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Fumigant toxicity of *Pistacia lentiscus* essential oil against *Tribolium castaneum* and *Lasioderma serricorne*

Olfa BACHROUCH[1,2], Jouda MEDIOUNI-BEN JEMÂA[3], Thierry TALOU[4], Brahim MARZOUK[2], Manef ABDERRABA[1]
[1]*Unité de Recherche de Physico-Chimie Moléculaire (URPCM). IPEST, La Marsa, Tunisie*
[2]*Unité de Plantes Aromatiques et Médicinales, Centre de Biotechnologie du Technopole de Borj Cedria, Hammam lif, Tunisie*
[3]*Laboratoire de Protection des Végétaux, INRAT, Ariana, Tunisie*
[4]*Laboratoire de Chimie Agro-industrielle, Unité de recherche mixte INRA/INP ENSIACET (UMR1010), Toulouse Cedex, France*

Abstract

This study reports fumigant toxicity of *Pistacia lentiscus* L. (Anacardiacae) essential oil against 1-7 day-old adults of *Tribolium castaneum* (Herbst) and *Lasioderma serricorne* (F.). The chemical composition of the essential oil was assessed via GC and GC-MS. Alpha-phellandrene (3.20%), alpha-pinene (9.48%) and limonene (19.11%) were the major compounds. The mortality of adults was tested in different concentrations ranging from 114 to 1023 µl/l air and different exposure times. Significant differences in insect mortality were observed within insect species, oil concentrations and exposure time. The fumigant toxicity potential of *P. lentiscus* on *L. serricorne* was greater (LC_{50} = 8.44 µl/l, LC_{95} = 43.68 µl/l) than on *T. castaneum* (LC_{50} = 28.03 µl/l, LC_{95} = 63.46 µl/l). Moreover, the median lethal time values (LT_{50}) were respectively 18.58 and 41.05 hours. The results suggested that *P. lentiscus* essential oil may have potential as a control agent against these two stored product beetles.

Key words: Anacardiacae, Anobiidae, lethal concentration, monoterpenoids, Stored-product insects, Tenebrionidae.

Introduction

Annual post- harvest losses resulting from insect damage and other bio-agents are estimated to be 10-40% of world agricultural production (Mohan and Fields, 2002). Insects are the main problem in stored grains because they affect their quantity and their quality (Madrid *et al*., 1990). Grain infestation by various storage-product pests may occur at various stages from harvest to consumption.

The rust-red flour beetle, *Tribolium castaneum* (Herbst) and the cigarette beetle, *Lasioderma serricorne* (F.) are cosmopolitan and polyphagous stored product pests. *T. castaneum* is one of the major pests of stored grains and stored products throughout the world (Sinha and Watters, 1985). *L. serricorne* is known to develop on a variety of grain-based products, spices, and tobacco, and infest these commodities during storage, and manufacturing (Dimetry *et al*., 2004). These two beetles are among the main stored-product pests in Tunisia and North Africa (Jarraya, 2003).

In many storage systems, the use of fumigants is the most economical tool for managing stored grain insect pests (Mueller, 1990). Phosphine and methyl bromide are the two most common fumigants used for stored product protection. However, the ozone depleting effect of methyl bromide has led to its restrictions and phasing out (MBTOC, 1998). As alternatives, older fumigants including carbon disulphide (Yonglin and Allen, 1999) and newer compounds like isothiocyanates (Shaya *et al*., 2003) have been investigated for stored-products protection. Moreover, carbon dioxide (CO_2) has been used for disinfesting storage commodities (Annis, 1987). Although, the effectiveness of these methods seems good, there are of global concerns about their negative effects on human health and environment (Kostyukovsky *et al*., 2002).

There is therefore an urgent need to find out an alternative strategy to control these pests. Among Integrated Pest Management tactics, plants have played a significant role because they constitute an important source of insecticides (Arroyo, 1995). In recent years, essential oils received a great deal of attention as pest control agents. They are characterized by a low toxicity to human and animals, high volatility, and toxicity to stored grain insect pests (Batish *et al*., 2008). Sahaf *et al*., (2008) reported that essential oils may be applicable to the protection of stored products. In addition, aromatic plants and their essential oils have been used since antiquity in flavour and fragrances, as condiment or spice, in medicines, as antimicrobial/insecticidal agents and to repel insect or protect stored products.

