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Essential oils as biological alternatives to protect date palm (*Phoenix dactylifera* L.) against *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae)

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The aims of this research were to determine the chemical composition of the essential oil of three Tunisian plants and to evaluate their biological activity against eggs, larvae, and adult insects of *Ectomyelois ceratoniae* Zeller. The essential oils extracted from leaves of *Thymus capitatus* (L.) Hoffmanns. & Link, *Rosmarinus officinalis* L. and needles of *Pinus halepensis* Mill. were analyzed by gas chromatography-mass spectrometry; 34, 16, and 56 constituents were identified, respectively. The major constituents were (*Z*)-caryophyllene (23.8%), β-myrcene (20.5%) and α-pinene (13.3%) in *P. halepensis* oil, carvacrol (66.9%), *p*-cymene (9.1%), and δ-terpinene (6.2%) in *T. capitatus* oil and 1,8-cineole (47.5%), camphor (14.9%), α-pinene (14.1%), and borneol (13.1%) in *R. officinalis* oil. The insecticidal effects of essential oils on eggs, larvae, and adults of *E. ceratoniae* were investigated. Ovicidal activity of oils was studied by spray on eggs while larvicidal and adulticidal activities were assessed by fumigation and spray. Number of hatched eggs was verified after 10 d, larva and adult mortalities were observed after 6, 12, and 24 h. Globally, eggs and larvae were the most resistant to the three different oils, needing higher doses to obtain a higher mortality. The spray method was most effective than fumigation. Essential oil extracted from *T. capitatus* proved to be very toxic towards *E. ceratoniae* on all three phases at the dose of 20 μL mL^{-1} (100% inhibition), followed by the oil from *R. officinalis* (90-100% inhibition), nevertheless, weak activity was obtained with *P. halepensis* oil (68.3-85% inhibition). Results obtained may suggest that the essential oils of *T. capitatus* and *R. officinalis* possess high insecticidal activity and therefore, can be used in biotechnological application as natural preservative in stored dates and could be useful in managing populations of *E. ceratoniae* in field.

Key words: Bio-pesticides, *Ectomyelois ceratoniae*, essential oils, fumigation, insecticidal activity.

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) sector constitutes an integral part of the Tunisian agricultural economy; it contributes significantly to the national gross domestic product. In addition, dates provide a major source of livelihood to the majority of the population in the Southern part of Tunisia. But, date palm in Tunisia is confronted with several biotic and abiotic problems. Among these constraints, pests and diseases are considered to be a major threat to the sector. The date moth (*Ectomyelois ceratoniae* Zeller; Lepidoptera: Pyralidae), also widely known as the carob moth, is a major pest in all countries producing date palm (Idder et al., 2009; Zouba et al., 2009). The pest causes significant damage on various crops throughout the Mediterranean basin, which varies by region, host plant, and plant variety. Infestation rates on dates can reach 18% to 20% in Tunisia and 30% in Algeria with 67.5% for 'Deglet Nour' (Idder et al., 2009; Zouba et al., 2009).

Carob moth represents a serious pest to stored products because of its rapid development under storage conditions; it is also the most significant phytosanitary problem of date production. Date infestations, at all levels, field, packing and storage houses, enormously depreciates the marketable quality of dates and risks to compromise exports in particular those of 'Deglet Nour' (Zouba et al., 2009). Tunisian authorities realize advantages of lower pesticide use to the health of their citizens and the quality of the environment (Hura et al., 1999). Actually, postharvest control of date moth is primarily dependent on methyl bromide treatment. Unfortunately, this chemical is a highly toxic gas and it poses several hazards to animals and human (Hallier et al., 1990). Since this potent chemical fumigant is being phased out, the replacement of this product becomes an urgent need. On the other hand,

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several phytochemicals, extracted from various botanical sources, have been reported to have antibiotic effects on insects (Enan, 2001; Andrade et al., 2004; Jiang et al., 2009; Yang et al., 2009). Plant essential oils have been recognized as an important natural source of insecticides and their use as mosquito control agents has been shown to minimize the impact that most pesticidal compounds impose on the environment. Natural pesticides are promising in that they are effective, environment friendly, easily biodegradable and also inexpensive (Enan, 2001). Rosemary (*Rosmarinus officinalis* L.) is a perennial herb that belongs to the *Lamiaceae* family. It is used as a food flavoring agent and known medicinally for its powerful antimutagenic properties, antibacterial and chemopreventive.

