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Composition and insecticidal activity of essential oil from *Pistacia lentiscus* L. against *Ectomyelois ceratoniae* Zeller and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae)

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ABSTRACT

This study reports investigations on the chemical constituents and fumigant toxicity of *Pistacia lentiscus* L. (Anacardiaceae) essential oil against two major stored-date insects, carob moth *Ectomyelois ceratoniae* Zeller and Mediterranean flour moth *Ephestia kuehniella* Zeller. Results showed that *P. lentiscus* essential oil contained terpinene-4-ol (23.32%), α-terpineol (7.12%) and β-caryophyllene (22.62%) as major compounds. Fumigant toxicity tests showed that *P. lentiscus* oil was more toxic to *E. kuehniella* (LC_{50} = 1.84 μl/l, LC_{95} = 5.14 μl/l) than *E. ceratoniae* (LC_{50} = 3.29 μl/l, LC_{95} = 14.24 μl/l). The fecundity and hatching rate of both insects decreased with increases in concentration or exposure time to the oil. At 136 μl/l air, fecundities and hatching rates were respectively 35 eggs/female and 42.86% for *E. ceratoniae* and 78 eggs/female and 29.49% for *E. kuehniella*.

1. Introduction

In the Maghreb countries, date palm is of great socio-economic importance (Majourhat et al., 2002). Date fruit is the main staple and date palm is the basis of survival for the inhabitants of the North African Sahara and the hot arid desert region (Botes and Zaid, 1999). They represent an important source of nutrients and energy (Boudries et al., 2007). Tunisia is currently the tenth largest world producer and the foremost exporter of dates in terms of value (Besbes et al., 2009). In South Tunisia, dates and their secondary products are the main agricultural products of the oases and have a major role in the local economy. The annual national production is estimated to be 145,000 tons including 7000 tons for export (GIFruits, 2009).

The carob moth, *Ectomyelois ceratoniae* Zeller and the Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) are the most important and destructive insects attacking dates in storage in Tunisia (Mediouni et al., 2004). These polyphagous species attack several other host plants stored locally such as pomegranate, almond and pistachio nut (Dhouibi, 1989) and cause major economic losses in the Mediterranean basin and Near East regions (Al-Izzi et al., 1985). The carob moth infests 20% of the harvestable date crop annually (GIFruits, 2009). The Mediterranean flour moth, one of the most important stored-product pests, especially in amylaceous products (Sedlacek et al., 1995; Athanassiou et al., 2008), also occurs widely on dates. Apart from direct infestation, the faeces and webbings of larvae spoil the product.

The use of fumigants is the most economical tool for managing these stored-date pests (Azelmat et al., 2006). Methyl bromide is still the primary insecticide used in post-harvest insect control for dates in Tunisia and in several other countries (Zare et al., 2002). However, the use of this pesticide is to be phased out because it is an ozone depleter (Bell, 2000). Recently, research has been focusing on the use of plant essential oils and their bioactive constituents as possible alternatives to methyl bromide (Negahban et al., 2007; Ogendo et al., 2008). In this context, essential oils may play a significant role as pest control agents. Regnault-Roger et al. (1993) reported that essential oil from Mediterranean plants were effective against *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). Furthermore, the fumigant toxicity of essential oil of *Artemisia*

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sieberi Besser has been demonstrated against two stored-product pests, *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) (Negahban et al., 2006). In Tunisia, the genus *Pistacia* (Anacardiaceae) is represented by *Pistacia lentiscus* L., *Pistacia atlantica* Desf., *Pistacia terebinthus* L. and *Pistacia vera* L. (Bonnier and Douin, 1990). *Pistacia lentiscus* locally known under the common name "dharw", is a perennial shrub commonly used as a popular cure for hypertension (Villar et al., 1987). In addition, Al-Said et al. (1986) reported that traditional healers used *P. lentiscus* mastic gum for the relief of abdominal discomfort, stomach aches, dyspepsia and peptic ulcer. Several studies have reported on the insecticidal activity or repellency of *P. lentiscus* essential oil (Pascual-Villalobos and Robledo, 1998; Lamiri et al., 2001). This study was undertaken to investigate *P. lentiscus* essential oil composition. In addition, this paper describes for the first time the fumigation activity of the oil against two stored-date moths: *E. ceratoniae* and *E. kuehniella*. The effects of essential oil on copulation rate, longevity, fecundity and hatching rate were evaluated.

2. Materials and methods

2.1. Insect rearing

For bioassays, 0–24 h old adult insects were collected from the rearing colony initiated in the laboratory of Plant Protection at the National Institute of Agricultural Research of Tunisia. Carob moth was reared on an artificial diet based on wheat bran, yeast, sucrose, salt mixture, vitamin C, aureomycine, methylparaben, lysine, glycerine and distilled water (Mediouni and Dhouibi, 2007). The rearing conditions were: 25 ± 1 °C, a photoperiod of 15:9h (L:D) and 65 ± 5% relative humidity (r.h.).

The Mediterranean flour moth was reared on bread wheat flour (*Triticum aestivum* L.), in plastic boxes in darkness at 25 ± 1 °C and 65 ± 5% r.h.

2.2. Plant material

Pistacia lentiscus leaves were collected from plants at the flowering stage from natural populations in the locality of Siliana (North-West, Tunisia) during May 2008. A voucher specimen (P.l.08003) was deposited in the Aromatic and Medicinal Plants laboratory (Borj Cedria Biotechnology Centre, Tunisia). The harvested material was air-dried at room temperature (20–25 °C) for one week and then stored in cloth bags.

