of *Swertia chirata* Buch-Ham. ex Wall. against *Helicoverpa armigera* Hubner and *Spodoptera litura* Fab. Social Science, 31: 1902-1905.

Batish, D. R., Sing, P. H., Kohli, K. R. and Kaur, S. 2008. Eucalyptus Essential Oil as a Natural Pesticide. Forest Ecology and Management, 256: 2166-2174.

Begum, A., Sandhya, S., Ali, S. S., Vinod, K. R., Reddy, S. and Banji, D. 2013. An in-depth review on the medicinal flora *Rosmarinus officinalis* (Lamiaceae). Acta Scientiarum Polonorum. Technologia Alimentaria, 12: 61-74.

Ben Chaaban, S., Hamdi, S. H., Mahjoubi, K. and Mediouni-ben Jemâa, J. 2018.

[DOR: 20.1001.1.22519041.2022.11.2.1.4]

Composition and insecticidal activity of essential oil from *Ruta graveolens*, *Mentha pulegium, and Ocimum basilicum* against *Ectomyelois ceratoniae* Zeller and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Journal of Plant Diseases and Protection, 5: 201-212.

Botes, A. and Zaid, A. 1999. The economic importance of date production and international trade. In: Zaid, A., Arias, E.J. (Eds.), Date Palm Cultivation. FAO Plant Production and Protection, 156: 45-57.

Boudries, H., Kefalas, P. and Hornero-Mendez, D. 2007. Carotenoid composition of Algerian date varieties (Phoenix dactylifera) at different edible maturation stages. Food Chemistry, 101: 1372-1377.

Boutekedjiret, C., Buatois, B.and Bessiere, J. M. 2005. Characterisation of Rosemary Essential Oil of Different Areas of Algeria. Journal of Essential Oil Bearing Plants, 8(1): 65-70.

Carvalhinho, S., Costa, A. and Coelho, A. 2012. Susceptibilities of *Candida albicans* mouth isolates to antifungal agents, essentials oils, and mouth rinses. Mycopathologia, 174: 69-76.

Chalchat, J. C., Garry, R. P., Michet, A., Benjilali, B.and Chabart, J. L. 2011. Essential oils of rosemary (*Rosmarinus officinalis* L.). The chemical composition of oils of various origins (Morocco, Spain, France). Journal of Essential Oil Research,5: 613-618.

Credland, P. F. 1992. The structure of bruchid eggs may explain the ovicidal effect of oils. Journal of Stored Products Research, 28: 1-9.

Da Silva, F. G. E., Mendes, F. R. S., Assunço, J. C. C., Pinheiro Santiago, G. M., Xavier Bezerra, M. A., Barbosa, F. G., Mafezoli, J., Rodrigues, J. C. C. and Rocha, R. R. 2014. Seasonal variation, larvicidal and nematicidal activities of the leaf essential oil of *Ruta graveolens* L. Journal of Essential Oil Research, 26(3): 1-7.

Dehliz, A., Lakhdari, W., Acheuk, F., Hammi, H., Soud, A. and M'lik, R. 2016. Potentiality of indigenous parasitoids in south-eastern Algeria against the date moth. Faunistic Entomology, 69: 75-79.

El-wakeil, N. E. 2013. Botanical Pesticides and Their Mode of Action. Gesunde Pflanz, 65: 125-149.

FAO: Food and Agriculture Organisation of the United Nations. 2016.[accessed 2019 Oct 14]. http://www.fao.org/corp/copyright/en.

Finney, D. J. 1971. Probit Analysis. 3rd ed. Cambridge University, London.

Fu, Y., Zu, Y., Chen, L., Shi, X., Wang, Z., Sun, S. and Efferth, T. 2007. Antimicrobial activity of clove and rosemary essential oils alone and in combination. PhytotherapyResearch, 21: 989-994.

Gezici, S., Sekeroglu, N. and Kijjoa, A. 2017. In vitro anticancer activity and antioxidant properties of essential oils from *Populus alba* L. and *Rosmarinus officinalis* L. from South Eastern Anatolia of Turkey. Indian Journal of Pharmaceutical Education and Research, 51: 498-503.

Hendel, N., Larous, L. and Belbey, L. 2016. Antioxidant activity of rosemary (*Rosmarinus officinalis* L.) and its in vitro inhibitory effect on *Penicillium digitatum*. International Food Research Journal, 23: 17-25.