The genus *Pistacia* includes many species widely distributed in the Mediterranean and Middle Eastern areas. *Pistacia lentiscus* L. (Anacardiacae) is a shrub encountered in mountainous areas of Tunisia (Bonnier and Douin, 1990). *P. lentiscus* is valued because it is used as a popular cure for hypertension (Villar *et al*., 1987). Mastic gum has been used by traditional healers for the relief of upper abdominal discomfort, stomachaches, dyspepsia and peptic ulcer (Al-Said *et al*., 1986). Moreover, *P. lentiscus* essential oil was characterized by the predominance of monoterpene hydrocarbons (Ben Douissa *et al*., 2005) which are typically volatile and rather lipophilic compounds and can rapidly penetrate into insects and interfere with their physiological functions (Lee *et al*., 2002). Furthermore, the insecticidal activity of *P. lentiscus* essential oil was reported on the fourth instar larvae of the mosquito *Culex pipens* L. (Traboulsi *et al*., 2002).

No studies have been reported previously concerning the activity of *P. lentiscus* as fumigant on insect pests. Thus, this work was undertaken to further investigate essential oil composition of *P. lentiscus* collected from

Jebel Mansour (North Tunisia) and to evaluate its fumigant toxicity against adults of *T. castaneum* and *L. serricorne*, two stored product beetles.

Materials and methods

Insect rearing

The rust-red flour beetle: *T. castaneum* and the cigarette beetle *L. serricorne* were reared on wheat flour. The rearing conditions were darkness in 25 ± 1 °C and 65% ± 5% (Relative Humidity). Adult insects, 1-7 days old, were used for fumigant toxicity tests.

Plant material

P. lentiscus leaves were collected from natural populations at the flowering stage during May 2008 in the region of Jebel Mansour in Tunisia. A voucher specimen (P.l.08003) was deposed in the Aromatic and Medicinal Plants laboratory (Borj Cedria Biotechnology Center-Tunisia). The harvested material was air-dried at room temperature (20-25 °C) for one week and then stored in cloth bags.

Extraction and analysis of essential oil

Essential oil was extracted from leaves (100 g of dry matter) subjected to hydrodistillation during 90 mm using a modified Clevenger-type apparatus. This time was fixed after a kinetic survey. Anhydrous sodium sulphate was used to remove water after extraction. The extracted oil was stored at 4 °C.

Essential oils were analysed by gas chromatography 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, U) was 1.6 ml/min and the split ratio 60:1. Analyses were performed using the following temperature program: oven kept isothermally at 35 ºC for 10 min, increased from 35 to 205 ºC at the rate of 3 ºC/min and kept isothermally at 205 ºC during 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 thickness) was used. The oven temperature was programmed to rise from 50 °C 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 1s and 40-300 *m/z* respectively.

Each volatile essential oil compound was identified by comparing its retention index (RI) relative to (C_9-C_{18}) *n*-alkanes with those available in literature of authentic compounds (Analytical reagents, Labscan, Ltd, Dublin, Ireland) and in our laboratory and by matching their mass spectra 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). Relative percentage amount of each identified compound was obtained from the electronic integration of its FID peak area.

The volatil compounds were ranged into groups (Monoterpene hydrocarbons, Oxygenated monoterpenes, Esters monoterpenes, Ether monoterpenes, Sesquiterpene hydrocarbons, Aliphatic aldehydes, Aliphatic alcohols, Aliphatic hydrocarbons and Ketons). Major compounds in each group were marked in bold form. The amount of each volatile compound was expressed in µg/g dry weight.

