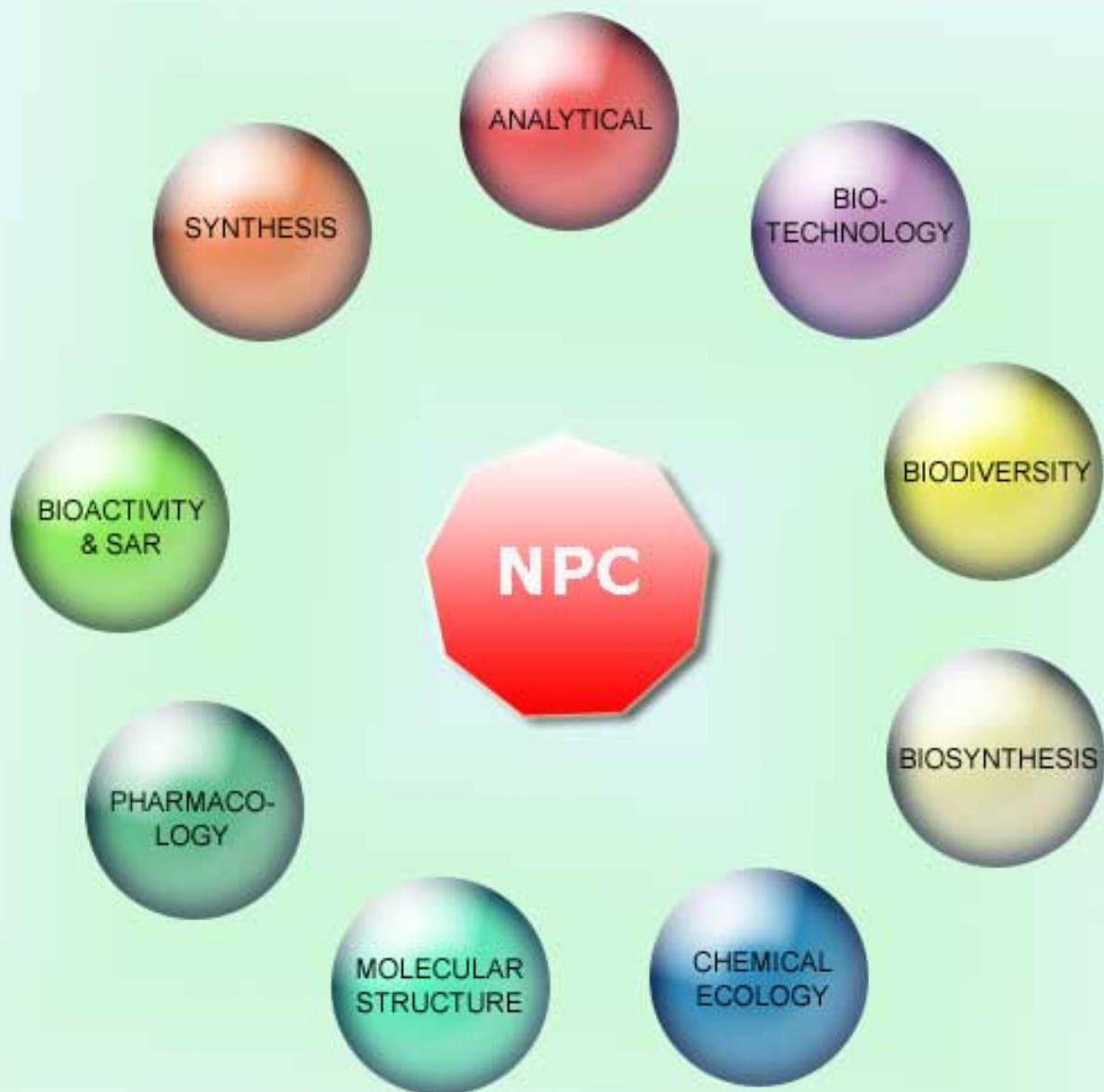


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Insecticidal and Insect-repellent Activities of Essential Oils from Verbenaceae and Anacardiaceae Against *Rhizopertha dominica*