Hough-Goldstein, J. A. 1990. Antifeedant Effects of Common Herbs on the Colorado Potato Beetle (Coleoptera : Chrysomelidae). Environmental Entomology, 19: 234-238.

Isman, M. B., Wilson, J. A. and Bradbury, R. 2008. Insecticidal activities of commercial rosemary oils (*Rosmarinus officinalis*) against larvae of *Pseudaletia unipuncta* and *Trichoplusia ni* in relation to their chemical compositions. Pharmaceutical Biology, 46(1): 82-87.

Lahlou,M. and Berrada, R. 2003. Composition and nematicidal activity of essential oils of three chemotypes of *Rosmarinus officinalis* L. acclimatised in Morocco. Flavour and Fragrance Journal, 18: 124-127.

Lamiri, A., Lhaloui, S., Benjilali, B. and Berrada, M. 2001. Insecticidal effects of essential oils against Hessian fly, *Mayetiola destructor* (Say). Field Crops Research, 71: 9-15.

Lawrence, B. M. 2000. Essential oils: from agriculture to chemistry.International Journal of Aromatherapy, 10: 82-98.

[DOR: 20.1001.1.22519041.2022.11.2.1.4]

Leonard, G. and Julius, J. M. 2000. Review Biopesticides: a review of their action, applications, and efficacy. Pest Management Science, 56: 651-676.

Majourhat, K., Bendiaba, K., Medraoui, L. and Baaziz, M. 2002. Diversity of leaf peroxidases in date palm (Phoenix dactylifera L.) as revealed in an example of marginal (seeding derived) palm groves. Scientia Horticulturae, 95: 31-38.

Mediouni-ben Jemâa, J. andDhouibi, M. H. 2007. Mass-rearing and field performance of irradiated carob moth *Ectomyelois ceratoniae* in Tunisia. Area-Wide Control Insect Pests, 265-273.

Mediouni-ben Jemâa, J., Fukova, I., Frydrychova, R. Dhouibi, M. H. and Marec, F. 2004. Karyotype, sex chromatin, and sex chromosome differentiation in the carob moth *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae). Caryologia, 57: 191-201.

Mehaoua, MS, Hadjeb, A., Lagha, M., Bensalah, M. K. and Ouakid, M. L. 2013. Study of the toxicity of Azadirachtinon larval mortality and fertility of Carob Moth's female *Ectomyelois ceratoniae* (Lepidoptera, Pyralidae) under controlled conditions. American Journal of Sustainable Agriculture, 7(1): 1-9.

Mothana, R., Hasson, S. and Schultze, W. 2011. Phytochemical composition and in vitro antimicrobial and antioxidant activities of essential oils of three endemic Soqotraen *Boswellia* species. Food Chemistry, 126: 1149-54.

Pare, P. W. and Tumlinson, J. H. 1999. Plant volatiles as a defense against insect herbivores. Plant Physiology, 121: 325-331.

Sahayaraj, K. 1998. Antifeedant effect of some plant extracts on Asian armyworm, *Spodoptera litura* (Fabricius). Current Science, 74(6): 523-525.

Sedra, M. H. 2003. The Date Palm is the basis of the development of oases in Morocco. INRA, Availablefrom:https://www.inra.org.ma/sites/default/files/publications/ouvrages/palmierdattier.pdf [Accessed 14th June 2019].

Silva, G. D., Pizarro, P., Casals, Y. and Berti, M. 2003. Evaluación de plantas medicinales en polvo para el control de *Sitophiluszeamais* Motschulsky en maíz almacenado. Revista Brasileira de Agrociência, 9(4): 383-388.

Stamopoulos, D. C., Damos, D. and Karagianidou, G. 2007. Bioactivity of five monoterpenoid vapors to *Tribolium confusum* (du Val) (Coleoptera: Tenebrionidae). Journal of Stored Products Research, 43: 571-577.

Van den Dool, H. and Kratz, P. D. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. Journal of Chromatography, 11: 463-471.

XLStat. 2007. Statistical Software for Excel. [https://www.xlstat.com].

