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Efficacy of Mediterranean Essential Oils to Control the Date Moth, Ectomyelois Ceratoniae Zeller (Lepidoptera: Pyralidae)

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Abstract: This research aimed to determine the chemical composition of two Eucalyptus (Myrtaceae) essential oils chemotypes from the Mediterranean region and to evaluate their biological activity against eggs, larvae, and adult insects of Ectomyelois ceratoniae Zeller. The essential oils were extracted by hydrodistillation and analysed by the gas chromatography-mass spectrometry technique (GC-MS). p-cymene (25.28%), cryptone (15.08%), spathulenol (14.64%), and Terpinen-4-ol (6.22%) were obtained as the majority compounds in the essential oil of E. globulus from Algeria, as well as the main essential oil compound of E. globulus from Spain was 1,8-cineole (82.34%). Adulticidal and larvicidal activities of oils were studied by fumigation and ingestion, while ovicidal activity was assessed by topical application. The Spanish E. globulus presented the highest toxic activity against eggs (75% inhibition), young larvae L1 (LC₅₀ = 7.33 mg/mL), and adults (LC₅₀ = 0.27 mg/mL). However, the Algerien E. globulus showed lower egghatching inhibition with 53.33% and fewer fumigant and antifeedant activities (LC₅₀ = 0.33 and 3.55 mg/mL for adults and first instar larvae, respectively). The results of this study indicated the efficacy of *Eucalyptus* essential oil as an alternative to synthetic insecticides for the control of E. ceratoniae.

Keywords: Insecticidal activity, Essential oils, Eucalyptus globulus, Ectomyelois ceratoniae, Lethal concentration LC₅₀.

1. Introduction

The date palm (*Phoenix dactylifera* L.) is one of the most valuable domesticated fruit trees cultivated in arid regions of the world. It is considered a key plant because of its productive capacity, ritual significance in human societies, health benefits, and range of subsistence products from its fruits and other parts of the palm (Johnson *et al.*, 2015). The size of the date crop is increasing continually in all parts of the world, it was about 9.5 million tons in 2020 (FAO, 2023). Nevertheless, the date sector faces many constraints, mainly related to phytosanitary problems (Allam, 2008), affecting the quantity and quality of production. The date moth *Ectomyelois ceratoniae* is considered the most dangerous pest of dates and the main export constraint (Doumandji, 1981). The damage begins on palm trees and continues in storage areas (Jacques, 1990), and could reach 90% of the harvestable crop annually (Bouka *et al.*, 2001).

The post-harvest control of this pest is exclusively based on the use of synthetic insecticides (Azelmat *et al.*, 2006). This chemical product had numerous harmful sides related to residual toxicity, insect resistance, and adverse effects on non-target organisms, human health, and environments (Oueld El Hadj *et al.*, 2003; Bisaad *et al.*, 2011). Thus, the search for efficient and eco-friendly alternatives is required. In this respect, Keita *et al.* (2001) pointed out that natural substance including essential oils have numerous effects on insect: fumigant and antifeedant activities, reduction in growth and fecundity, inhibition of molting and respiration.

The Mediterranean basin, like many regions of the world, covers an areaof 1.6%, which present a floristic heritage with high levels of plant diversity and endemism. It has about 25,000 plant species, but only 10% are known. Almost 60% of these species are found nowhere else (Schönfelder, 2014). *Eucalyptus* (Myrtaceae) has been praised as a rich source of essential oil. Various biological properties have already been attributed to the genus *Eucalyptus*, among them insecticidal effect against several harmful insects (Cimanga *et al.*, 2002).

This study aims to evaluate the insecticidal toxicity of two Mediterranean *Eucalyptus* oil; *E. globulus* from Algeria and *E. globulus* from Spain, against the different life stage of *E. ceratoniae*, in order to find new bioactive natural products.

2. Materials and methods

2.1. Plant material

Leaves of *E. globulus* grew spontaneously were collected from Ziban steppes $(35^{\circ}13'00''N, 5^{\circ}42'37''E)$ situated in Southern Algeria, during Marsh 2019. Leaves from *E. globulus* were obtained in April 2019 from the province of Murcia $(37^{\circ}59'00''N, 1^{\circ}08'00''W)$ located in South-East Spain. The harvested material was air-dried at room temperature $(25 \, ^{\circ}C)$ in darkness for further use.

