

## Chemical composition of four essential oils from *Eupatorium* spp. Biological activities toward *Tribolium castaneum* (Coleoptera: Tenebrionidae)

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\*\*INTEQUI-CONICET

### Composición química de cuatro aceites esenciales provenientes de *Eupatorium* spp. y su toxicidad para *Tribolium castaneum* (Coleoptera: Tenebrionidae)

■ **RESUMEN.** Se evaluaron las propiedades tóxicas y repelentes de los aceites esenciales de cuatro especies del género *Eupatorium* (Asteraceae): *E. buniifolium* Hook. et Arn, *E. inulaefolium* Kunth, *E. arnottii* Baker y *E. viscidum* Hook. & Arn, en diferentes concentraciones frente a adultos de *Tribolium castaneum* Herbst. Los aceites esenciales se aislaron de las partes aéreas de las plantas, mediante técnicas de hidrodestilación y se analizaron por los métodos GC-FID y GC-MS. Los ensayos de toxicidad por contacto demostraron que todos los aceites fueron tóxicos y la mortalidad fue, en todos los casos, dependiente de la dosis. El aceite esencial de *E. buniifolium* presentó la mayor actividad repelente.

**PALABRAS CLAVE.** *Tribolium castaneum*. *Eupatorium*. Monoterpenos. Sesquiterpenos. Aceites esenciales. Repelencia. Toxicidad.

■ **ABSTRACT.** Toxic and repellent properties of whole essential oils from four *Eupatorium* (Asteraceae) species (*E. buniifolium* Hook. et Arn, *E. inulaefolium* Kunth, *E. arnottii* Baker, and *E. viscidum* Hook. & Arn) were investigated in different concentrations toward *Tribolium castaneum* Herbst adults. The essential oils were isolated by hydrodistillation techniques from the aerial parts. The analysis was performed by GC-FID and GC-MS methods. Contact toxicity assays showed that all the evaluated essential oils were toxic. Furthermore, in all the cases mortality was dose dependent. The main repellency was observed for the essential oil recovered from *E. buniifolium*.

**KEY WORDS.** *Tribolium castaneum*. *Eupatorium*. Monoterpenes. Sesquiterpenes. Essential oils. Repellency. Toxicity.

### INTRODUCTION

Insect pests are one of the main causes of extensive damage in stored grains and their products. Since 1961 (Parkin *et al.*, 1962), the resistance to insecticide in many strains

of *Tribolium castaneum* Herbst have been reported. The malathion-specific resistance has been intensively studied (Assié *et al.*, 2007). Therefore, the use of several kinds of safe insecticides or repellents in food grains storage is necessary, and the interest

for the development of the pesticides with natural products extracted from plants has recently been growing. The interference of plant natural products with the feeding, development and survival of insects has been extensively studied (Sosa & Tonn, 2008). It is well known that the presence of some monoterpenes and sesquiterpenes in plants could help them against predators through some protection mechanism. Thus, volatile terpenes with low molecular-weight have been reported as insect behavior modifiers as well as growth regulators (Hick *et al.*, 1999).

The insecticidal activity of a large number of plant essential oils has been assayed, exhibiting acute toxic effects against several stored-grain insect pests (Liu & Ho, 1999; Tunç *et al.*, 2000; Padin *et al.*, 2000; Lee *et al.*, 2001; Kostyukovsky *et al.*, 2002; Papachristos *et al.*, 2004; Wang *et al.*, 2005; Tapondjou *et al.*, 2005; Rozman *et al.*, 2007). It has been suggested that essential oils are less hazardous than synthetic compounds and rapidly degraded in the environment (Isman, 2000; Moretti *et al.*, 2002).

As part of a program aimed at studying the effects of plant secondary metabolites (García *et al.*, 2003; Pungitore *et al.*, 2004a, b; Juan H. *et al.*, 2008; Sosa & Tonn, 2008) and essential oils (García *et al.*, 2005, 2007) isolated from plants growing in Argentina toward insect pests, we have investigated the chemical composition and biological effects of the essential oils isolated from four South American *Eupatorium* species toward *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), a worldwide pest of stored grains.

The *Eupatorium* genus includes small herbs and shrubs and comprises nearly 600 species distributed in North, Central and South America, Eastern Asia, Taiwan and the Philippines (Herz, 2001). In Argentina there are 82 species growing naturally, and some of them are used in popular medicine (Cabrera *et al.*, 1997). Essential oils, some isolated secondary metabolites, and extracts prepared from several species of the *Eupatorium* genus have shown biological activities including antibacterial (Bailac *et al.*, 2000; El-Seedi *et al.*, 2002), trypanocidal (Sülzen *et al.*, 2006),

and cytotoxic (Mongelli *et al.*, 2000). The essential oil of *Eupatorium betonicaeforme* (D.C.) Baker has been reported due to its larvicidal properties toward *Aedes aegypti* (L.) larvae (Albuquerque *et al.*, 2004). *Eupatorium buniifolium* Hook. et Arn. (known as "romerillo", "romerillo colorado" or "chilca") spreads from northern to central Argentina, and decoctions of the aerial parts are used for antirheumatic, antiseptic or digestive treatments. In addition, free-radical inhibition and cytotoxicity have been reported (Miño *et al.*, 2005). *Eupatorium inulifolium* Kunth ("sanalotodo" or "yerba de Santa María") grows in the northeast of Argentina, and it is used externally for the treatment of skin infections due to its antimicrobial properties (Ferraro *et al.*, 1977).

