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Insecticidal Properties of *Peumus boldus* Mol. Essential Oil on the House Fly, *Musca domestica* L.

[Propiedades insecticidas del aceite esencial de *Peumus boldus* Mol. sobre la mosca doméstica, *Musca domestica* L]

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Abstract

The composition of the essential oil (EO) obtained by hydro distillation from dry leaves of *Peumus boldus* was analyzed using gas chromatography (GC) and gas chromatography/mass spectroscopy (GC/MS). The insecticidal activity of the oil against the house fly *Musca domestica* was evaluated by placing flies in a sealed glass jar containing a piece of EO-treated cotton yarn. The dose necessary to kill 50% of flies (LC_{50}) in 1 h was determined at 26±1°C. The essential oil from *Peumus boldus* showed potent insecticidal properties (LC_{50} = 6.26 mg/dm^3). According to GC and GC/MS analysis, 1,8-cineol (36.72%); *p*-cymene (26.79%); ascaridol (6.25%); sabinene (5.10%); 4-terpineol (4.39%); β-pinene (4.16%) and limonene (2.68%) were the principal components of the EO. The EO from *Peumus boldus* seems promising as a natural insecticide against houseflies. The peumus boldus essential oil reported in this paper is different to that reported in other publications. .The most important differences are the low content of ascaridol and the high content of both 1,8-cineol and *p*-cimene which can be attributed to the time of year and the geographic location of the samples plants.

Keywords: Musca domestica; natural insecticide; essential oil composition; *Peumus boldus*
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Resumen

La composición del aceite esencial (AE), obtenido por hidrodestilación de hojas secas de *Peumus boldus* se analizó mediante cromatografía de gases (CG) y cromatografía de gases / espectrometría de masas (CG / EM). La actividad insecticida del aceite contra la mosca doméstica, *Musca domestica* se evaluó colocando las moscas en un frasco de vidrio sellado con un trozo de hilo de algodón tratado con diferentes cantidades de AE. La dosis necesaria para matar el 50% de las moscas (LC_{50}) en 1 hora se determinó a 26 ± 1ºC. El aceite esencial de *Peumus boldus* mostró potentes propiedades insecticidas (LC_{50}= 6.26 mg/dm3). De acuerdo con los análisis de GC y CG / EM, 1,8-cineol (36,72%), *p*-cimeno (26.79%); ascaridol (6,25%); sabineno (5.10%), 4-terpineol (4,39%), β-pineno (4,16%) y limoneno (2,68%) fueron los componentes principales del AE. El AE de *Peumus boldus* parece prometedor como un insecticida natural contra moscas. La composición del aceite esencial de *Peumus boldus* encontrado en este trabajo es diferente a lo reportado en otras publicaciones. Las diferencias más importantes son el bajo contenido de ascaridol y el alto contenido de ambos 1,8-cineol y *p*-cimeno que puede atribuirse a la época del año y la ubicación geográfica de las plantas recolectadas.

Palabras claves Musca domestica; *Peumus boldus;* Composición del aceite esencial; Insecticida natural
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Introduction

Musca domestica L. (Diptera: Muscidae) is one of the most common insects associated with man. Flies are mechanical vectors of several human and animal diseases (Malik at al. 2007). Integrated pest management programs (IPM) seem to be a good alternative for the control of house flies. These programs combine different control methods that include the use of botanical insecticides (Tripathi et al., 1973; Malik et al., 2007). Among the botanical insecticides, plant essential oils (or their components) have been evaluated as they show a broad spectrum of biological activities including toxicity, repellant, oviposition and feeding deterrence (Isman and Machial 2006; Batish et al., 2008; Rosell et al., 2008).

In our continuing interest in the potential of essential oils (EOs) from Chilean flora as insecticides against *Musca domestica* (Urzúa et al., 2010), we present an evaluation of the insecticidal properties of a widespread species endemic to Central Chile, *Peumus boldus* Mol. (Boldo) (Riedeman and Aldunate 2001) . In addition to its status as an important species in large areas (Riedeman and Aldunate 2001) , a key factor in its selection was that powdered *P. boldus* leaves are an effective control of *Sitophilus zeamais* in stored grains (Silva et al., 2006).

EXPERIMENTAL

General

1,8-Cineole; limonene; α-pinene; β-pinene; Bornyl acetate; α-Terpineol and 4-terpineol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dimethyl-2,2-dichlorovinyl phosphate (DDVP) was provided as a gift by Professor H. Masuh from the Center of Investigation on Pests and Insecticides, CONICET, Argentina. The essential oil component analysis was performed using gas chromatography (GC) and gas chromatography/mass spectroscopy (GC/MS). Qualitative analysis was performed using a Hewlett-Packard 5891 gas chromatograph linked to a Hewlett-Packard 5972 mass spectrometric detector with an integrated data system (Hewlett Packard, Palo Alto, CA, USA); quantitative analysis was carried out using a Shimadzu GC-9A gas chromatograph fitted with a FID-9 detector (Shimadzu Corporation, Kyoto, Japan). The same capillary column (SPB-5, film thickness 0.25 μm, 30m x 0.25 mm, Supelco, Deerfield IL, USA) was used in both instruments.

