

Insecticidal Effect of *Schinus latifolius* Essential Oil on the Housefly, *Musca domestica* L.

[Efecto Insecticida del Aceite Esencial de *Schinus latifolius* en Mosca Doméstica, *Musca domestica*]

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Abstract

The composition of essential oil (EO) from *Schinus latifolius* obtained by hydro distillation of dry leaves was analyzed using gas chromatography (GC-FID) and gas chromatography/mass spectroscopy (GC/MS). The insecticidal effect of the oil on 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 (LC₅₀) in 0.5 and 1 h was determined at 26±1°C. The essential oil from *Schinus latifolius* showed significant insecticidal properties [LC₅₀ = 31.98 mg/dm³ (0.5 h) and LC₅₀ = 19.20 mg/dm³ (1 h)]. According to GC-FID and GC/MS analysis a total of 54 compounds were identified accounting for 99.45% of the EO, with limonene (50.23%); α-pinene (15.01%); β-pinene (11.81%); sabinene (4.71%) and α-thujene (2.18%) as the main components. The EO from *Schinus latifolius* appears promising as a natural insecticide against houseflies. The composition of the *Schinus latifolius* essential oil reported in this study is different to that reported in other publications. The most important characteristic is the high content of limonene (50.23%), which can be attributed to the time of year and the geographic location of the sampled plants.

Keywords: *Musca domestica*; *Schinus latifolius*; essential oil composition; natural insecticide; antagonist effect among monoterpenes

Resumen

La composición del aceite esencial (AE), obtenido por hidrodestilación de hojas secas de *Schinus latifolius* se analizó mediante cromatografía de gases (CG-FID) 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 (LC₅₀) en 0,5 y 1 hora se determinó a 26 ± 1°C. El aceite esencial de *Schinus latifolius* mostró un buen efecto insecticida [DL₅₀ = 31,98 mg/dm³ (0,5 h) y DL₅₀ = 19,20 mg/dm³ (1 h)]. De acuerdo con los análisis de GC-FID y CG/EM, un total de 54 compuestos fueron identificados lo que representa el 99,45% del AE, siendo limoneno (50,23%); α-pineno (15,01%); β-pineno (11,81%); sabineno (4,71%) y α-tujeno (2,18%) los componentes principales del AE. El AE de *Schinus latifolius* parece prometedor como un insecticida natural contra la mosca doméstica. La composición del aceite esencial de *Schinus latifolius* encontrado en este trabajo es diferente al informado en otras publicaciones. La característica mas importante es el alto contenido de limoneno (50,23%), que podría atribuirse a la época del año y la ubicación geográfica de las plantas recolectadas.

Palabras Clave: *Musca domestica*; *Schinus latifolius*; composición del aceite esencial; insecticida natural; efecto antagonístico entre monoterpenos

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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 *et al.*, 2007).

Many insecticides have been used for housefly control, however, their use can affect human health, agricultural ecosystems and the environment in general (Reed, 2002).

In addition, houseflies develop resistance to insecticides and one of the major mechanisms of resistance is a change in the target site (Kozaki *et al.*, 2009).

Integrated pest management programs (IPM) appear 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 botanical insecticides, plant essential oils (or their components) have been evaluated as they show a broad spectrum of biological activity including toxicity, repellent, oviposition and feeding deterrence (Isman, 2001; Isman and Machial, 2006; Batish *et al.*, 2008; Rosell *et al.*, 2008; Palacios *et al.*, 2009a; Kumar *et al.*, 2011). In our continuing interest in the potential of essential oils (EO's) from Chilean flora as insecticides against *Musca domestica* (Urzúa *et al.*, 2010a; Urzúa *et al.*, 2010b), we present an evaluation of the insecticidal property of a widespread species endemic to Central Chile, *Schinus latifolius* (Gilles ex Lindl.) Engler, (Riedeman and Aldunate, 2001). In addition to being a medicinal plant with antiseptic properties (Muñoz *et al.*, 1981), a key factor in its selection was that the citrus-scented leaves of *S. latifolius* are used to repel insects and that the EO extracted from leaves of the close *Schinus molle* L. is a well known house fly repellent (Wimalaratne *et al.*, 1996) with proven insecticidal properties (Palacios *et al.*, 2009a).

EXPERIMENTAL

General

Limonene; α -pinene; β -pinene; bornyl acetate; α -terpineol; 4-terpineol; 1-octanol and octanoic acid 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-FID) and gas chromatography/mass spectroscopy (GC/MS). Qualitative analysis was performed using a Thermo Scientific Trace GC Ultra linked to a ISQ quadrupole mass spectrometric detector with an integrated data system (Xcalibur 2.0, Thermo Fisher Scientific Inc. 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 (Rtx-5MS, film thickness 0.25 μ m, 60m x 0.25 mm, Restek Corporation, Bellefonte, PA. USA) was used in both instruments.