2.2. Insect rearing

A laboratory rearing of *E. ceratoniae* was established in the regional station of plant protection-Biskra (S.R.P.V) from infested field-collected dates. The date moth was reared in plastic boxes ($15 \times 20 \times 30$ cm), on an artificial diet based on wheat bran (44%), yeast (4%), citric acid (1%), vitamin mixture (1%), methylparaben (0.5%), salt mixture (1%), ascorbic acid (0,5%), sugar (5%), gluten (3%) and distilled water (40%) (Mediouni-Ben Jemâa and Dhouibi, 2007), under constant environmental conditions (26 ± 2 °C, $65 \pm 10\%$ RH, and L: D 15:9h) (Al-izzi *et al.*, 1987). Adults, fresh eggs, and first instar larvae (L1) (0-24 h) were collected and used for bioassays.

2.3. Extraction and analysis of essential oils

The essential oils were obtained by hydro distillation of the dried leaves (100 g of each sample in 1000 mL of distilled water) using a clevenger-type apparatus for approximately 3-4 h. The collected oils were dried over anhydrous sodium sulphate and conserved at 4°C prior to analysis. The quantitative and qualitative analysis of volatile profile in essential oil was carried out using an Agilent GC7890 gas chromatograph (GC), equipped with an Agilent MS5975 mass spectrometry detector (MSD). Essential oils were diluted 1:100 with isooctane in vials of 1.5 ml with mandrel inserts of 250 µl. The GC system was equipped with a low and polar bleed capillary fused-silica column, Supelco-Wax10, with 15 m (length)× 0.1 mm (internal diameter) x 0.1 µm (film thickness). The carrier gas used was hydrogen (0.6ml/min), generating a head pressure of 38.17 psi and produced with an electrolytic Parker-Domnik-Hunter generator. Sandwich injections: 0.2 µl isooctane, plunger to needle: 0.2 µl air, 0.2 µl air, 0.3 µl sample, and 0.2 µl air, were performed bu the use of a Gerstel sampler MPS2XT. Other conditions of the injections: injector temperature 260 °C, split valve 100:1 and, septum purge 3 ml/min. Oven temperature was controlled from 50 °C to 250 °C at 30 °C/min, and kept constant at 250 °C for 5 min. MSD operated with transfer line, ion source, and quadrupole temperatures at 250 °C, 230 °C, and 150 °C, respectively. Other MSD conditions: electron multiplier voltage 1129, electron ionization energy 70 eV, 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.

2.4. Contact toxicity

In this assay, tests were performed at 27 °C and 75% RH, and several pilot tests were carried out to select the doses to be used. Thus, twenty fresh eggs were transferred to a Petri dish (Ø=90mm), 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. Three repetitions were performed for each dose with a corresponding control sprayed only with diluted Tween 20 (1%). After three days of incubation, the hatched eggs were counted by using a binocular loupe for one week.

2.5. Fumigant toxicity

In these experiments, ten newly emerged adults (< 24 h old) were placed into a plastic bottle of 500 mL capacity, which served as fumigant chambers containing a piece of cotton soaked by 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.The bottles were tightly closed and covered with parafilm, and three replicates of each control and treatment were set up. The mortality of the insect was recorded after 3, 6, 12, and 24 h of the test completion.

2.6. Antifeedant toxicity

To evaluate both essential oils antifeedant toxicity, the solution of each sample dissolved in 1 mL diluted Tween 20 correspondst othe following concentrations: 1, 4, 7, and 10mg/mL was applied to a Petri dishes (Ø=90 mm) containing a thinlayer (25 g) of the artificial diet. Then respectively, ten newly emerged first instar larvae (L1) were placed in each Petri dish. Mortality was recorded every three days by the use of a binocular loupe. For 12 days, the feeding trial was conducted in 3 replicates besides to the control. When no movements were observed after excitation, the larva was considered dead.

2.7. Statistical analysis

Abbott correction formula (Abbott, 1925) was applied to assess insect mortality. Data were analysed using Probit analysis method (Finney, 1971). The LC₅₀ was determined for established regression lines. The percentage mortality value for different concentrations was subjected to analysis of variance (ANOVA) using XLstat (2016). Differences between means were compared through Fisher's test, and values of P <0.05 were considered significantly different.