Plant material

Leaves of *P. boldus* Mol. (Monimiaceae), were collected in November 2009 (spring) from Chillan (VIII Region, Chile). The leaves were dried in the shade at room temperature for 10 days. Voucher specimens were deposited in the Herbarium of the National Natural History Museum, Santiago, Chile

Essential oil extraction and analysis

Essential oil was extracted from 100 Kg of dry crushed leaves for 4 h by hydrodistillation in a semi pilot Clevenger-type apparatus. A sample of the EO was dried over anhydrous sodium sulfate. The EO component analysis was performed by gas chromatography (GC) and gas chromatography/mass spectroscopy (GC/MS) using the instrumentation described above. The operating conditions were as follows: on-column injection; injector temperature, 250°C; detector temperature, 280°C; carrier gas, He at 1.25 ml/min; oven temperature program: 35 ºC for 5 min, increase to 260 ºC at 5 ºC/min, and then 260º C for 5 min. The mass detector ionization employed an electron impact of 70 eV. Recording conditions employed a scan time of 1.5 s and a mass range of 50 to 500 amu. Compounds in the chromatograms were identified by comparison of their mass spectra with those in the NIST 2002 library database, and by comparison of their retention index with those reported in the literature (Adams,2007), for the same type of column or those of commercial standards, when available.

Fly collection and maintenance

The colonies of *M. domestica* used in this study originated from adults collected in the experimental field of the Universidad Católica of Córdoba, in Córdoba, Argentina, using a sweep net. The flies were transferred to a small cage and then reared in entomological cages (30×30×30 cm) at 26 (±1) °C under a 12:12 light:dark cycle and 70% humidity. Adult flies were provided with water and fed a 1:1 (v/v; approximately) mixture of granulated sugar and powdered milk. Bran and milk were prepared at a

weight ratio of 1:3 and 100 g of this mixture was placed on a plastic plate as an oviposition site.

Bioassay

The bioassay was designed so the flies would have high probability of coming into contact with volatile compounds within the one hour test period; therefore, the flies were allowed access to the total space within the exposure vessel. Ten 4-5 day old adult house flies, of both sexes, were placed in a glass jar (1.2 dm^3) fitted with a screw cap that had a 7-cm length of cotton yarn suspended from the center of its inner face. Different dosages of pure EO (without solvent) were applied to the yarn. The control vessel had no compound on the cotton yarn. The jars were sealed tightly and maintained at temperature of 26 ± 1 °C. Each test was repeated three times. The assay was also conducted with the cotton yarn enclosed in a breathable cloth bag to prevent direct contact. Dimethyl 2, 2-dichlorovinyl phosphate (DDVP), a volatile organophosphate, was used as a positive control. Mortality in each group was assessed after one hour of exposure.

Data analysis

The mean mortality data of the three repeated assays per dose (4-6 doses) was used to calculate the LC_{50}. Probit analysis (Harvard Programming; Hg1, 2) was used to analyze the dose-mortality response.

Results and Discussion

From the dry leaves of *P. boldus* (100 Kg), 2.00 Kg (2%) of EO was obtained. The composition of the EO is listed in Table 1. 1,8-Cineol (36.72%) (**1**); *p*-cymene (26.79%) (**2**); ascaridol (6.25%) (**3**); sabinene (5.10%) (**4**); 4-terpineol (4.39%) (**5**); β-pinene (4.16%) (**6**) and limonene (2.68%) (**7**), were the principal components of *P. boldus* EO.

Table 1 Composition of the essentials oil of leaves of *Peumus boldus*

Compound	R.I	(%)	Identification
α-Pinene	938	4.16	RI, MS, Co-I
Camphene	940	0.52	RI, MS
Sabinene (**4**)	973	5.10	RI, MS
β-Pinene (**6**)	993	1.74	RI, MS, Co-I
3-Carene	1015	1.24	RI, MS
p-Cymene (**2**)	1024	29.79	RI, MS, Co-I
Limonene (**7**)	1029	2.68	RI, MS, Co-I
1,8-Cineol (**1**)	1036	36.62	RI, MS
Linalool	1105	1.51	RI, MS
Fenchol	1126	1.09	RI, MS
4-Terpineol (**5**)	1158	4.39	RI, MS, Co-I
α-Terpineol	1165	1.93	RI, MS, Co-I
Myrtenal	1169	tr	RI, MS
Bornyl acetate	1259	tr	RI, MS, Co-I
Ascaridol (**3**)	1273	6.25	RI, MS
β-Elemene	1371	tr	RI, MS
Methyleugenol	1373	tr	RI, MS
trans-Caryophyllene	1393	tr	RI, MS

RI: Retention index; MS: Mass spectrum; Co-I: standard; tr: trace amounts (< 0.03%).