The plant is also known for its antioxidant activity (Ibañez et al., 2003). Aleppo pine (*Pinus halepensis* Miller) is the most widely distributed tree throughout the entire Mediterranean region. The Aleppo pine tree is known to have aphrodisiac characteristics as well as medicinal properties (Uncini et al., 2001; Said et al., 2002). Thyme (*Thymus capitatus* [L.] Hoffmanns. & Link) is a perennial, herbaceous shrub commonly used as a spicy herb that belongs to the *Lamiaceae* family. Antimicrobial, antifungal, antioxidant, and radical-scavenging properties that are mediated by thymol and carvacrol as the major phenolic components of the thyme oil, have been previously reported (Bounatirou et al., 2007). The main objectives of this study were to determine the chemical composition of the essential oils extracted from the aerial parts of *R. officinalis*, *T. capitatus*, and *P. halepensis*, and to study their insecticidal activity on the different developmental stages of *Ectomyelois ceratoniae* in order to promote them as biopesticides.

MATERIALS AND METHODS

Plant material and essential oil isolation

The needles of Aleppo pine (*Pinus halepensis*), leaves of *Thymus capitatus* and *Rosmarinus officinalis* were collected in September 2009 from the arboretum of Korbous, Tunisia. The experimental site is located in the Nabeul region (north-east Tunisia, 36°39' N, 10°42' E) had an area of 16 ha. The site was characterized by an annual mean rainfall of 540 mm. Trees were planted on a soil-rich sand, sandstone and clay. Temperature recorded during the hottest moth was about 32.7 °C whereas for the coldest moth, it fell to 6.8 °C. Five samples collected from more than 10 different plants (randomly chosen but at least 5 m apart) were harvested, mixed for homogenization, and used in three replicates for essential oil extractions. The voucher specimens of plants were submitted to the herbarium division of the National Institute of Researches on Rural Engineering, Water and Forests (Tunisia) and identification was performed in the Laboratory of Forest Ecology.

Air-dried and finely grounded raw materials (100 g) were submitted to hydrodistillation for 3 h within 500 mL distilled water using a Clevenger type apparatus according to the standard procedure described in the European Pharmacopoeia (2004). The oil obtained was collected and dried over using anhydrous sodium sulfate and stored in sealed glass vials in a refrigerator at 4 °C prior to analysis.

Essential oil analysis

The essential oils were analyzed using a gas chromatography analysis with Hewlett Packard 5890 II GC equipped with Flame Ionization Detector (FID) and HP-5 MS capillary column (5% phenyl/95% dimethylpolysiloxane: 30 m × 0.25 mm id, film thickness 0.25 µm). Injector and detector temperature were set at 250 and 280 °C, respectively. Oven temperature was kept at 50 °C for 1 min then gradually raised to 250 °C at 5 °C min^{-1} and subsequently held isothermal for 4 min. Nitrogen was the carrier gas at a flow rate of 1.2 mL min^{-1}. Diluted samples (1/100 in hexane, v/v) of 1.0 µL were injected manually and in the splitless mode. Quantitative data were obtained electronically from FID area percent data without use of correction factors.

Oil analysis was performed using a gas chromatography analysis/mass spectrometry analysis (GC/MS), Hewlett Packard 5890 II GC, equipped with a HP 5972 mass selective detector and a HP-5 MS column (30 m × 0.25 mm id, film thickness 0.25 µm). For GC/MS detection, an electron ionization system with ionization energy of 70 eV, a scan time of 1.5 s and mass range of 40-300 amu, was used. Helium was the carrier gas at a flow rate of 1.2 mL min^{-1}. Injector and transfer line temperatures were set at 250 and 280 °C, respectively. Oven program temperature was the same with GC analysis. Diluted samples (1/10 in hexane, v/v) of 1.0 µL were injected manually following the splitless mode. The identification of compounds was based on mass spectra (compared with Wiley 275.L, 6^{th} edition mass spectral library) or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds or as in data published in literature as described by Adams (2001). Further confirmation was done from Retention Index data generated from a series of n-alkanes retention indices (relative to C9-C28 on the HP-5 MS column).

