Hindawi Publishing Corporation
ISRN Entomology
Volume 2014, Article ID 175827, 5 pages
http://dx.doi.org/10.1155/2014/175827

Research Article

Composition, Repellent, and Insecticidal Activities of Two South American Plants against the Stored Grain Pests *Tribolium castaneum* and *Tribolium confusum* (Coleoptera: Tenebrionidae)

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Received 8 November 2013; Accepted 24 December 2013; Published 20 February 2014

Academic Editors: C. Abramson and M. V. Micieli

As part of a screening program to evaluate the biological activity of indigenous plants, we report the composition and the bioactivity of essential oils (EOs) extracted from Té de Burro *Aloysia polystachya* [(Griseb.) Moldenke] and Lemon Verbena *Aloysia citriodora* [Palau] against two of the most widespread secondary pests of stored products, the red flour beetle *Tribolium castaneum* [Herbst] and the confused flour beetle *Tribolium confusum* [Jacqueline du Val]. Analysis by gas chromatography-mass spectrometry of the EOs led the identification of their major constituents and their relative proportions. EO of *A. citriodora* showed the highest repellent activity against both beetles (>70%). On the other hand, both plants showed fumigant toxicity only against *T. confusum*, without significant differences between them (LC_{50} values of 5.92 and 5.53 mg/L air for *A. polystachya* and *A. citriodora*, resp.). For contact toxicity (topical applications) the EO of *A. polystachya* was more effective (LD_{50} = 7.35 μg/insect) than the EO of *A. citriodora* (LD_{50} = 13.8 μg/insect) only against *T. castaneum*. On the other hand, *T. confusum* was not susceptible by contact to any of these EOs. These results provide important tools for the development of an Integrated Pest Management (IPM) program.

1. Introduction

The red flour beetle *Tribolium castaneum* (Herbst) and the confused flour beetle *Tribolium confusum* (Duval) (Coleoptera: Tenebrionidae) are the most widespread and destructive secondary pests of stored grains and grain-derived products. They have been reported as serious pests in Argentina. Particularly, *T. castaneum* has been found as one of the most prevalent secondary pests in port areas of Buenos Aires province [1]. Control of these insects is primarily dependent upon continuous application of synthetic insecticides, which produce disturbance in the environment, increasing costs of application, pest resurgence, pest resistance, and lethal effects on nontarget organisms, in addition to direct toxicity to users. Thus, the risks associated to the use of these products have led to the growth of environmentally sustainable alternatives.

In the last couple of decades, agrochemical companies have promoted the study of natural products for the development of new insecticides. An evidence of that is the number of organic agriculture products that reached the market [2, 3]. Thus, insecticides of natural origin are proposed as rational alternatives to synthetic ones and, among the biopesticides, essential oils (EOs) are growing rapidly on the botanical pesticide markets [4]. The diversity in their composition and mixture of compounds enhance their insecticide efficacy and reduce the evolution of tolerance and resistance to these products. For this purpose, biological activities of diverse plants have been recorded by several authors [5–9]. EOs and their constituents exert insecticidal effects, repel or deter insect food consumption, or reduce and disrupt insect growth. Several species of Verbenaceae have such EOs with biological properties [10–12]. In particular, the genus *Aloysia* includes approximately 200 species of herbs, shrubs, and small trees. *Aloysia polystachya* (Griseb.) Moldenke, and *Aloysia citriodora* (Palau) are native from Argentina and are distributed throughout South and Central America and tropical Africa. The EOs and extracts from these species are valued medicinally, and also their aromatic properties in culinary and cosmetic industries, due to the presence of phenolic compounds (flavonoids) [13]. Moreover, terpenes extracted from these plants have been shown to have important ecological roles in plant defense and attract pollinators, and they have been reported as repellents and insecticides against several pests [4, 14].

As part of a screening program to evaluate the bioactivity of native plants as insect control agents, we report the composition, repellent activity, and fumigant and contact toxicity of EOs of *A. polystachya* and *A. citriodora* against adults of *T. confusum* and *T. castaneum*.