3. Results and Discussion

3.1. Essential oil composition

GC-MS analysis of both essential oils were respectively reported in Tables 1 and 2. The oil yields based on dry matter weight were 0,600 g (0.60%) for *E. globules* from Algeria and 1.040 g (1.04%) for *E. globules* from Spain. Results showed that a total of 39 compounds were identified in

the essential oil of *E. globulus* from Algeria. This oil profile was characterised mainly by p-cymene (25.28%), cryptone (15.08%), spathulenol (14.64%), and Terpinen-4-ol (6.22%). For *E. globulus* from Spain, 10 components were identified. The chemical composition of this essential oil was dominated by 1,8-cineole (82.34%). Components such as D-Limonene (6.40%) and α -pinene (3.67%) were identified in trace amounts. Significant differences have been found in the chemical composition of the two analysed samples, *outstanding* that essential oil's quantity and quality vary with the plant species (Ali et al., 2010). Similar results were reported by Farah et al. (2002) which indicated that the CG-SM analyses of the various samples of Eucalyptus come from the region of Mechraâ El Kettane (Morocco) presented a homogeneous chemical composition with α -pinene, p-cymene and 1.8-cineole as major constituents. The percentages of α -pinene and p-cymene oscillated between 0.30 and 23.2% respectively for the samples of essential oils from E. globulus and E. grandis. According to Bruneton (1999), one of the interests of the Eucalyptus genus is the diversity of the composition of essential oils provided by the leaves. This result confirms once again that the geographical origin (Marotti et al., 1994), genetic characteristics, climatic conditions (Da Silva et al., 2014), harvest period, and extraction technique (Isman et al., 2008) influenced the chemical composition of the essential oil. Monoterpenoids such as p-cymene and 1,8-cineole as the majority components are characteristic of the *Eucalyptus* genus and is mainly responsible of its insecticidal proprieties (Batish *et al.*, 2008).

Ν	RI (min)	LRI	Compound	Concentration (%)
1	1.355	1025	alpha-Pinene	0.39
2	1.381	1027	alpha-Thujene	1.30
3	1.581	1069	Camphene	0.11
4	1.808	1110	beta-Pinene	0.06
5	1.889	1122	Sabinene	0.69
6	1.941	1122	Thuja-2,4(10)-diene	0.13
7	2.147	1168	alpha-Phellandrene	1.18
8	2.231	1178	alpha-Terpinene	0.17
9	2.341	1198	D-Limonene	1.14
10	2.400	1211	beta-Phellandrene	5.44
11	2.606	1245	gamma-Terpinene	0.47
12	2.755	1270	p-Cymene	25.28
13	2.811	1282	alpha-Terpinolene	0.11
14	3.565	1423	alpha-Thujone	1.13
15	3.657	1440	beta-Thujone	0.57
16	3.894	1491	alpha-Copaene	0.06
17	3.963	1508	Chrysanthenone	0.30
18	4.013	1515	Camphor	0.27
19	4.079	1543	Linalool	1.16
20	4.172	1564	cis-Sabinene hidrate acetate	1.11
21	4.349	1601	Terpinen-4-ol	6.22
22	4.446	1614	p-Menth-2-en-1-ol	0.95
23	4.566	1664	alpha-Himachalene	1.48
24	4.691	1675	Cryptone	15.08
25	4.724	1694	alpha-Terpineol	0.69
26	4.821	1708	Germacrene-D	0.17

 Table 1. Essential oil composition of E.globulus from Algeria

27	4.889	1724	Phellandral	2.39
28	4.910	1735	Bicyclogermacrene	1.74
29	4.979	1756	delta-Cadinene	0.10
30	5.105	1784	Cuminaldehyde	2.73
31	5.294	1848	p-Cymen-8-ol	0.79
32	5.552	1853	Linalyl isovalerate	0.95
33	5.849	1986	Caryophyllene oxide	0.20
34	5.980	2039	Ledol	0.28
35	6.110	2079	Elemol	0.54
36	6.175	2101	p-Cymen-7-ol	0.67
37	6.272	2127	Spathulenol	14.64
38	6.512	2164	Thymol	0.46
39	6.895	2301	alpha-Sinesal	0.86
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RI: values of calculated retention indices.

LRI: Linear retention index (Van den Dool and Kratz, 1963), agree with NIST or Wiley library.

Ν	RT (min)	LRI	Compound	Concentration (%)
1	1.357	1025	alpha-Pinene	3.67
2	1.808	1110	beta-Pinene	0.25
3	2.148	1168	alpha-Phellandrene	0.99
4	2.234	1178	alpha-Terpinene	0.17
5	2.349	1198	D-Limonene	6.40
6	2.419	1211	1,8-Cineole	82.34
7	2.607	1245	gamma-Terpinene	1.02
8	2.751	1270	p-Cymene	3.56
9	4.409	1620	Aromadendrene	0.13
10	4.728	1694	alpha-Terpineol	0.26

 Table 2. Essential oil composition of E. globulus from Spain

RI: values of calculated retention indices.

LRI: Linear retention index (Van den Dool and Kratz, 1963), agree with NIST or Wiley library.