Fumigant toxicity bioassays

To determine the fumigant toxicity of *P. lentiscus* essential oil, 2 cm diameter filter papers (Whatman No.1) were impregnated with the tested oil doses calculated to give equivalent fumigant concentrations of 114-1023µl/l air. The impregnated filter paper was then attached to the screw caps of a 44 ml Plexiglas bottle. Caps were screwed tightly on the vials, each of which contained separately 10 adults (1-7 days old) of each species. Each treatment and check was replicated five times. Mortality was recorded after 3, 6, 9, 12 and 24 h of exposure. When no leg or antennal movements were observed, insects were considered dead. The mortality was calculated using the Abbott correction formula (Abbott, 1925).

Lethal doses bioassays

An experiment was designed to assess 50% and 95% lethal doses. A series of dilutions was prepared to evaluate mortality of insects after an initial screening experiment. Ten adult insects were put into 44 ml Plexiglas bottles with screw lids. Oil amounts tested on *T. castaneum* and *L. serricorne* were 5, 10, 15, 20, 25, 30, 40, 45µl corresponding to concentrations of 114, 227, 341, 455, 569, 682, 910, 1023 µl/l air. Control insects were kept under the same conditions without any essential oil and each dose was replicated five times. The number of dead and live insects in each bottle was counted 24 h after initial exposure. The mortality was evaluated by direct observation of the insects every hour until total mortality. Probit analysis (Finney, 1971) was used to estimate LC_{50} and LC_{95} values.

Lethal time bioassays

To be effective, fumigation trials should be based on the use of lower fumigant concentrations leading to high mortality in short time. Therefore, a bioassay was designed to determine median effective time to cause mortality of 50% and 90% of test insects (LT_{50}, LT_{90} values) at the three lowest doses (114, 227 and 341 µl/l air).

The mortality was assessed by direct observation of insects every hour until all the insects were dead. Data showing time mortality for each experiment were analyzed by Finney's method (1971).

Results

Essential oil composition of *Pistacia lentiscus* leaves

GC and GC/MS analysis of *P. lentiscus* essential oil leaves collected from Jebel Mansour (North of Tunisia) permitted to identify forty compounds (table 1). The oil yield was 0.02% on the basis of dry matter weight.

Essential oil was rich in monoterpene hydrocarbons (45.12%) followed by oxygenated monoterpenes (19.38%) and sesquiterpenes (2.71%). Its main constituents were limonene (19.11%), α-pinene (9.48%), α-phellandrene (3.20%) and Δ-3-carene (2.75%).

Table 1. Volatile compound percentages (%) and amounts (µg /g dry weight) of leaf essential oil from *P. lentiscus*.

Volatile compound*	RI[a]	RI[b]	Identification[c]	%	(µg /g dry weight)
Monoterpene hydrocarbons					
Tricyclene	924	1014	GC/MS, Co GC	1.75	0.49
α-Thujene	928	1035	GC/MS, Co GC	1.08	0.25
α-Pinene	**939**	**1032**	**GC/MS, Co GC**	**9.48**	**2.07**
Camphene	954	1076	GC/MS	1.00	0.28
Sabinene	975	1132	GC/MS	1.42	0.31
β-Pinene	980	1118	GC/MS	3.27	0.76
Myrcene	991	1174	GC/MS	1.12	0.23
α-Phellandrene	1006	1176	GC/MS	3.20	4.07
Δ-3-Carene	1011	1059	GC/MS	2.75	0.77
p-Cymene	1026	1280	GC/MS	0.06	0.08
Limonene	**1030**	**1203**	**GC/MS**	**19.11**	**4.07**
E-β-Ocimene	1050	1266	GC/MS	0.29	0.06
γ-Terpinene	1053	1243	GC/MS	0.16	0.03
Terpinolene	1092	1290	GC/MS	0.37	0.08
Oxygenated monoterpenes					
Z-linalool oxyde	1074	1450	GC/MS	0.45	0.13
E-linalool oxyde	1088	1475	GC/MS	0.19	0.04
Linalool	1098	1553	GC/MS	0.53	0.14
Borneol	1165	1702	GC/MS	0.88	0.24
Terpinene-4-ol	1178	1611	GC/MS	1.53	0.37
α-Terpineol	1189	1709	GC/MS	2.13	0.24
Geraniol	1255	1857	GC/MS	0.07	0.02
Esters monoterpenes					
Bornyl acetate	1295	1597	GC/MS	0.13	0.03
Linalyl-proprionate	1325	1597	GC/MS	1.53	2.26
α-Terpenyl acetate	1344	1706	GC/MS	2.19	0.60
Ether monoterpenes					
1,8-Cineole	1033	1213	GC/MS	2.04	0.43
Sesquiterpene hydrocarbons					
α-Cubebene	1351	1472	GC/MS	tr	tr
Copaene	1372	1490	GC/MS	0.29	0.07
β-elemene	1391	1600	GC/MS	0.28	0.06
β-caryophyllene	1434	1594	GC/MS, Co GC	1.11	0.29
α-humulene	1454	1687	GC/MS	–	–
Allo-aromandrene	1474	1661	GC/MS	–	–
Δ-muurolene	1476	1675	GC/MS	0.29	0.07
Germacrene-D	1480	1726	GC/MS, Co GC	0.38	0.08
Aliphatic aldehydes					
E-2-hexenal	850	1219	GC/MS	1.29	0.30
Aliphatic alcohols					
Hexanol	865	1354	GC/MS	0.23	0.06
E-2-hexenol	856	1356	GC/MS, Co GC	0.54	0.12
Z-3-hexenol	855	1370	GC/MS	0.85	0.23
Aliphatic hydrocarbons					
Tridecane	1300	1300	GC/MS	0.09	0.01
Nonadecane	1900	1900	GC/MS	0.36	0.02
Ketons					
Camphor	954	1076	GC/MS, Co GC	0.17	0.050