Insect rearing and food substrate

Ectomyelois ceratoniae was reared on an artificial diet based on wheat bran (60%), sucrose (12%), salt mixture (2%), yeast (1.3%), lysine (1.23%), methyl paraben (0.13%), vitamin C (0.67%), aureomycine (0.67%), glycerine (150 mL), and distilled water (150 mL) (Mediouni and Dhouibi, 2007). Rearing was conducted in plastic boxes in a rearing room where photoperiod (15:9 h L:D), temperature (28 ± 2 °C) and relative humidity (65 ± 5%) were automatically regulated. Eggs new laid, newly

emerged adults (0-24 h old), and last instar larvae (fifth instar larvae) were collected and used for bioassays.

Insecticidal activity bioassays

In the *in vitro* tests on *E. ceratoniae* eggs, aqueous solutions of the three different oils were used at the concentrations of 0 (control), 4, 8, 12, and 20 μL mL^{-1}. Thirty eggs were transferred to Petri dish containing a thin layer of food substrate. Tests were performed at 30 °C and 80% RH and the eggs were sprayed with 1 mL each oil solution. The hatched larvae were counted after 10 d.

To determine the essential oils fumigation capacity towards *E. ceratoniae* larvae and adult stages, a solution of each oil dissolved in 1 mL water with Tween 20 was applied to filter paper placed in the bottom of a Petri dish in the following concentrations: 0 (control), 4, 8, 12, and 20 μL mL^{-1} for each sample, and then respectively, 20 larvae and 20 insects of *E. ceratoniae* were placed in each Petri dish containing a thin layer of food substrate (artificial diet). In these experiments, the parameters observed were larvae and insect mortality after 6, 12, and 24 h. All treatments were replicated three times. To determine the direct contact larvicidal and insecticidal effects of the oils, respectively, 20 larvae and 20 insects were placed in Petri dish containing a thin layer of food substrate and sprayed with 1 mL essential oil solutions in the following concentrations: 0 (control), 4, 8, 12, and 20 μL mL^{-1} for each sample. The larvae and insects mortality was evaluated after 6, 12, and 24 h. In this experiment, four oil concentrations with three replicates per concentration were used.

Statistical analysis

Data of hatched larvae and mortality assays were subjected to one-way ANOVA using the SPSS 13.0 software package. Differences between means were compared through Student-Newmans-Keuls test and values of $p \leq 0.05$ were considered significantly different (Sokal and Rohlf, 1995).

RESULTS AND DISCUSSION

Essential oils chemical composition

Yields based on dried weight of *P. halepensis*, *T. capitatus*, and *R. officinalis* were respectively 0.93%, 1.14%, and 0.71%. Table 1 lists the linear retention indices and percentage composition of the mentioned essential oils. Fifty six constituents were identified in *P. halepensis* sample representing 98.69% of the total oil. Aleppo pine oil was characterized by a high percentage of monoterpene hydrocarbons (44.24%), followed by sesquiterpene hydrocarbons (31.36%), oxygenated sesquiterpenes (11.81%) and oxygenated monoterpenes (11.28%). The major components of *P. halepensis* essential oil were (*Z*)-caryophyllene (23.8%), β-myrcene (20.5%), α-pinene (13.3%); with presence of α-terpinolene (6.7%), α-cadinol (6.05%) and β-pinene (5.1%) at relatively high percentages. The data analysis shows that the chemical profile of our essential oil differs from those of Morocco, Italy, Greece, and Algeria, with quantitative and qualitative differences of individual components (Dob et al., 2005). For *R. officinalis* oil, the major components were 1,8-cineole (47.5%), camphor (14.9%), α-pinene (14.1%), and borneol (13.15%). Similar results (Zaouali and Boussaid, 2003) showed the same major components of the essential oil from Tunisian *R. officinalis* but with different levels. *Thymus capitatus* essential oil contained 34 compounds accounting for 99.28% of the total oil composition; the major components were carvacrol (66.9%), *p*-cymene (9.1%), and δ-terpinene (6.22%). It was characterized by high content of monoterpenes (95.98%), while sesquiterpenes were found only in low amounts (3.3%). The monoterpene fraction consisted mainly of oxygenated monoterpenes (79.43%), while, monoterpene hydrocarbons were present in smaller proportions (16.55%). The chemical composition of Tunisian *T. capitatus* essential oil was previously investigated and it was shown that carvacrol, δ-terpinene, and *p*-cymene were the major components of the oil which is in agreement with our results (Bounatirou et al., 2007).