CH ₃ CH ₃ CH ₃	H ₃ C	HO HO H ₃ C H H ₃ C H H ₃ C H H ₃ C H ₃ C	H ₃ C CH ₃
p-Cymene (25.28)	Cryptone (15.08)	Spathulenol (14.64)	1,8-Cineole (82.34)
	<i>E. globulus</i> from Spain		

3.2. Insecticidal activity effect

Results showed toxic effects of the two essential oils on eggs which increased significantly with increases in the concentration (P < 0.05). The highest activity was found with the Spanish *E. globulus*, which caused 75% of the hatching rate inhibition at the concentration of 2.5 mg/mL. With the same concentration, the inhibition of hatching rates was 53.33% when eggs were exposed to Algerian *E. globulus* oil (**Figure 1**). The findings of ovicidal potential corroborate those of Bachrouch *et al.* (2010)

who indicated that *Pistacia lentiscus*essential oil caused 57.1% of mortality for *E. ceratoniae* eggs at 136 mL/L air. Amri *et al.* (2014), showed that there is significant difference between the effect of essential oils of *Thymus capitatus, Pinus halepensis and Rosmarinus officinalis* on the eggs of *E. ceratoniae* treated by contact. These oils induced 100, 84 and 100% inhibition of hatching eggs respectively at 20 μ l/mL. The ovicidal effect of essential oils had been attributed to the monoterpenoids, which penetrated into the eggs, through the chorion, blocking as result the embryogenesis process (Credland, 1992).

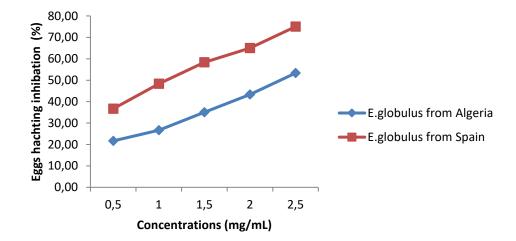


Figure 1. Eggs-hatching inhibition of *E. ceratoniae* exposed to various essential oil concentrations from Spanish *E. globules* and Algerian *E. globulus*.

About adulticidal activity, essential oil of *E. globulus* from Algeria caused only 56.67% of adults mortality at a dose of 0.4 mg/mL, after 24 hours of exposure. Under the same conditions, mortality was 85.93% for treatment with *E. globulus* oil from Spain (**Figure 2**). These results showed that both essential oils are toxic to adults by fumigation, although that Spanish *E. globulus* was more effective and allowed to record the lowest LC_{50} (0.27 mg/mL), after 24 hours (**Table 3**).

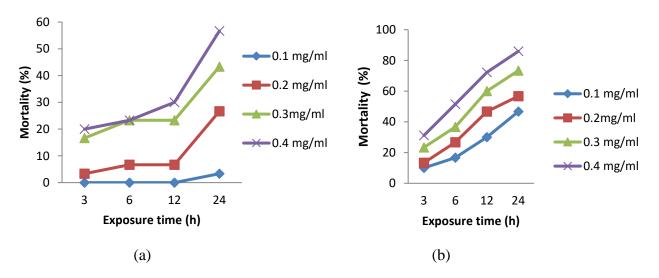


Figure 2. Mortality of *E. ceratoniae* adults exposed to various periods and concentrations of essential oil from Algerian (a) and Spanish (b) *E. globulus*.

Exposure time	E. globulus	LC ₅₀ (mg/mL)	S (Slope)	Р
(hours)	essential oil	(CL95)		
3	Spanish	0.36(0.31-0.45)	5.88	0.03
	Algerian	0.44(0.41-0.48)	1.38	0.00
6	Spanish	0.31(0.30-0.35)	5.92	0.02
	Algerian	0.41(0.38-0.45)	1.36	0.03
12	Spanish	0.28 (0.16-0. 29)	4.33	0.02
	Algerian	0.34(0.32-0.37)	1.29	0.03
24	Spanish	0.27(0.25-0.29)	3.45	0.01
	Algerian	0.33(0.28-0.40)	1.99	0.02

 Table 3. LC₅₀ (mg/mL) value of Spanish and Algerien *E.globulus* essential oils against adults of *E.ceratoniae*

 LC_{50} lethal concentration causes 50% mortality in the exposed insect population.