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Essential oils extracted from leaves of *Aloysia polystachya* and *A. citriodora* (Verbenaceae) and from leaves and fruits of *Schinus molle* var. *areira* (Anacardiaceae) were tested for their repellent and toxic activities against adults of *Rhizopertha dominica* (Coleoptera: Bostrichidae). Topical application and filter paper assays were employed for contact toxicity studies; filter paper impregnation was also used for fumigant and repellent assays. In topical tests *A. polystachya* was as effective as *S. molle* leaves. In the case of repellent assays, *A. citriodora* was the most effective oil based on the class scale. *A. polystachya* was the most toxic plant on contact toxicity by filter paper assay (LC_{50} 26.6 mg/cm²). Fumigant toxicity was only evaluated with fruits and leaves of *S. molle*, and no significant differences were found between them. Published data are included to compare the fumigant toxicity of *S. molle* with that of *A. citriodora* and *A. polystachya*.

Keywords: *Aloysia polystachya*, *Aloysia citriodora*, *Schinus molle*, lesser grain borer, fumigant toxicity, contact toxicity, repellency.

Chemical control has been used for a long time against stored grain pests, but this has had serious drawbacks. The indiscriminate use of chemical pesticides has given rise to many serious problems, including genetic resistance of pest species, toxic residues, increasing costs of application, and environmental pollution [1a]. There is an urgent need for safe but effective, biodegradable pesticides. Higher plants may provide new sources of natural pesticides, safe to apply, easily processed, potentially less expensive and of broad spectrum [1b].

The genus *Aloysia* includes approximately 200 species of herbs, shrubs and small trees [1c]. They are distributed throughout South and Central America and tropical Africa. Most are traditionally used as gastrointestinal and respiratory remedies, and as insect control agents [2a-2e]. *A. polystachya* is a shrub with aromatic leaves, native of Argentina. The infusions have tonic, carminative and digestive properties, indicated for stomach aches and slow

digestion [2a]. *A. citriodora* is a shrub used as an analgesic, anti-inflammatory, sedative, diuretic and antispasmodic. It is distributed in North Carolina, California, Central and South America, Spain, India and China [1c]. *Schinus molle*, an evergreen tree, native in tropical and sub-tropical America, is cultivated as an ornamental and for its antipyretic, anti-inflammatory and diuretic properties [3a].

The lesser grain borer, *R. dominica*, is arguably the most important insect pest of stored grains worldwide, causing large economic losses [3b]. This pest has displaced *Sitophilus oryzae* in wheat, due to the fact that it is able to develop in drier environments.

The present study is part of a research program on bioactive compounds and their use as insect control agents. Here we report the composition of the essential oils of *A. polystachya*, *A. citriodora* and *S. molle*; the contact toxicity and repellence of the

Table 1: Major identified constituents of *A. polystachya*, *A. citriodora* and *S. molle* essential oils and their relative proportions in the oils.

<i>A. citriodora</i>		<i>A. polystachya</i>	
Constituents	corr.%	Constituents	corr.%
Citronellal	51.3	Carvone	83.5
Sabinene	22.9	Limonene	16.5
α -Cucumene	9.6		
Limonene	7.4		
Caryophyllene	2.4		
α -Pinene	2.3		
γ -Cedrene	2.3		
<i>p</i> -Cymene	1.8		
<i>S. molle</i>			
Leaves		Fruits	
Constituents	corr.%	Constituents	corr.%
Limonene	15.7	Limonene	40.3
α -Phellandrene	13.8	α -Phellandrene	24.5
Elemol	9.0	β -Myrcene	16.3
β -Cubebene	7.3	1-Terpinen-4-ol	3.3
Camphepane	5.3	α -Pinene	3.0
δ -Cadinene	5.3	β -Phellandrene	2.4
γ -Eudesmol	3.6	Caryophyllene	1.6
α -Pinene	3.6	3-Carene	1.3
β -Eudesmol	2.8	Methyl octanoate	1.3
β -Pinene	2.1	Camphepane	1.2
β -Myrcene	1.7	β -Pinene	0.6
Sabinene	1.5	2-Carene	0.5
Caryophyllene	1.4	α -Humulene	0.2
Tricicleine	0.8	Copaene	0.1
Bornyl acetate	0.8		

leaf essential oils of *A. polystachya* and *A. citriodora*; of the leaf and fruit oils of *S. molle* against *R. dominica*; and the fumigant toxicity of *S. molle*.