–: compound not detected; RI[a], RI[b]: Retention indices calculated using respectively an apolar column (HP-5) and polar column (HP-Innowax); tr = traces (≤ 0.05%).

Fumigant toxicity

P. lentiscus essential oil was toxic to the two beetle species (figure 1). It was more toxic to *L. serricorne* than to *T. castaneum*. The lowest concentration (114 µl/l air) of the oil led to no mortality of *T. castaneum* and 45% mortality of *L. serricorne*, after 24 h of exposure. Meanwhile at 455 µl/l air, *P. lentiscus* essential oil achieved respectively 10% and 60% mortality after 9 h exposure. After 24 h exposure, 22.5% and 70% mortality were obtained at this concentration respectively for

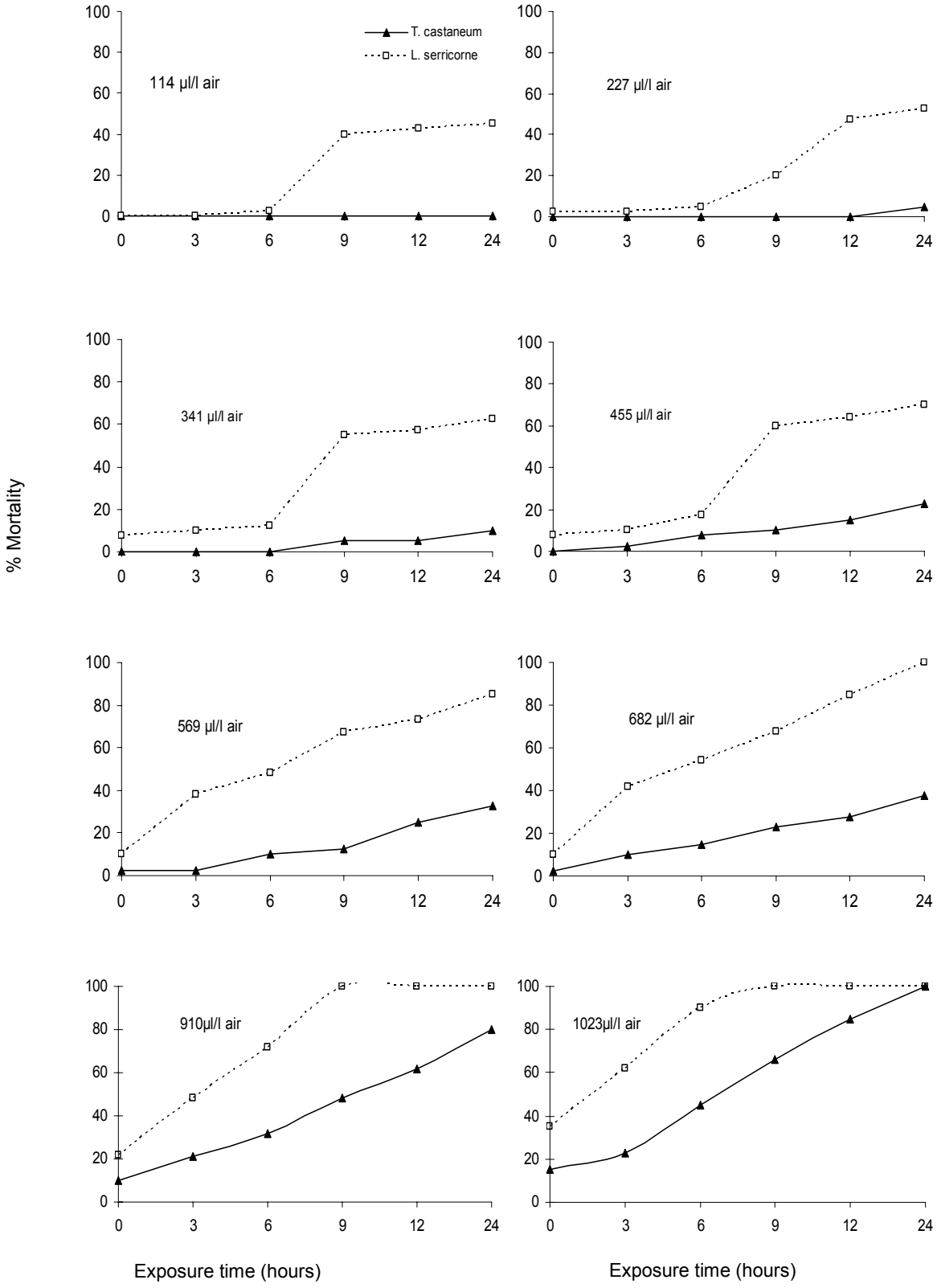

Figure 1. Percentage of mortality of *T. castaneum* and *L. serricorne* exposed for various periods of time to essential oil from *P. lentiscus*.

T. castaneum and *L. serricorne*. At the highest concentration (1023µl/l air), 85% and 100% mortality were recorded for *T. castaneum* respectively after 12 h and 24 h exposure whereas, the mortality rate for *L. serricorne,* was 100% for both exposure period (figure 1).