CI₉₅ 95% confidence interval

Our results seem to be moderate compared to those obtained by Ben Chaaban*et al.* (2019) who had shown that at 14.54 μ L/L air, *Mentha pulegium* essential oils had a fumigant effect on *E. ceratoniae* adults. 100% of mortality was recorded after only 7 h of exposure. While at the same concentrations and atthe same time of exposure, *Ocimum basilium* showed only 14%. Regnault-Roger *et al.* (2008), reported that monoterpenes have a rapid toxic action by inhalation type on insects adults. Moreover, monoterpenoids are generally volatile and rather lipophilic compounds which can penetrated the respiratory system of the insect and interfered with their physiological functions, leading to asphyxiation and final death (Lee *et al.*, 2002). Regarding our antifeedant activity, a moderate to high toxic activity was clearly shown in the results of the figure 2 below with the oils from the two samples studied. The mortalities of young larvae L1 caused oscillated between 10 to 70% for *E. globulus* from Spain and 4 to 40% for *E. globulus* from Algeria (**Figure 3**).

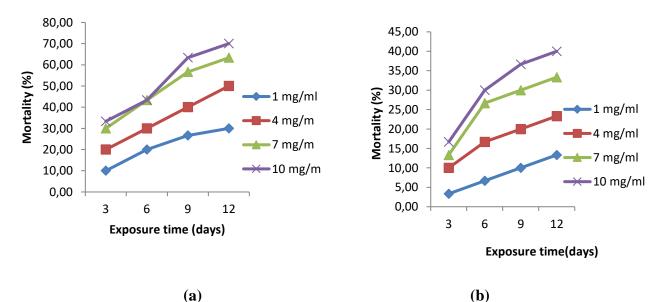


Figure 3. Mortality of *E. ceratoniae* first instar larvae L1 exposed to various periods and concentrations of essential oil from Spanish(a) and Algerian (b) *E. globulus*.

Indeed, an increase in mortality rates was observed depending on the dose used and the duration of exposure. Thus, after the 9th day, stability in the mortality rate in almost all concentrations were noted. This could be explained by the fact that the persistence of the active principle did not last beyond the 9th day. Our results agree with those reported by Arasu *et al.* (2013), who pointed out that essential oils caused disgusting and reductions in the feeding of insect. Volatile essences have antifeedant effects, thus affect the growth, fecundation, molting, and development of insects (Bessah and Benyoussef, 2015).

Exposure time	E. globulus	LC50 (mg/mL)	S	Р
(days)	essential oil	(CL95)	(Slope)	
3	Spanish	31.54(16.21-61.36)	13.90	0.006
	Algerian	129.38(66.35-252.31)	14.03	0.001
6	Spanish	16.15(7.19-36.25)	24.49	0.027
	Algerian	31.55(17.68-56.30)	9.87	0.004
9	Spanish	5.11(2.79-9.36)	10.97	0.027
	Algerian	26.12(14.00-48.75)	11.79	0.010
12	Spanish	3.55(2.04-6.19)	08.98	0.019
	Algerian	7.33(05.00-10.75)	04.55	0.017
	1115011011	1.55(05.00 10.15)	07.55	

Table 4. LC₅₀ (mg/mL) value of Spanish and Algerian *E. globules* essential oils against first instar larvae (L1) of *E.ceratoniae*

 LC_{50} lethal concentration causes 50% mortality in the exposed insect population.

CI95 95% confidence interval

These results can be explained by the richness of the chemical composition containing heteroatoms (O, N,...) near double/triple bonds, aromatic rings ... Literature shows that *E. Globulus* plays an excellent insecticide against two major cotton pests, Pectinophora gossypiella and Thaumatotibia leucotreta in order to protect human health and safeguard the environment (Rekioua *et al.* 2022; Nadio *et al.* 2023). *E. Globulus* was also tested as corrosion inhibitor of steel in acid media (Bouyanzer *et al.* 2006; Rekkab *et al.* 2012).

The evaluation of the current literature and data underlines the complex and holistic modes of action of 1,8-Cineole in inflammatory processes was summarized by several researchers (Pries *et al.*, 2023; Cai *et al.*, 2021; Juergens *et al.*, 2003). There is tremendous evidence that 1,8-Cineole causes manifold anti-inflammatory and health-promoting effects in different human diseases; it's really the source, biological activities (Khammassi *et al.*, 2023; Cai *et al.*, 2021; Lima *et al.*, 2013; Juergens *et al.*, 2003).

4. Conclusion

Results of the present work showed that both Mediterranean Eucalyptus essential oils have ovicidal, adulticidal, and antifeedant effects against *E. ceratoniae*. They could be good bio-insecticide alternatives to chemical control while preserving the environment and human health. Those natural molecules are biodegradable and less likely to cause the resistance of the target insect. Further, it is important to identify the bioactivity of individual compounds and their mode of action and investigate the effect of *E. globulus* essential oil on date quality.

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Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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