Table 1: Repellency scale from the less to the most repellent = 0 to V.

Class	DC (%)
0	<0.1
I	0.1 to 20
II	20.1 to 40
III	40.1 to 60
IV	60.1 to 80
V	80.1 to 100

DC: average distribution coefficient.

Table 2: Major identified constituents of *Aloysia polystachya* and *Aloysia citriodora* essential oils and their relative proportion in the oils.

A. citriodora		*A. polystachya*	
Constituents	corr.%	Constituents	corr.%
Citronellal	51.29	Carvone	83.5
Sabinene	22.93	Limonene	16.5
α-curcumene	9.57		
Limonene	7.44		
Caryophyllene	2.37		
α-pinene	2.28		
γ-cedrene	2.27		
p-cymene	1.82		

2. Materials and Methods

2.1. Insects. *T. castaneum* and *T. confusum* are both insecticide-susceptible strains that have been reared in laboratory culture since 1995 and 2005, respectively. Adults of both species were reared in incubators at 28 ± 1°C and 70–80% R.H. in the dark and fed on a mixture of wheat, yeast, and milk in dust (13 : 1 : 1 w : w·w), in glass containers of 500 cm^3.

2.2. Extraction and Analysis of the Composition of Essential Oils. Young fresh leaves from *A. polystachya* were collected from wild plants, during the summer period at Lamarque city, Rio Negro Province, Argentina (39° 24′S. 65° 42′W.). Young fresh leaves from *A. citriodora* were collected from wild plants in Bahía Blanca, Buenos Aires Province, Argentina (38° 44′S. 62° 16′W.) Leaves from both plants were collected into plastic bags and kept in the freezer until the extraction. EOs were extracted using a Clevenger-type apparatus by hydro-distillation for 4 h. After the extraction, the EOs were dried using anhydrous sodium sulphate and stored in the dark at 4°C. Plant oils yields were 0.54% (mg g^{-1}) for *A. polystachya* and 0.34% (mg g^{-1}) for *A. citriodora.*

The essential oils composition was determined by gas chromatography-mass spectrometry (GC-MS) using a Hewlett-Packard 5890 Series II gas chromatograph, equipped with a HP-5972 (EI-70 eV) mass selective detector and a 25 m × 0.25 mm i.d., 0.25 μm film thickness HP-5MS capillary column with the injection block set 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. The carrier gas was He (1 mL min^{-1}, split ratio 1 : 50). Aliquots of the essential oils were dissolved in diethyl ether (injection of 2 μL) for the analysis. Oil components were identified by comparison of their Kobats retention indices with those of known compounds and also by comparison of their mass spectra with those stored in the NBS75K.LMS 86 database.

2.3. Bioassays. All bioassays were set up in laboratory under natural light and controlled temperature and humidity (28 ± 1°C and 70–80% R.H). Ten Adults of each species of 3–5 days old were used for each treatment and control. Five independent replicates were conducted, and all the experimental units were placed at 28 ± 1°C and 70–80% R.H., in the dark.

2.4. Repellency. Repellency test was conducted according to Talukder and Howse [15]. Filter papers (Whatman N° 1, diameter 9 cm) were divided into two halves. One half was impregnated with 0.5 mL of either EO diluted in *n*-hexane (treatment) or *n*-hexane (control). The concentrations evaluated were 90, 120, and 314 $\mu g/cm^2$. Paper disks were air dried, and then placed inside a Petri dish. Ten adult insects were released in the middle of each disk and covered with plastic tape with some holes to prevent insects from escaping. The number of insects on each half of the paper was counted at hourly intervals for 5 h. The distribution coefficient (DC) was calculated using the formula $DC = [(N_c - N_t)/(N_c + N_t)] \times 100$, where N_c and N_t are the number of insects found on the control and treated zone, respectively [16]. The average values were then categorized according to the scale in Table 1.