Biological activities of essential oils against *E. ceratoniae*

Results on ovicidal, larvicidal, and adulticidal activities of three plants essential oils against *E. ceratoniae* at different doses and different times of exposure are presented in Tables 2 - 6. All assays showed toxic effects of essential oils on eggs, larvae and adults of *E. ceratoniae* after 6, 12, and 24 h of exposure. The highest activities were found with *T. capitatus* and *R. officinalis* essential oils, followed by *P. halepensis*. The ovicidal effects of essential oils against *E. ceratoniae* eggs showed that *T. capitatus* and *R. officinalis* essential oils induced 100% inhibition of hatching eggs at the concentration of 20 μL mL^{-1}; however, Aleppo pine oil was the less potent sample with 84.44% inhibition at of 20 μL mL^{-1} (Table 2). The results of the larvicidal effects of oils tested in direct contact also displayed a notable effect; their ability to kill larvae was enhanced with increased concentrations and time of treatment (Table 3). *Thymus capitatus* oil showed 100% effectiveness at 12 μL mL^{-1} after 24 h or 20 μL mL^{-1} after 12 h, while at the same concentrations and the same time of exposure, *R. officinalis* showed only 88.33% and 95%, respectively, and *P. halepensis* oil showed 58.33% and 73.33% effectiveness, respectively (Tables 3 and 4). The adulticidal activity of the oils against *E. ceratoniae* at different doses and different exposure times was evaluated. *Thymus capitatus* oil was the most effective, induced at the dose of 8 μL mL^{-1} after 24 h, 100% and 100% mortality, respectively, followed by *R. officinalis* (with 65% and 83.33%, respectively) and *P. halepensis* (with 55% and 53.33%, respectively) (Tables 5 and 6).

Table 1. Chemical composition of essential oils *Pinus halepensis*, *Rosmarinus officinalis*, and *Thymus capitatus*.