Gas chromatography-mass spectrometry revealed that the essential oil from leaves of *A. polystachya* contain mainly monoterpenoids as limonene (16.5%) and carvone (83.5%). *A. citriodora* is characterized by the presence of citronellal (51.3%) and sabinene (22.9%) as the major components; and the fruit and leaf oils of *S. molle* contain limonene and α -phellandrene, but in different concentrations (Table 1).

In contact toxicity bioassays, the essential oils from leaves of *S. molle* and *A. polystachya* showed the highest effect against adults of *R. dominica*, with LD₅₀ values of 0.88 and 0.61 mg/cm², respectively (Table 2). Similar data were obtained earlier with the essential oil of *Curcuma longa* against *R. dominica* [4a], and with the major components of the essential oils of *Ocimum kilimandscharicum* and *O. kenyense* against the lesser grain borer [4b].

Table 3 shows the average repellency values for each of the tested oils. The essential oil of *A. citriodora* at 120 and 314 μ g/cm² showed the highest repellent activity against *R. dominica*, based on class scale. On the other hand, the essential oils from fruits and leaves of *S. molle* significantly differed ($p < 0.05$) in their repellent effect at hours two and four after treatment, respectively. We found a moderate

Table 2: Contact toxicity of *A. polystachya*, *A. citriodora* and *S. molle* essential oils determined by topical application to *R. dominica*.

EO	PP	LD ₅₀	95% CI	LD ₉₅	95% CI	Slope \pm SE	X ²
(1)	leaves	0.61	(0.02-1.6)	78.7	(25.2-4893)	0.7 \pm 0.2	6.2
(2)	leaves	3.57	(2.2-4.2)	7.1	(5.9-11.9)	5.4 \pm 1.5	0.06
(3)	leaves	0.88	(0.1-1.6)	36.1	(12.2-2342)	1.02 \pm 0.3	0.9
	fruits	8.88	(5.3-14.4)	115	(39.1-37472)	1.4 \pm 0.5	0.3

Numbers in the same column followed by the same letters do not differ significantly.

LD₅₀: Lethal Dose 50 (mg/cm²); LD₉₅: Lethal Dose 95 (mg/cm²); CI 95%: Confidence Interval of 95%; SE: Standard error. EO: essential oils, PP: plant part used. (1) *A. polystachya* (2) *A. citriodora* (3) *S. molle*.

Table 3: Average repellency of *A. polystachya*, *A. citriodora* and *S. molle* essential oils to adults of *R. dominica*.

Plant	C	Average repellency rate (PR) ¹					MR	RC
		1	2	3	4	5		
<i>A. polystachya</i>	90	33a	33a	40a	47a	20a	34.6	II
	120	33a	40a	60a	27a	73a	46.6	III
	314	33a	13a	53a	40a	53a	38.4	II
<i>A. citriodora</i>	90	80a	50a	40a	50a	20a	48	III
	120	87a	80a	70a	70a	53a	72	IV
	314	80a	80a	80a	80a	80a	80	IV
<i>S. molle</i> (l)	90	53a	60a	53a	67b	40a	54.6	III
	120	67a	40a	47a	13a	27a	38.8	II
	314	60a	-20a	-27a	0a	20a	6.6	I
<i>S. molle</i> (f)	90	53a	60a	53a	67b	40a	54.6	III
	120	67a	40a	47a	13a	27a	38.8	II
	314	60a	-20a	-27a	0a	20a	6.6	I

Numbers in the same column of each plant followed by the same letters do not differ significantly in ANOVA test.

¹ PR%: [(N_c-N_t)/(N_c+N_t)] x 100

C: concentration (μ g/cm²); RC: repellency class; MR: Mean repellence.

repellent effect for *A. polystachya* and *S. molle* (PR \leq 60); something similar was found with *A. polystachya* against *S. oryzae* [5], and with *S. molle* against *T. confusum* [3a]. *A. polystachya* showed the highest toxicity against *R. dominica* in impregnated filter paper contact bioassays (Table 4).