Plant material

Leaves of *Schinus latifolius* were collected from Huaquén del Mar (V Región, Chile, 32° 18' 49.57"S, 71° 28' 16.03"W) at an altitude of 35 m over the average sea level during the flowering season, November 2010. Voucher specimens were deposited in the Herbarium of the National Natural History Museum, Santiago, Chile. The leaves were dried in an oven with circulating air at 40° C for 24 h.

Essential oil extraction and analysis

Essential oil was extracted from 387 g of dry milled leaves for 4 h by hydro distillation (2.5 L, H₂O) in a Clevenger-type apparatus. The EO was dried over anhydrous sodium sulfate. The EO component analysis was performed by gas chromatography (GC-FID) 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: 40 °C for 5 min, increase to 260° C at 5° C/min, and then 260° 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 40 to 400 amu. Compounds in the chromatograms were identified by comparison of their mass spectra with those in the NIST08 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 dm³) 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 LC₅₀. Probit analysis (Harvard Programming; Hg1, 2) was used to analyze the dose-mortality response.

RESULTS AND DISCUSSION

From the dry leaves of *S. latifolius* (387g), 1.33 g (0.34%) of EO was obtained. The composition of the EO is listed in Table 1. Limonene (50.23%)(1); α -pinene (15.01%)(2); β -pinene (11.81%)(3); sabinene (4.71%)(4) and α -thujene (2.18%)(5) were the principal components of *S. latifolius* EO.

Table 1: Composition of the essential oil of leaves of *Schinus latifolius*.

Compound	RI	%	Identification	Compound	RI	%	Identification
2-Hexanal	860	0,05	RI, MS	5-Methyl-3-hexen-2-one	1334	0,08	RI, MS
Tricyclene	929	0,20	RI, MS	α -Copaene	1394	0,44	RI, MS
α -Thujene (5)	934	2,18	RI, MS	Decyl acetate	1412	0,22	RI, MS
α -Pinene (2)	943	15,01	RI, MS, Co-I	Dodecanal	1414	0,13	RI, MS
Camphene (7)	958	1,16	RI, MS	(E)-Caryophyllene	1445	0,10	RI, MS
Sabinene (4)	982	4,71	RI, MS	<i>trans</i> - α -Bergamotene	1452	0,05	RI, MS
β -Pinene (3)	986	11,81	RI, MS, Co-I	2-Dodecenal	1479	0,36	RI, MS
β -Myrcene	994	0,82	RI, MS	Aromadendrene	1487	0,42	RI, MS
δ -3-Carene	1018	0,13	RI, MS	Germacrene D	1505	0,29	RI, MS
α -Terpinene	1024	0,07	RI, MS	α -Muurolene	1521	0,41	RI, MS
ρ -Cimene	1034	1,55	RI, MS	NI	1532	0,09	
Limonene (1)	1040	50,23	RI, MS, Co-I	γ -Cadinene	1538	0,18	RI, MS
β -Ocimene	1054	0,09	RI, MS	δ -Cadinene	1544	1,02	RI, MS
γ -Terpinene	1067	0,08	RI, MS	δ -Amorphene	1560	0,06	RI, MS
1-Octanol	1074	0,08	RI, MS, Co-I	Elemol	1571	0,06	RI, MS
Terpinolene	1096	0,06	RI, MS	β -Calacorene	1589	0,07	RI, MS
NI	1333	0,12		Viridiflorol	1598	0,05	RI, MS
<i>trans</i> -Pinocarveol	1154	0,09	RI, MS	Spathulenol	1607	0,56	RI, MS

Octanoic acid	1174	0,52	RI, MS, Co-I	Ledol	1615	0,08	RI, MS
4-Terpineol	1190	1,09	RI, MS, Co-I	Guaiol	1623	0,28	RI, MS
<i>p</i> -Cymen-8-ol	1195	0,05	RI, MS	NI	1627	0,24	
Myrtenal	1212	0,13	RI, MS	Cubenol	1657	0,06	RI, MS
NI	1238	0,10		α -Eudesmol	1661	0,07	RI, MS
Carvone	1259	0,07	RI, MS	τ -Cadinol	1668	0,79	RI, MS
1-Decanol	1276	0,55	RI, MS	α -Cadinol	1682	1,30	RI, MS
2,5-Dimethyl-2,5-hexanediol	1284	0,23	RI, MS	Bulnesol	1695	0,11	RI, MS
Bornyl acetate (6)	1299	0,59	RI, MS	Guaiol acetate	1704	0,15	RI, MS

RI: Retention index; MS: Mass spectrum; Co-I: standard; NI: not identified.