Probit analysis showed that *L. serricorne* was more susceptible to *P. lentiscus* essential oil than *T. castaneum*. The corresponding LC_{50} and LC_{95} were respectively 8.44 and 43.68 µl/l for *P. lentiscus* and 28.03 and 63.46µl/l for *T. castaneum* (table 2).

The LT_{50} values for *L. serricorne* ranged from 18.58 h for the lowest dose (114 µl/l air) to 7.21 h for the highest dose (341 µl/l air). With *T. castaneum*, the LT_{50} values ranged from 41.05 h to 14.79 h for the lowest and the highest doses respectively (table 3).

Table 2. LC_{50} and LC_{95} values of *P. lentiscus* essential oil against *T. castaneum* and *L. serricorne*.

	L. serricorne	*T. castaneum*
LD_{50}[a,b] (LC_{50})	8.44 (5.33-11.02)	28.03 (38.29-67.87)
LD_{95}[a,b] (LC_{95})	43.68 (30.01-94.72)	63.46 (48.56-105.97)
Slope ± SEM	2.30 ± 0.4	4.64 ± 0.69
Degree of freedom	10	10
χ^2	21.6	22.52

[a]Units LD_{50} and LD_{95} = µl/ air, applied for 24 h at 25 °C.
[b]95% lower and upper confidence limits are shown in parenthesis.