Essential oils from fruits and leaves of *S. molle* had fumigant toxicity adequate to kill 50% of the insects at concentrations between 0.6 and 0.8 mg/cm². Based on LC₅₀ values, there were no significant differences between them. However, the essential oils of *A. polystachya* and *A. citriodora* significantly differed from those of *S. molle* [6a] (Table 5). More studies must be undertaken to elucidate the differences found. These results are in agreement with those obtained with essential oils of *Ocimum basilicus*, *Carum carvi* and *Coriandrum sativum* against adults of *R. dominica* [6b], and using the essential oil of *S. molle* against *Nezara viridula* [6c]. On the basis of our bioassay results it can be concluded that: (1) the essential oils of *A. polystachya*, *A. citriodora* and *S. molle* had insecticidal and repellent effects against *R. dominica*; (2) these essential oils were toxic by penetrating the insect body through the spiracles and

Table 4: Contact toxicity to *R. dominica* of *A. polystachya*, *A. citriodora* and *S. molle* essential oils, determined by impregnated filter paper assay.

EO	MP	LC ₅₀	95% CI	LC ₉₅	95% CI	Slope ± SE	X ²
(1)	leaves	26.5	(24.1- a 28.8)	43.8	(38.7- 54.09)	7.55±1. 1	0.7
(2)	leaves	216b	(181- 249)	447	(369- 620)	5.2±0.8	4.7
(3)	leaves	41.2	(16.18- c 68.65)	888	(309.9- 36961)	1.23±0. 36	0.7
	fruits	236.	(191- 4 d 275)	464	(386- 639)	5.61±1. 02	0.7

Numbers in the same column followed by the same letters do not differ significantly.

LC₅₀: Lethal Concentration 50 (mg/cm²) ; LD₉₅: Lethal Concentration 95 (mg/cm²) ; CI 95%: Confidence Interval of 95%; SE: Standard error. EO: essential oils, MP: material plant. (1) *A. polystachya* (2) *A. citriodora* (3) *S. molle*.

Table 5: Fumigant toxicity to adults of *R. dominica* of *A. polystachya*, *A. citriodora* and *S. molle* essential oils.*

EO	MP	LC ₅₀	95% CI	LC ₉₅	95% CI	Slope ± SE
(1)	leaves	0.2a	(0.2-0.3)	1.6	(0.8- 31.1)	2.15±0.6
(2)	leaves	0.2a	(0.1-0.2)	1.3	(0.6- 30.1)	2.11±0.7
(3)	leaves	0.6b	(0.4-1.3)	8.3	(2.7- 744)	1.48±0.4
	fruits	0.8b	(0.5-4.6)	4.1	(1.5- 1535)	2.34±0.8

Numbers in the same column followed by the same letters do not differ significantly.

LC₅₀: Lethal Concentration 50 (mg/cm²) ; LC₉₅: Lethal Concentration 95 (mg/cm²) ; CI 95%: Confidence Interval of 95%; SE: Standard error.

EO: essential oil, MP: material plant. (1) *A. polystachya* (2) *A. citriodora* (3) *S. molle*.

*data published in the Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas.

the integument; (3) of the plant species tested, *A. polystachya* showed the highest toxicity against *R. dominica*.

Experimental

Insects: *R. dominica* (F.), an insecticide normal-susceptible strain, was maintained in glass flasks at 28±1°C and 70–80% r.h. in the dark, and reared on whole wheat. Adults of both sexes and 3- 4 days old, were used in toxicity and repellent assays.

Plant material: *Aloysia polystachya* (Griseb.) Moldenke was collected during the summer period at Lamarque city (39° 24' lat. 65° 42' long.), Río Negro Province, Argentina (Herbarium Voucher Number, BBB MGM 452). *A. citriodora* Palau was collected in Bahía Blanca (38° 44' lat. 62° 16' long.), Buenos Aires province, Argentina (Herbarium Voucher Number, BBB MGM 480), and leaves and fruits of *Schinus molle* L. var. *areira* (L.) DC. were collected in Bahía Blanca (38° 44' lat. 62° 16' long.), Buenos Aires province, Argentina (Herbarium Voucher Number, BBB CV 10444). Essential oils were extracted from fresh samples using a Clevenger-type apparatus by hydro-distillation for 4 h. Essential oils

were dried over anhydrous sodium sulfate and stored in the dark at 4°C in nitrogen. Plant oil yields were 0.54% (w/w) for *A. polystachya*, 0.22% (w/w) and 0.42% (w/w) for leaves and fruits of *S. molle*, respectively, and 0.34% (w/w) for *A. citriodora*.