Nº	Compounds (%)	RI	*P. halepensis*	*R. officinalis*	*T. capitatus*	Identification
1	Tricyclene	926	0.10	-	-	MS.RI
2	α-Thujene	931	0.42	-	1.20	MS.RI
3	α-Pinene	939	13.30	14.10	0.63	MS.RI, Co-inj
4	Camphene	953	0.10	2.10	0.22	MS.RI, Co-inj
5	Thuja-2,4(10)-diene	958	0.10	-	-	MS.RI
6	Sabinene	976	1.20	-	0.49	MS.RI, Co-inj
7	β-Pinene	980	5.10	1.90	0.31	MS.RI, Co-inj
8	β-Myrcene	988	20.5	0.10	1.76	MS.RI
9	α-Phellandrene	1005	0.14	0.30	0.22	MS.RI
10	δ-3-Carene	1011	1.03	-	0.15	MS.RI
11	α-Terpinene	1018	0.34	0.21	0.10	MS.RI
12	p-Cymene	1026	0.31	-	9.10	MS.RI
13	1,8-Cineole	1021	-	47.50	0.30	MS.RI
14	Limonene	1031	1.60	-	2.52	MS.RI, Co-inj
15	Phenyl acetaldehyde	1041	0.56	-	-	MS.RI
16	(*E*)-β-Ocimene	1050	0.10	-	-	MS.RI
17	δ-Terpinene	1062	0.66	-	6.22	MS.RI
18	(*Z*)-Sabinene hydrate	1068	0.10	-	0.10	MS.RI
19	α-Terpinolene	1088	6.70	-	1.10	MS.RI
20	α-Pinene oxide	1094	0.40	-	-	MS.RI
21	β-Thujone	1115	0.10	-	-	MS.RI
22	Camphor	1143	0.10	14.90	0.10	MS.RI
23	Pinocarvone	1161	0.20	-	0.10	MS.RI
24	Umbellulol	1168	0.10	-	0.40	MS.RI
25	α-Terpinen-4-ol	1177	0.61	3.31	0.53	MS.RI
26	Myrtenol	1176	-	0.10	0.10	MS.RI
27	α-Terpineol	1189	0.32	-	0.12	MS.RI
28	Borneol	1201	-	13.15	0.11	MS.RI
29	Verbenone	1203	0.12	0.10	0.22	MS.RI
30	(*Z*)-Carveol	1209	-	-	1.10	MS.RI
31	Iso-dihydrocarveol	1213	0.30	-	0.12	MS.RI
32	(*E*)-Sabinene hydrate acetate	1219	0.20	-	1.35	MS.RI
33	Citronellol	1229	0.10	-	0.10	MS.RI
34	Pulegone	1234	-	-	0.11	MS.RI
35	Geraniol	1248	-	-	0.12	MS.RI
36	Isobornyl acetate	1279	0.61	0.90	0.10	MS.RI
37	Carvacrol	1298	-	-	66.9	MS.RI
38	δ-Elemene	1332	0.30	-	-	MS.RI
39	α-Terpinyl acetate	1349	0.49	-	0.10	MS.RI
40	β-Bourbounene	1363	0.20	-	-	MS.RI
41	α-Copaene	1376	0.34	-	-	MS.RI
42	β-Cububene	1383	0.20	-	-	MS.RI
43	Langifolene	1397	0.10	-	-	MS.RI
44	(*Z*)-Caryophyllene	1418	23.80	0.20	2.17	MS.RI, Co-inj
45	(*E*)-Caryophyllene	1419	-	-	1.13	MS.RI
46	(*Z*)-Muurrola-4-(14),5-diene	1444	0.20	-	-	MS.RI
47	α-Humulene	1454	2.58	-	-	MS.RI, Co-inj
48	(*Z*)-β-Farnesene	1459	0.20	-	-	MS.RI
49	Bicyclogerrnacrene	1495	1.55	-	-	MS.RI
50	(*E*)-Eudesma-6,1,1-diene	1490	0.90	-	-	MS.RI
51	*epi* Zonarene	1501	0.50	-	-	MS.RI
52	Cubebol	1509	0.87	-	-	MS.RI
53	β-Bisabolene	1511	0.10	-	-	MS.RI
54	δ-Cadinene	1523	0.60	-	-	MS.RI
56	Itacilene ether	1536	0.50	-	-	MS.RI
57	β-Calacorene	1546	0.50	-	-	MS.RI
58	(*Z*)-Muurola-5-en-4-ol	1559	0.68	-	-	MS.RI
59	Caryophyllene oxide	1581	0.41	0.1	-	MS.RI
60	Cedrol	1596	0.35	-	-	MS.RI
61	Humulene peroxide II	1606	0.20	-	-	MS.RI
62	α-Acorenol	1630	0.70	-	-	MS.RI
63	β-Acorenol	1634	0.52	-	-	MS.RI
64	α-Cadinol	1654	6.05	-	-	MS.RI
65	Abietatriene	2054	0.33	-	-	MS.RI
Total identified, %			98.69	98.97	99.28	
Monoterpene hydrocarbons, %			44.24	18.71	16.55	
Oxygenated monoterpenes, %			11.28	79.96	79.43	
Sesquiterpene hydrocarbons, %			31.36	0.30	3.30	
Oxygenated sesquiterpenes, %			11.81	-	-	

RI: Retention index; MS: mass spectrometry; Co-inj: Co-injection; -: not detected.

The potential insecticide capability of essential oils of *Thymus* sp., *R. officinalis*, and *Pinus* sp. has been previously reported by several authors (Lee et al., 2001a; Hori, 2003; Prajapati et al., 2005; Pavela, 2009) but no one deals with the carob moth. Insecticidal activities of several essential oils regularly appear to be associated with the presence

Table 2. Ovicidal effect of *Thymus capitatus*, *Rosmarinus officinalis* and *Pinus halepensis* essential oils on *Ectomyelois ceratoniae*.