Discussion

P. lentiscus is an evergreen shrub with a strong smell and green leaves (Arista *et al*., 1990). It's widely distributed in the Mediterranean and Middle Eastern area. In Tunisia, it occurs and grows especially in North and North-western mountain region with different climatic stages (Bonnier and Douin, 1990). Our study has shown that the chemical composition of *P. lentiscus* obtained from Jebel Mansour (North Tunisia) is a limonene/pinene chemotype with 19.11% and 9.48% limonene and α-pinene respectively. The chemical variation of the essential oil could be due to many factors such as geographic areas, individual chemotypes, harvest time and plant part (Perry *et al*., 1999).

These results were different from those obtained in Tunisian essential oil by Ben Douissa *et al.* (2005) where only twenty seven compounds were identified. The α-pinene (17%), δ-terpinene (9%) and terpinene-4-ol (12%) were the main compounds.

Zrira *et al.* (2003) reported that in Moroccan essential oil, 45 constituents were identified where α-pinene, β-myrcene (10.2-11.5%) and limonene (6.8-9.8%); while terpinene-4-ol (32.7-43.8%), α-pinene (7.1-13.5%) and bornyl acetate (6.8-10.3%) were the main constituents. On the other hand, *P. lentiscus* essential oil from Italy present β-pinene (18.71%), β-phellandrene (12.83%) and β-caryophyllene (13.22%) as the dominating compounds (Congiu *et al.*, 2002). According to Fernandez *et al.* (2000), Spanish essential oil was characterized by the predominance of the sesquiterpenes hydrocarbons fraction. Furthermore, β-caryophyllene (13.1%), γ-cadinene (8.1%) and germacrene-D (6.8%) were the major compounds.

In this work, *P. lentiscus* essential oil demonstrated fumigant toxicity to *T. castaneum* and *L. serricorne*. It showed strong species-specific toxicity that was highly dependent upon the concentration and exposure time. The fact that the oil at concentrations of 114-227 µl/l air, was effective enough to achieve 52.5% mortality of *L. serricorne* within 24 h of exposure, and 100% mortality after 24 h exposure at concentration 682 µl/l air, offers practical potential in controlling this pest. *T. castaneum* was more resistant to fumigation; 80-100% mortality was achieved within 24 h exposure at concentrations of 910-1023 µl/l air. Results of our study compare favourably with other investigations in which *P. lentiscus* essential oil produced significant activity against pest insects. In this context, Traboulsi *et al.* (2002) indicated that significant effects were detected with the combination of *P. lentiscus* + *Mentha microphylla* C. Kock and *P. lentiscus* + *Myrtus communis* L. essential oils, where total mortality was achieved

Table 3. LT_{50} values of *P. lentiscus* essential oil against *T. castaneum* and *L. serricorne*.

Insect species	Concentration (µl per l air)	LT_{50} (h)[1]	LT_{90} (h)[1]	χ^2
T. castaneum	114	41.05 (37.51-46.21)	79.89 (65.44-121.73)	2.35
T. castaneum	227	21.44 (18.23-26.87)	75.95 (48.95-182.042)	5.98
T. castaneum	341	14.79 (12.27-18.65)	64.99 (38.70-113.40)	1.87
L. serricorne	114	18.58 (13.37-27.7)	51.46 (105.22-829.58)	1.82
L. serricorne	227	12.12 (10.85-16.95)	45.14 (54.02-540.13)	0.96
L. serricorne	341	7.21 (4.82-11.75)	36.60 (35.57-242.114)	2.46

[1]95% lower and upper confidence limits are shown in parenthesis.

against the mosquito *C. pipiens* when applied by contact. In addition, Pascual-Villalobos and Robledo (1998) indicated that *P. lentiscus* aerial parts extracts had a repellent effect on larvae of *T. castaneum*. *P. lentiscus* had index values over 50 indicating that larvae were repelled by the treated diet in comparison with the control after 2 and 24 hours. Furthermore, essential oil of another specie of *Pistacia* genus, *Pistacia terebinthus* L., had a low toxicity against the bean weevil *Acanthoscelides obtectus* Say (Papachristous and Stamopoulos, 2002). Therefore, it can be concluded that essential oil products are generally broad-spectrum, due to the presence of several active ingredients that operate via several modes of actions (Chiasson *et al*., 2004).