As far as we can determine, the composition of only two EO samples from *Schinus latifolius* have been investigated. Barroso et al., 1991, reported as major compounds: β -pinene (35%) (3), sabinene (24%) (4) and α -pinene (21%) (2) and in the sample studied by Niemeyer and Teiller, 2007, they reported: β -pinene (24.2%) (3), bornyl acetate (19.7%) (6), α -pinene (16.6%) (2) and camphene (19.7%) (7). The

composition of the *Schinus latifolius* essential oil found in this study is different to that previously reported. The most important differences are the high content of limonene (50.23%) (1) and the low content of sabinene (4), bornyl acetate (6) and camphene (7), which can be attributed to the time of year and the geographic location of the sampled plants (Shoonhoven et al., 2005).

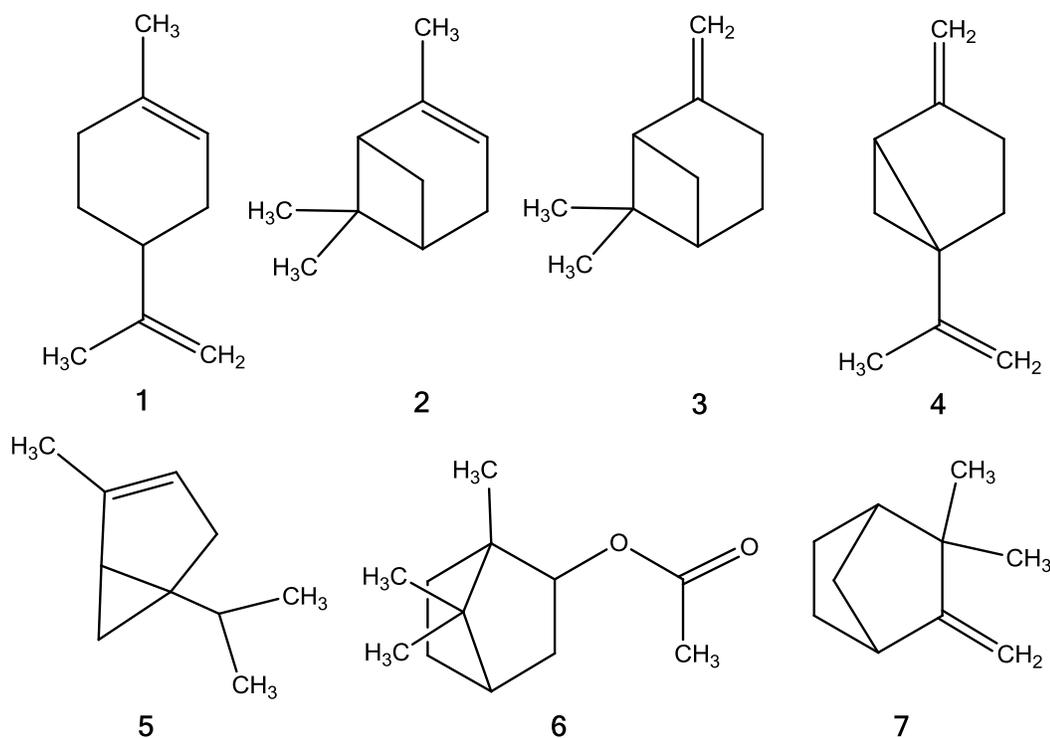


Figure 1: Principal monoterpenoids in the essential oil of *Schinus latifolius*

The fumigant effects of EO against adult *M. domestica* were evaluated by determining the LC₅₀ values, which are presented in Table 2.

Table 2: LC₅₀ of *Schinus latifolius* essential oil on *Musca domestica*

Time (h)	Mean LC ₅₀ in mg/dm ³ (95%CI)
0.5	31.98 (10.34–98.96)
1	19.20 (7.71–47.67)

Time: 1 h; t: 26 ± 1° C

The insecticidal properties of some monoterpenoids have been determined using the same bioassay, in which the LC₅₀ in a 0.5 h experiment was 12.1 mg/dm³ for α-pinene, 6.2 mg/dm³ for limonene and 6.4 mg/dm³ for β-pinene (Palacios et al., 2009b). The insecticidal properties of an essential oil may be related in principle to its individual components. The proportion of limonene in the *S. latifolius* EO was 50.23%, which means the LC₅₀ dose of *S. latifolius* EO (31.98 mg/dm³) contains approximately 16 mg of limonene. This amount is around 2.6 times higher than the LC₅₀ of pure limonene (6.4 mg/dm³). These results demonstrate that the insecticidal property of limonene is affected through an antagonistic mechanism probably produced by other major monoterpenes present in the *S. latifolius* essential oil.

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