Doses (μL mL^{-1})	Inhibition of eggs hatching (%) (mean percentage ± SE)		
	T. capitatus	*R. officinalis*	*P. halepensis*
Control	14.4 ± 2.9A	14.4 ± 2.9A	14.4 ± 2.9A
4	46.6 ± 1.9Ba	32.2 ± 1.1Bb	18.8 ± 1.1Ac
8	63.3 ± 5.7Ca	49.9 ± 1.9Cb	35.5 ± 1.1Bc
12	88.8 ± 4.0Da	78.8 ± 2.2Da	55.5 ± 5.8Cb
20	100 ± 0.0Ea	100 ± 0.0Ea	84.4 ± 2.9Db

Lower-case letters compare means in the lines and uppercase letters in the columns. Means with the same letter are not significantly different at $p \leq 0.05$.

of monoterpenoids and sesquiterpenes (Kiran and Devi, 2007). The essential oils of *T. capitatus, R. officinalis*, and *P. halepensis* contains mainly monoterpenes such as carvacrol, 1,8-cineole, α-pinene, camphor, borneol, β-myrcene and sesquiterpenes such as (*Z*)-caryophyllene, which are known to possess insecticidal activity (Lee et al., 2001b; Rozman et al., 2007; Kordali et al., 2008; Silva et al., 2008; Lucia et al., 2009; Qin et al., 2010) (Table 1). Generally, the insecticidal activity is related to the major components of the essential oils.

Previous reports showed that *Thyme* sp. and *Origanum* sp. oils possessed a potent insecticidal activity against *Culex quinquefasciatus*, *Sitophilus granarius* and *Tribolium confusum*; the test of individual components of such oils show that carvacrol, its major component, was found to be the principal toxic constituent (Kordali et al., 2008; Pavela, 2009). Our results agree with previous reports about insecticidal activity of *R. officinalis* essential oil. Yi et al. (2007) have shown the insecticidal effect of rosemary vapor oil against *Plutella xylostella* larvae (*Lepidoptera*). Other reports show that the insecticidal activity of *R. officinalis* oil rich in 1,8-cineole (58.8%) against *Mayetiola destructor* by fumigation causes 66.66% mortality after 120 min, but *Eucalyptus globulus* with 74.5% 1,8-cineole causes 63.33% mortality after the same time of exposure at the same dose, which explain that 1,8-cineole was the first responsible for this activity. In the case of *P. halepensis*, there is no available report in the literature about the insecticidal activity of its essential oils, but several studies have demonstrated the insecticidal activity of individual pure oil components against different stage of development of various insects and showed the potential insecticide effects of α-pinene and (*Z*)-caryophyllene, which are the major components of Aleppo pine oil (Lucia et al., 2009; Qin et al., 2010).

Our results indicate that the method of direct contact was more effective than fumigation, which can explain that oils act mainly in the respiratory system with the fumigation while acting in the cutaneous and respiratory systems with the direct contact. The egg stage of *E. ceratonia* was the most resistant to the action of oils, followed by larvae and adult. Similar results were obtained with the *Eucalyptus* sp. oils on eggs, larvae and adults of *Lutzomyia longipalpis* (Maciel et al., 2010). Our results can explain the effectiveness of essential oils on the three biological stages of *E. ceratoniae*. Although, the mechanism of action of the essential oils was not investigated in this study, it is known that some terpenoids such (*Z*)-caryophyllene inhibits the activity of several enzymes such as acetylcholinesterase, glutathione s-transferase, carboxyl esterase, which are important detoxification enzyme of insect body, involved in the detoxification of antifeedant, fumigant, and pesticides. When the activities of these enzymes were inhibited, the function of detoxification would be decreased, limited or even blocked, other enzyme, such as sodium

Table 3. Larvicidal effect of *Thymus capitatus*, *Rosmarinus officinalis*, and *Pinus halepensis* essential oils after 6, 12, and 24 h on *Ectomyelois ceratoniae* by direct contact.