Fumigant toxicity of essential oil from different aromatic plants was investigated against the rust-red floor beetle and the cigarette beetle. *T. castaneum* was the less susceptible to essential oil from *Ocimum gratissimum* L. (Ogendo *et al*., 2008), *Vitex pseudo-negundo* (Hausskn.) (Sahaf *et al*., 2008), *Artemisia siberi* Besser (Negahban *et al*., 2006), *Lavandula angustifolia* Mill., *Rosmarinus officinalis* L., *Thymus vulgaris* L. and *Laurus nobilis* L. (Rozman *et al*., 2007). These results are similar to our finding that *T. castaneum* was tolerant to *P. lentiscus* essential oil. On the basis of the LC_{50} values, *P. lentiscus* essential oil from Jebel mansour (LC_{50} = 28.03 µl/l air), may be considered more toxic than *Vitex pseudo-negundo* essential oil (LC_{50} = 47.27µl/l air) (Sahaf *et al*., 2008). However, the essential oils of *Artemisia sieberi* Besser collected either from Qom province or Karaj (Iran) were more toxic to *T. castaneum* than *P. lentiscus* essential oil with respectively LC_{50} values of 16.76 and 20.31 µl/l air (Negahban *et al*., 2006; 2007).

Little work has been done to manage *L. serricorne* by using medicinal plants despite their excellent pharmacological actions (Namba, 1993). Bullington (1998) observed that the vapour toxicity of neem oil was efficient on *L. serricorne*. Moreover, biologically active constituents of *Foeniculum vulgare* Mill. essential oil : (E)-anethole, estragole and (+) Fenchone gave respectively 100, 90 and 60 % mortality at 0.105 mg cm^{-2} against *L. serricorne* adults (Kim and Ahn, 2001). In addition, essential oils from horseradish (*Cocholeria aroracia* L.), and mustard [*Brassica juncea* (L.)] used at the dose of 3.5 mg cm^{-2} were effective against *L. serricorne* adults (Kim *et al*., 2003). Our results demonstrated that *P. lentiscus* essential oil achieved 100% mortality against *L. serricorne* adults at the dose of 4.64 mg cm^{-2}.

Our research confirms the insecticidal proprieties of *P. lentiscus* essential oil as fumigant against the cigarette beetle.

Significant differences were observed in the time response mortality of *T. castaneum* exposed to the dose of 111.1 µl/l air of *V. pseudo-negundo* (LT50 = 13.43 hours) (Sahaf *et al*., 2008) compared to the dose of 114 µl/l air of *P. lentiscus* essential oil (41.05 hours). This result suggested that our tested essential oil was less toxic to *T. castaneum* than *V. pseudo-negundo*.

Conclusion

Tunisia is situated in Mediterranean basin region comprising largely of arid and semi-arid areas. It has many indigenous aromatic plants from different families. It therefore seems very worthwhile to conduct a comprehensive screening program to determine the insecticidal efficacy of such plants.

Our results demonstrated that the major constituents of *P. lentiscus* essential oil, primarily monoterpenes, are of particular interest to industrial markets due to their fumigant activity against the two stored- product beetles: *T. castaneum* and *L. serricorne* . Thus, the possibility of employing this natural fumigant to control insects in stored products may warrant further investigation.

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Authors' addresses: Olfa BACHROUCH (corresponding author, bachrouch_olfa@yahoo.fr), Manef ABDERRABA, Unité de Recherche de Physico-Chimie Moléculaire, IPEST, BP51, 2070 La Marsa, Tunisia; Jouda MEDIOUNI BEN JEMAA, Laboratoire de Protection des Végétaux, INRAT, 2049 Ariana, Tunisia; Thierry TALOU, Laboratoire de Chimie Agro-industrielle, Unité de recherche mixte INRA/INP.ENSIACET (UMR1010), 118 route de Narbonne, 31077 Toulouse Cedex France; Brahim MARZOUK, Unité de Plantes Aromatiques et Médicinales, Centre de Biotechnologie du Technopole de Borj Cedria, BP901, 2050 Hammam lif, Tunisie.

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