The composition of the *Peumus boldus* essential oil reported in this paper is different to that reported in other publications. (Bittner et al., 2008; Miraldi et al., 1996; Niemeyer and Teiller, 2007; Vogel et al., 1997).The most important differences are the low content of ascaridol and the high content of both 1,8-cineol and *p*-cymene that can be attributed to the time of year and the geographic location of the sampled plants (Shoonhoven et al., 2005).

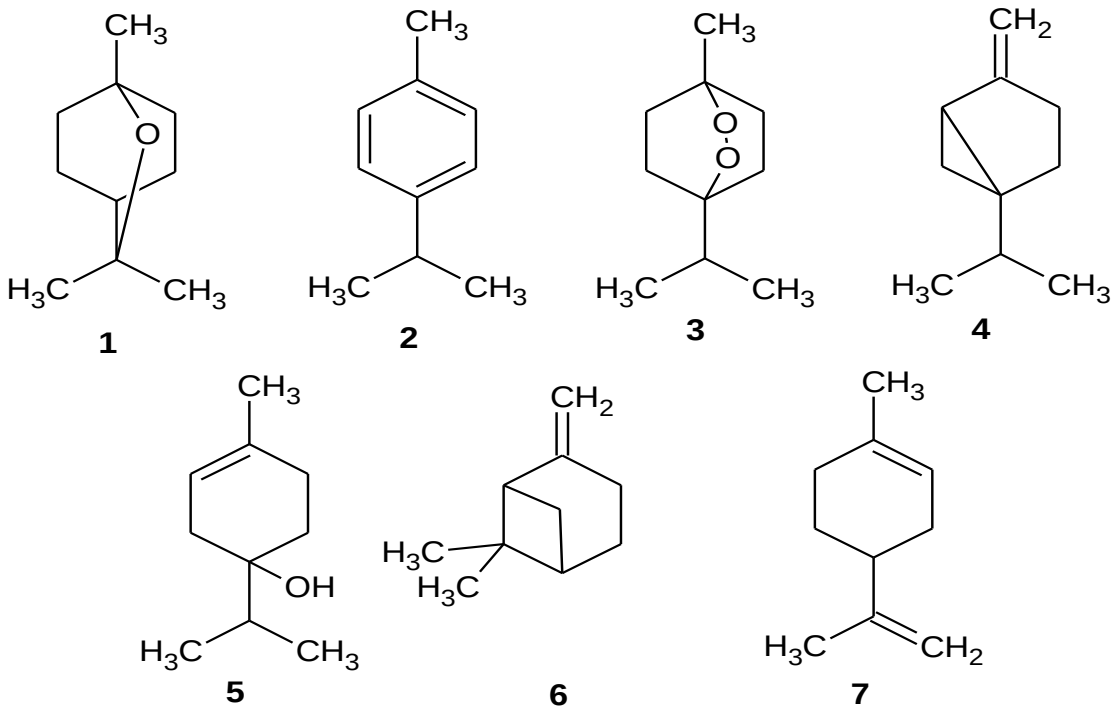

Figure 1: Principal terpenoids in the essential oil of *Peumus boldus*

In addition a direct comparison is difficult, with the exception of data reported by Niemeyer (2007), as the other authors did not report retention indices (RI) and the component identification of essential oils made using the GC-MS equipment database should only be considered as tentative (Adams, 2007).

The fumigant effects of EO against adult *M. domestica* were evaluated by determining the LC_{50} values, which are presented in Table 2.

Table 2: LC_{50} of essential oil against *Musca domestica*

Essential oil	Mean LC_{50} in mg/dm^3 (95%CI)
Peumus boldus	6.26 (2.69 – 14.54)

Time : 1 h; t : 26 ± 1º C

The EO from *P. boldus* contains 36.72% of 1,8-cineol. The insecticidal properties of some monoterpenoids have been determined using the same bioassay, and the LC_{50} in a 0.5 h experiment were 3.35 mg/dm^3 for 1,8-cineol, 12.1 mg/dm^3 for α-pinene, 6.2 mg/dm^3 for (4*R*)(+)-limonene, 36.8 mg/dm^3 for 4-terpineol and 5.0 mg/dm^3 for (4*S*)(-)-limonene (Palacios et al., 2009). The insecticidal properties of an essential oil may be related in principle to its individual components. The proportion of 1,8-cineole in the *P. boldus* EO was

36.72%, which means the LC_{50} dose of *P. boldus* EO contains approximately 2.3 mg of 1,8-cineole. This suggests that 1,8-cineole is the primary active component in *P. boldus* EO, however, the possibility of synergistic/antagonistic effects cannot be ruled out.

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