2.5. Fumigant Toxicity. To determine the fumigant toxicity, filter papers (5 cm^2 area) were impregnated with EOs diluted

Table 3: Average repellency of EOs of *A. polystachya* and *A. citriodora* against *T. castaneum* and *T. confusum*.

Plant	Insect	Average distribution coefficient (DC%)[1] C^a	Hours after treatment 1	2	3	4	5	MR[c]	RC[b]
A. polystachya	*T. castaneum*	90	−13[a]	20[a]	13[a]	40[a]	20[a]	16	I
		120	−6[a]	−46[a]	−60[a]	−66[a]	−80[a]	−51.6	0
		314	46[a]	33[a]	66[a]	53[a]	46[a]	48.8	III
	T. confusum	90	93[a]	86[a]	73[a]	93[a]	93[a]	87.6	V
		120	86[a]	40[a]	40[a]	20[ab]	33[a]	43.8	III
		314	80[a]	60[a]	53[a]	46[b]	46[b]	57	III
A. citriodora	*T. castaneum*	90	80[a]	93[a]	93[a]	80[a]	100[a]	89.2	V
		120	93[a]	86[a]	73[a]	100[a]	80[a]	74.4	IV
		314	80[a]	80[a]	66[a]	93[a]	93[a]	82.4	V
	T. confusum	90	93[a]	100[a]	100[a]	100[a]	86[a]	95.8	V
		120	80[a]	100[a]	93[b]	100[a]	100[a]	94.6	V
		314	93[a]	86[a]	80[b]	80[a]	67[a]	81.2	V

[1]DC% = $[(N_c - N_t)/(N_c + N_t)] \times 100$; [a]C: concentration ($\mu$g cm^{-2}); [b]RC: repellency class; [c]MR: mean rate.
Numbers in the same column of each plant followed by the same letters do not differ significantly in ANOVA test.

Table 4: Fumigant activity of EOs of *A. polystachya* and *A. citriodora* against *T. confusum*.

Essential oil	LC_{50}[a]	95% CI[c]	LC_{95}[b]	95% CI[c]	Slope ± SE[d]	X^2
A. polystachya	5.92[a]	(5.2–6.5)	11.9	(10.08–15.9)	5.37 ± 0.75	2.75
A. citriodora	5.53[a]	(2.6–6.8)	13.2	(10.5–30.6)	4.35 ± 1.39	0.12

[a]LC_{50}: lethal concentration 50 (mg litre^{-1} air); [b]LC_{95}: lethal concentration 95 (mg liter^{-1} air); [c]CI 95%: confidence interval of 95%; [d]SE: standard error. Numbers in the same column followed by the same letters do not differ significantly.

in *n*-hexane and controls with *n*-hexane alone. Each filter paper was attached to a glass vial, covered with a fine mesh cloth. These vials were then introduced inside a glass flask of 40 mL. Ten adults of each species were placed inside the flask. Concentrations evaluated were from 1 to 12 mg/L_{air}. Mortality was evaluated 72 h after treatment [17].

2.6. Contact Toxicity. For topical applications, aliquots (0.2 μL per insect) were applied ventrally to the thorax of adults using a microapplicator. Controls were determined using *n*-hexane. Both treated and control insects were then transferred to glass vials (10 insects per vial). Ten insects were used for each concentration and control. Concentrations evaluated were from 4 to 18 μg/insect. Five independent replicates were conducted. Mortality was registered after 72 h.

2.7. Statistical Analysis. Data from repellency assays (DC) were analyzed using analysis of variance (ANOVA) and minimum significant difference (MSD). Probit analysis was used to estimate LC_{50} and DL_{50} values by MicroProbit 3.0. Mortality values were corrected with Abbott's formula [18] to eliminate natural mortality of control.

3. Results and Discussion

Analysis by gas chromatography-mass spectrometry of the steam distilled oils showed that the major components of *A. polystachya* were carvone (83.5%) and limonene (16.5%), while *A. citriodora* was mainly made up of citronellal (51.29%) and sabinene (22.93%) (Table 2).