Essential oils: Chemical composition of the essential oils was determined by gas chromatography-mass spectrometry (GC-MS). GC analysis was performed with a Hewlett-Packard 5890 Series II gas chromatograph, equipped with a HP-5972 (EI-70eV) mass selective detector and capillary column HP-5MS (25 m × 0.25 mm i.d., 0.25 µm film thickness). The temperature of the injection block was 250°C. The GC oven temperature was held at 50°C for 2 min, programmed at 5°C min⁻¹ to 200°C and then held at this temperature for 10 min. The FID detector temperature was set at 300°C. Helium was used as carrier gas with a flow rate of 1 mL min⁻¹. Aliquots of the essential oils were dissolved in diethyl ether for the analysis. Oil components were identified by comparison of their retention indices (Kovats Indices) with those of known compounds and also by comparison of their mass spectra with those stored in the MS data base (NBS75K.LMS DATA).

Bioassay: The repellency test was conducted according to [7a]. Papers (Whatman No 1, diameter 9 cm) were divided into two halves. One was impregnated with 0.5 mL of either the essential oil in *n*-hexane (treated) or *n*-hexane (control). The concentrations evaluated were 90, 120 and 314 µg/cm². Paper disks were air dried for 1 h and then placed inside a glass Petri dish (diameter 9 cm). In different replicates, the orientation of the Petri dish was changed to avoid the effects of any external directional stimulus affecting the distribution of the insects. Ten adult insects were released in the middle of each filter paper disk and then covered with a plastic tape with some holes to prevent the insects from escaping. Five independent replicates were set up. All the experiment was performed at 28±1°C and 70–80% r.h., in the dark. The number of insects on each half of the paper was counted at hourly intervals for 5 h. The percentage of repellency (PR) was calculated using the formula of [7a], PR= [(N_c-N_t)/(N_c+N_t)] × 100, where N_c is the number of insects found on the control zone and N_t is the number of insects found on the treated zone. Positive values expressed repellency and negative values attractancy. Data (PR) were analyzed using ANOVA and DMS. The average values were then categorized according to the following scale [7b]:

class	Repellency rate (%)		Repellency rate (%)
0	>0.01 to <0.1	III	40.1 to 60
I	0.1 to 20	IV	60.1 to 80
II	20.1 to 40	V	80.1 to 100

The contact activity of the essential oils of *A. polystachya*, *A. citriodora* and *S. molle* against adults of *R. dominica* were determined in an impregnated-paper assay and by topical application. To determine toxicity using the filter paper assay, aliquots of 0.7 mL of the essential oils were applied to filter papers (Whatman Nº 1.9 cm diameter). The solvent (*n*-hexane) was allowed to evaporate for 10 mins. Each filter paper was then placed inside a glass Petri dish with 10 adults per concentration. The insects were covered with a plastic tape with some holes to prevent them from escaping and kept at 29°C, 70% r.h and in darkness. Five independent replicates per concentration and control treatments were performed. Mortality was recorded after 24, 48 and 72 h. For topical application, aliquots of 0.2 µL per insect were applied ventrally to the thorax of adults using a micro applicator. Controls were determined using *n*-hexane. Ten insects were used for each concentration and

control. Five independent replicates were conducted. Both treated and control insects were then transferred to glass vials (10 insects per vial) and kept in incubators at 29 ± 1°C, 70% r. h. and in darkness. Mortality was observed after 24 h. Probit analysis was used to estimate LD₅₀ and LD₉₅ values by Micro Probit 3.0. To determine the fumigant toxicity of essential oils, filter papers (5 cm² area) were impregnated with 0.16, 0.24, 0.32, 0.48 and 0.64 mg/cm² of *n*-hexane solutions. Then each filter paper was attached to a glass vial (0.8 cm diameter and 2.8 cm length). Each vial was introduced inside a glass flask of 40 mL with a top. Ten adults of *R. dominica* were placed inside the flask. Mortality was evaluated at 24, 48 and 72 h [6b]. Probit analysis was used to estimate LC₅₀ and LC₉₅ values by Micro Probit 3.0.

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