Yari, M., Masoudi, S. and Rustaiyan, A. 2014. Essential oil of *Haplophyllum tuberculatum* (Forssk.) A. Juss. Grown Wild in Iran. Journal of Essential Oil Research, 12: 69-70.

[DOR: 20.1001.1.22519041.2022.11.2.1.4]

پتانسیل اسانس رزماری به‌عنوان حشره‌کش زیستی برای محافظت از خرمای انباری در برابر کرم گلوگاه انار *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae)

Selma Adouane[1, 2*], Yamina Bouatrous[2], Mohamed Seghir Mehaoua[1, 3], Jose Tudela[4], Sara Mechaala[2] and Virginia Tomas[5]

۱- گروه علوم کشاورزی، دانشگاه محمد خدر، بیسکرا، الجزایر.
۲- آزمایشگاه ژنتیک، بیوتکنولوژی، و ارزش‌گذاری منابع زیستی، دانشکده علوم دقیق و علوم طبیعت و حیات، دانشگاه محمد خیدر، بیسکرا، الجزایر.
۳- آزمایشگاه تنوع اکوسیستم‌ها و دینامیک سیستم‌های تولید کشاورزی در مناطق خشک، دانشکده علوم دقیق و علوم طبیعت و حیات، دانشگاه محمد خدر، بیسکرا، الجزایر.
٤- گروه بیوشیمی و زیست‌شناسی مولکولی، پردیس منطقه‌ای بین‌المللی "Campus Mare Nostrum"، دانشگاه مورسیا، اسپیناردو، مورسیا، اسپانیا.
٥- گروه شیمی تجزیه، پردیس منطقه‌ای بین‌المللی"Campus Mare Nostrum" ، دانشگاه مورسیا، اسپیناردو، مورسیا، اسپانیا.
پست الکترونیکی نویسنده مسئول مکاتبه: adouane.selma7@gmail.com
دریافت: ۲ آذر ۱۳۹۹؛ پذیرش: ۱۷ تیر ۱٤۰۱

چکیده: پژوهش حاضر به‌منظور مقایسه فعالیت حشره‌کشی دو تیپ اسانس رزماری (*Rosmarinus officinalis* L. (Lamiaceae از منطقه مدیترانه در برابر مراحل مختلف زندگی کرم گلوگاه انار (*Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae در شرایط محیطی ثابت انجام شد. اسانس‌ها به‌روش تقطیر با آب استخراج و با روش کروماتوگرافی گازی- طیفسنجی جرمی (GC-MS) آنالیز شدند. الفاپینن (۲۹/٤۲ درصد)، کامفن (۲٤/٦۲ درصد) و کافور (۲۰/۹٥ درصد) به‌عنوان ترکیبات غالب موجود در اسانس رزماری تیپ الجزایر و هم‌چنین ترکیبات اصلی اسانس رزماری تیپ اسپانیا آلفاپینن (۲٥,٦۲ درصد)، ۱،۸ سینِول (۲۱/۰٦ درصد)، کافور (۱۸/۳۹ درصد) بود. اثر تخم‌کشی روغن‌ها با کاربرد تماسی و اثرات بالغ‌کشی و لاروکشی به ترتیب به‌روش گازی و گوارشی بررسی شدند اسانس زرماری تیپ اسپانیا بالاترین اثر سمی را علیه تخم‌ها (۷۸/۳۳ درصد)، لاروهای جوان (LC_{50} = 3.40 mg/ml) و افراد بالغ (LC_{50} = 0.17 mg/ml) داشت. از سوی دیگر، رزماری تیپ الجزایر، مهار تخم‌کشی کم‌تری را با ٦۰ درصد و اثرات ضدتغذیه کم‌تری داشت که به‌ترتیب LC_{50} = 0.30 و LC_{50} = 4.97 میلی‌گرم در میلی‌لیتر برای افراد بالغ و لاروهای جوان را نشان داد. نتایج این مطالعه حاکی از کارایی اسانس رزماری به‌عنوان جایگزینی برای حشره‌کش‌های شیمیایی در مرحله پس از برداشت برای کنترل کرم گلوگاه انار دارد.

واژگان کلیدی: فعالیت حشره‌کشی، اسانس، رزماری، کرم گلوگاه انار، حشره‌کش زیستی

[DOR: 20.1001.1.22519041.2022.11.2.1.4]