	Larvae mortality (%) (mean percentage ± SE)								
	T. capitatus			*R. officinalis*			*P. halepensis*		
Doses (μL mL^{-1})	6 h	12 h	24 h	6 h	12 h	24 h	6 h	12 h	24 h
Control	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
4	20.0 ± 2.8b	28.3 ± 1.6b	45.0 ± 2.8b	23.3 ± 3.3b	31.6 ± 1.6b	40.0 ± 2.8b	13.3 ± 1.6b	21.6 ± 1.6b	31.6 ± 3.3b
8	40.0 ± 2.8c	61.6 ± 3.3c	73.3 ± 1.6c	31.6 ± 1.6c	43.3 ± 1.6c	55.0 ± 1.6c	23.3 ± 1.6c	30.0 ± 2.8c	51.6 ± 3.3c
12	71.6 ± 1.6d	88.3 ± 4.4d	100 ± 0.0d	63.3 ± 1.6d	71.6 ± 1.6d	88.3 ± 1.6d	40.0 ± 2.8d	50.0 ± 2.8d	58.3 ± 1.6c
20	91.6 ± 6.0e	100 ± 0.0e	100 ± 0.0d	81.6 ± 3.3e	95.0 ± 2.8e	98.3 ± 1.6e	61.6 ± 1.6e	73.3 ± 1.6e	73.3 ± 1.6d

Means in the same column with the same letter are not significantly different at $p \leq 0.05$.

Table 4. Larvicidal effect of *Thymus capitatus*, *Rosmarinus officinalis*, and *Pinus halepensis* essential oils after 6, 12, and 24 h on *Ectomyelois ceratoniae* by fumigation.

	Larvae mortality (%) (mean percentage ± SE)								
	T. capitatus			*R. officinalis*			*P. halepensis*		
Doses (μL mL^{-1})	6 h	12 h	24 h	6 h	12 h	24 h	6 h	12 h	24 h
Control	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
4	21.6 ± 3.3b	40.0 ± 2.8b	56.6 ± 1.6b	16.6 ± 1.6b	28.3 ± 1.6b	50.0 ± 2.8b	11.6 ± 1.6b	21.6 ± 1.6b	35.0 ± 2.8b
8	25.0 ± 2.8b	45.0 ± 2.8b	70.0 ± 2.8c	23.3 ± 1.6c	43.3 ± 4.4c	58.3 ± 1.6c	13.3 ± 3.3b	23.3 ± 3.3b	43.3 ± 3.3b
12	58.3 ± 1.6c	68.3 ± 1.6c	86.6 ± 1.6d	43.3 ± 1.6d	55.0 ± 2.8d	70.0 ± 2.8d	26.6 ± 4.4c	38.3 ± 4.4c	53.3 ± 1.6c
20	75.0 ± 2.8d	91.6 ± 3.3d	100 ± 0.0e	68.3 ± 1.6e	78.3 ± 1.6e	90.0 ± 2.8e	45.0 ± 2.8d	56.6 ± 3.3d	68.3 ± 4.4d

Means in the same column with the same letter are not significantly different at $p \leq 0.05$.

Table 5. Adulticidal effect of *Thymus capitatus*, *Rosmarinus officinalis*, and *Pinus halepensis* essential oils after 6, 12, and 24 h on *Ectomyelois ceratoniae* by direct contact.

	Insect mortality (%) (mean percentage ± SE)								
	T. capitatus			*R. officinalis*			*P. halepensis*		
Doses (μL mL^{-1})	6 h	12 h	24 h	6 h	12 h	24 h	6 h	12 h	24 h
Control	5.0 ± 2.8a	8.3 ± 4.4a	10.0 ± 5.0a	5.0 ± 2.8a	8.3 ± 4.4a	10.0 ± 5.0a	5.0 ± 2.8a	8.3 ± 4.4a	10.0 ± 5.0a
4	41.6 ± 4.4b	46.6 ± 1.6b	75.0 ± 2.8b	20.0 ± 2.8b	36.6 ± 6.0b	51.6 ± 4.4b	21.6 ± 1.6b	35.0 ± 2.8b	36.6 ± 1.6b
8	63.3 ± 4.4c	73.3 ± 1.6c	100 ± 0.0c	31.6 ± 1.6c	48.3 ± 1.6c	65.0 ± 2.8c	23.3 ± 1.6b	40.0 ± 5.0b	55.0 ± 2.8c
12	100 ± 0.0d	100 ± 0.0d	100 ± 0.0c	71.6 ± 1.6d	90.0 ± 2.8d	98.3 ± 1.6d	48.3 ± 1.6c	58.3 ± 1.6c	70.0 ± 2.8d
20	100 ± 0.0d	100 ± 0.0d	100 ± 0.0c	96.6 ± 1.6e	100 ± 0.0d	100 ± 0.0d	63.3 ± 1.6d	75.0 ± 2.8d	78.3 ± 1.6d

Means in the same column with the same letter are not significantly different at $p \leq 0.05$.