Both EOs had repellent effects against *T. castaneum* and *T. confusum* (Table 3). However, based on the repellency scale, the activity was stronger with *A. citriodora*, and it was higher than 70% to all concentrations evaluated (Table 3). The repellent effect of *A. citriodora* is possibly due to the presence of citronellal, which is one of the main constituents (>50%) and is a botanical compound use in commercial insect repellents [19]. Similar results were found by Olivero-Verbel et al. [20] when tested the repellent effect of *Eucalyptus citriodora* against *T. castaneum,* which main component is citronellal (40%). Indeed, the repellent effect of these plants has been also tested against other insects; for instance, Gillij et al. [11] found *A. citriodora* as one of the most promising EO of the fourteen plants test against *Aedes aegypti*. Moreover, Gleiser et al. [21] showed a dose-dependent activity of the EO of *A. polystachya* against the same mosquito. Considering that the EO of *A. citriodora* contains also other compounds such as limonene (7.4%) with potential repellent effects, synergistic phenomena should not be discarded [22].

For fumigant assay, both EOs were toxic against *T. confusum* but there were no significant differences ($P > 0.05$) between them. However, there were not lethal effects against *T. castaneum* (Table 4). More studies must be undertaken to elucidate the differences found between the susceptibility of both beetles, but a possible explanation can be based on the differences in the respiration rates of insects. *T. castaneum*

Table 5: Contact activity of EOs of *Aloysia polystachya*, and *Aloysia citriodora* determined by topical application to *T. castaneum*.

Essential oil	LD_{50}[a]	95% CI[c]	LD_{95}[b]	95% CI[c]	Slope ± SE[d]	X^2
A. polystachya	7.35[a]	(6.38–8.5)	21.95	(16.6–34.5)	3.46 ± 0.47	7.37
A. citriodora	13.8[b]	(9.5–43.7)	124	(41–158.2)	1.71 ± 0.59	0.4

[a]LD_{50}: lethal dose 50 (μg insect^{-1}); [b]LD_{95}: lethal dose 95 (μg insect^{-1}); [c]CI 95%: confidence interval of 95%; [d]SE: standard error. Numbers in the same column followed by the same letters do not differ significantly.

has a lower rate of air exchange and consequently a smaller diffusion of toxic compounds into the insect [23, 24].

For contact toxicity, no lethal effects were found against adults of *T. confusum*. However, significant differences were found between EOs, against *T. castaneum* ($P < 0.05$). *A. polystachya* showed the highest toxicity (Table 5). Carvone, the main compound of *A. polystachya*, has been found to be toxic against diverse insects, for example, Fang et al. [25] found strong contact toxicity of carvone and limonene, against *Sitophilus zeamais* and *T. castaneum*. Moreover, carvone caused the highest mortality against larvae of *T. castaneum* among the several monoterpenes evaluated (LC_{50} = 19.8 μg/cm^2) [26]. On the other hand, the concentration of these products has been found as an important factor that determines their toxicity, and it is directly proportional to the rate of penetration of a substance [27–29]. Carvone found in *A. polystachya* is denser than citronellal found in *A. citriodora*, thus this can be related with the differences found in the contact activity of both EOs. Similar results were found earlier with the EOs of *A. polystachya* against *R. dominica* (Fabricius) and *Nezara viridula* (L.) [12, 30].

4. Conclusions

This is the first report on the repellent and insecticidal activities of EOs of *A. citriodora* and *A. polystachya* (Verbenaceae) against *T. castaneum* and *T. confusum*, two of the most widespread secondary pests in the world. The results present herein indicate that EOs extracted from these indigenous plants showed repellent and bioinsecticides properties. The EO of *A. polystachya* had the greatest contact toxicity against *T. castaneum*. Both EOs had the same fumigant activity against *T. confusum*.

In summary, these EOs are good options for the control of stored grain pests but they need to be studied under commercial storage conditions.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

Financial assistance was provided by SECYT-UNS CONICET.

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