2.3. Extraction and analysis of essential oil

Essential oil was extracted from leaves (100 g of dry matter) subjected to hydrodistillation for 90 min using a modified Clevenger-type apparatus. Anhydrous sodium sulphate was used to remove water after extraction. The extracted oil was then stored at 4 °C.

Essential oil was analysed by gas chromatography (GC) using a Hewlett-Packard 6890 gas chromatograph (Agilent Technologies, Palo Alto, California, USA) equipped with a flame ionization detector (FID) and an electronic pressure control (EPC) injector. A polar HP-Innowax (PEG) column (30 m × 0.25 mm, 0.25 μm film thickness) and an apolar HP-5 column (30 m × 0.25 mm coated with 5% phenyl methyl silicone, and 95% dimethyl polysiloxane, 0.25 μm film thickness) from Agilent were used. Carrier gas flow (N_2) was 1.6 ml/min and the split ratio 60:1. Analyses were performed using the following temperature program: oven kept isothermally at 35 °C for 10 min, increased from 35 to 205 °C at the rate of 3 °C/min and kept isothermally at 205 °C for 10 min. Injector and detector temperatures were held, respectively, at 250 and 300 °C. The GC/MS analyses were made using an HP-5972 mass spectrometer with electron impact ionization (70 eV) coupled with an HP-5890 series II gas chromatograph. An HP-5MS capillary column (30 m × 0.25 mm coated with 5% phenyl methyl silicone, and 95% dimethyl polysiloxane, 0.25 μm film thicknesses) was used. The oven temperature was programmed to rise from 50 to 240 °C at a rate of 5 °C/min. The transfer line temperature was 250 °C. Helium was used as carrier gas with a flow rate of 1.2 ml/min and a split ratio of 60:1. Scan time and mass range were 1 s and 40–300 *m*/*z* respectively.

Essential oil volatile compounds were identified by calculating their retention index (RI) relative to (C9-C18) n-alkanes (Analytical reagents, Labscan, Ltd, Dublin, Ireland) and data for authentic compounds available in the literature and in our data bank, and also by matching their mass spectrum fragmentation patterns with corresponding data stored in the mass spectra library of the GC-MS data system (NIST) and other published mass spectra (Adams, 2001). The relative percentage amount of each identified compound was obtained from the electronic integration of its FID peak area.

Table 1
Essential oil composition (%) of *Pistacia lentiscus* L. leaves. (Means of 3 replicates ± S.E).

Volatile compound*	RI[a]	RI[b]	Identification	%
Z-3-hexenol	855	1370	GC/MS	1.68 ± 0.93
E-2-hexenol	856	1356	GC/MS, Co GC	0.36 ± 0.40
Tricyclene	924	1014	GC/MS, Co GC	2.09 ± 2.09
α-thujene	928	1035	GC/MS, Co GC	0.58 ± 0.65
α-pinene	939	1032	GC/MS, Co GC	0.67 ± 0.69
Camphor	954	1076	GC/MS, Co GC	0.72 ± 0.11
Camphene	954	1076	GC/MS	1.33 ± 1.29
Sabinene	975	1132	GC/MS	0.32 ± 0.36
β-pinene	980	1118	GC/MS	1.84 ± 2.08
Myrcene	991	1174	GC/MS	0.35 ± 0.39
α-phellandrene	1006	1176	GC/MS	0.45 ± 0.50
Δ-3-carene	1011	1059	GC/MS	0.38 ± 0.33
Limonene	1030	1203	GC/MS	0.34 ± 0.16
1-8-cineole	1033	1213	GC/MS	0.80 ± 0.90
(*E*)-β-Ocimene	1050	1266	GC/MS	0.13 ± 0.15
γ-terpinene	1053	1243	GC/MS	0.35 ± 0.39
Cis-linalool oxide	1074	1450	GC/MS	0.07 ± 0.08
Trans-linalool oxide	1088	1475	GC/MS	0.36 ± 0.21
Linalool	1098	1553	GC/MS	0.58 ± 0.06
Borneol	1165	1702	GC/MS	0.60 ± 0.20
Terpinene-4-ol	1178	1611	GC/MS	23.32 ± 18.41
α-terpineol	1189	1709	GC/MS	7.22 ± 1.65
Geraniol	1255	1857	GC/MS	0.28 ± 0.06
Bornyl acetate	1295	1597	GC/MS	0.84 ± 0.18
Linalyl-proprionate	1325	1597	GC/MS	1.70 ± 1.91
α terpenyl Acetate	1344	1706	GC/MS	1.19 ± 0.002
α-cubebene	1351	1472	GC/MS	tr
Copaene	1372	1490	GC/MS	1.48 ± 0.08
Beta-caryophyllene	1434	1594	GC/MS, Co GC	22.62 ± 24.15
α-humulene	1454	1687	GC/MS	1.14 ± 0.03
Allo-aromandrene	1474	1661	GC/MS	0.27 ± 0.02
Delta muurolene	476	1675	GC/MS	0.97 ± 0.50
GermacreneD	1480	1726	GC/MS, Co GC	0.85 ± 0.02

*Components are listed in order of elution in polar column (HP-Innowax); RI[a], RI[b]: Retention indices calculated using respectively an apolar column (HP-5) and polar column (HP-Innowax). tr = traces (≤0.05%).

2.4. Fumigant toxicity

Whatman N°1 filter papers (2 cm dia) were impregnated with doses of *P. lentiscus* oil calculated to release fumigant concentrations between 20 and 160 μl/l air. Each impregnated filter paper was attached to the screw cap of a 44 ml Plexiglas bottle. Ten adults of one or other of the insect species were added to each bottle and caps were screwed on tightly. Each concentration and control was