Table 6. Adulticidal effect of *Thymus capitatus*, *Rosmarinus officinalis*, and *Pinus halepensis* essential oils after 6, 12, and 24 h on *Ectomyelois ceratoniae* by fumigation.

	Insect mortality (%) (mean percentage ± SE)								
	T. capitatus			*R. officinalis*			*P. halepensis*		
Doses (μL mL^{-1})	6 h	12 h	24 h	6 h	12 h	24 h	6 h	12 h	24 h
Control	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a	0.0 ± 0.0a
4	38.3 ± 4.4b	76.6 ± 4.4b	88.3 ± 1.6b	13.3 ± 4.4b	38.3 ± 1.6b	58.3 ± 1.6b	18.3 ± 1.6b	21.6 ± 1.6b	30.0 ± 5.0b
8	73.3 ± 1.6c	90.0 ± 5.0c	100 ± 0.0c	40.0 ± 5.0c	65.0 ± 2.8c	83.3 ± 1.6c	33.3 ± 1.6c	41.6 ± 6.0c	53.3 ± 7.6c
12	100 ± 0.0d	100 ± 0.0c	100 ± 0.0c	81.6 ± 3.3c	100 ± 0.0d	100 ± 0.0d	43.3 ± 4.4cd	46.6 ± 4.4c	55.0 ± 5.0c
20	100 ± 0.0d	100 ± 0.0c	100 ± 0.0c	100 ± 0.0d	100 ± 0.0d	100 ± 0.0d	51.6 ± 6.0d	63.3 ± 6.6d	85.0 ± 5.0d

Means in the same column with the same letter are not significantly different at $p \leq 0.05$.

potassium-ATPase, which is the Na^+ and K^+ ion pump of epicyte of insect playing a crucial role in keeping the ionic equilibrium and nerve impulse of insect body, and the inhibition of this pump cause a leading of metabolic disturbance and nerve impulse of insect body (Qin et al., 2010). Stamopoulos et al. (2007) explained that the greater tolerance of the eggs was related to the neurotoxic action of these compounds, which acts only after nervous system of the embryo begins to grow. Another possible explanation is the lesser permeability of eggs surface at the beginning of embryogenesis. Other reports indicate that monoterpenes act by inducing changes in cyclic AMP production and blockage of octopamine receptor binding activity as it was demonstrated under *in vitro* treatment. Other biogenic microamines such as β-phenylethylamine, phenylethanolamine, *m*, *p*-tyramine and tryptamine are possible targets for monoterpenoids, whether the toxic action of monoterpenoids is mediated through other pathways such as GABA receptors, muscarinic and nicotinic acetylcholine receptors, β1-, β2-, and α-adrenergic receptor sites, Ca stores and ions-channels (Enan, 2001). Another hypothesis is that the monoterpenes act on other vulnerable sites, such as cytochrome P450 (Lee et al., 2001b), but understanding the real mechanism of action of these oils will require further investigation (Tsukamoto et al., 2005).

CONCLUSIONS

Chemical composition of essential oils varied according to species, variability that explained the difference in insecticidal activity. *Thymus capitatus* essential oil displayed the most effective activity and toxic effect against date moth (*Ectomyelois ceratoniae*). Our results suggest that the essential oils of *T. capitatus*, *Rosmarinus officinalis*, and *Pinus halepensis* possess insecticidal activity and therefore, could be useful in biotechnological application as natural preservative in stored dates and also for managing populations of *E. ceratoniae* in field. Therefore, it can be used as a potential source of sustainable eco-friendly botanical pesticide, after successful completion of wide range trials. However, further studies on the identification of the active principals involved their mode of action and field trials are needed to recommend *T. capitatus* essential oil as an insecticidal product used to protect date in a control program.

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