Taking into account the widespread distribution of *E. buniifolium* var. *buniifolium*, *E. inulifolium*, *E. arnottii* Baker, and *E. viscidum* Hook. & Arn. as well as the yield of the essential oils recovered from stream distillation, the purpose of this work was to investigate the toxic and repellent activities of the isolated essential oils toward the insect-pest *T. castaneum*.

## MATERIAL AND METHODS

### Biological material

#### Plants

Aerial parts of *Eupatorium arnottii*, *E. viscidum* and *E. buniifolium* were collected near San Luis City (33° 15' S, 66° 20' W) and in Quebrada de los Cóndores (33° 14' S, 66° 14' W), San Luis Province, Argentina. Voucher specimens were deposited at the Herbarium of the Universidad Nacional de San Luis (voucher numbers UNSL # 499-Del Vitto, 497-Del Vitto and 495-Del Vitto, respectively). *Eupatorium inulifolium* was collected near Corrientes city (27° 35' S, 58° 50' W), Corrientes Province, Argentina. A voucher sample, identified by Professors Aurelio Schinini (IBONE, Corrientes) and Luis Del Vitto (UNSL, San Luis), was deposited at the Herbarium of the Universidad Nacional

de San Luis (voucher number UNSL # 491-Del Vitto).

### Insects

All experiments were conducted in the laboratory using established colonies from an insecticide-susceptible strain of *Tribolium castaneum*. Adults used in the experiments, were reared on a mixture of flour, starch and yeast (3:3:1) at  $25 \pm 1$  °C, 65% RH and a photoperiod of 16:8 (L:D) h.

### Extraction of essential oil

Fresh aerial parts of *Eupatorium arnottii* (2.95 kg), *E. buniifolium* (3.45 kg), *E. viscidum* (3.20 kg), and *E. inulaefolium* (3.00 kg) were cut into small pieces and subjected to steam-distillation at 96 °C for 3 h using a Clevenger-type apparatus. The recovered essential oils were dried over anhydrous sodium sulfate and stored in cold (4°C). Yield of dried essential oils was 1.81, 2.97, 1.11 and 1.35 g/kg, respectively. The essential oil composition was determined gas chromatography-mass spectrometry (GC-MS). The GC-MS analyses were carried out using a Shimadzu QP 5000 mass spectrometer coupled with a Shimadzu GC-17A gas chromatograph. Analyses were performed using Ultra-2, 30 m, 0.25 mm i.d., 0.25 µm film thickness, fused-silica capillary column. The oven temperature program was 50°C for 4 min, rising to 180°C at 2°C/min, then to 290 °C at 6 °C/min; the injector temperature was 240 °C; the carrier gas was helium at 10 PSI; the injection mode was splitless for 2 min and then it was split with a ratio of 1:40; the sample volume injected was 0.2 µL (1.0 mg/ml, ethyl acetate); the interface temperature 250 °C, and the acquisition mass range was 40–700 m/z at 70 eV. Mass spectral data were compared with the MS instrument library and NIST library. Relative percentages of the major components were calculated by integrating the registered peaks.

### Bioassays

#### Contact toxicity

From a mother solution (4 mg/ml) four essential oil solutions were prepared using

*n*-hexane as solvent. One ml of each solution was applied to the bottom surface of a 125 ml Erlenmeyer flask and uniformly dispersed to give, after one hour of evaporation at r.t., final doses ranging between 0.028, 0.056, 0.141, 0.169, 0.212 mg/cm<sup>2</sup>. These doses were selected after a screening between 0.010 to 0.5 mg/cm<sup>2</sup>. After that, 0.5 g of rearing food were spread in each flask and ten randomly selected and unsexed adults of *Tribolium castaneum*, were introduced. Treated Erlenmeyer flasks were sealed with a cotton plug and kept at  $25 \pm 1$  °C with a photoperiod of 16:8 (L:D) h. Each treatment was independently replicated five times. Insect mortality was recorded at 24, 48 and 72 hs, and mortality (%) was corrected according to Abbott (1925). Data were analyzed using Two Way ANOVA Test at  $P \leq 0.05$  to determine significant differences among treatments and time of exposure (Graph Pad InStat, Version 3.0). ED<sub>50</sub> values were determined from linear regression.

### Repellency

The experiments employed a two-choice bioassay. Test arenas were two joined Erlenmeyer flasks of 125 ml with a glass tube of 1,5 cm long and 0,5 cm diameter fused to the base of the side wall of each flask. Five *n*-hexane solutions with increasing oil concentrations were prepared to give final doses ranging between 0.028, 0.056, 0.141, 0.169, 0.212 mg/cm<sup>2</sup>.

Each solution was homogeneously distributed on the bottom of the flasks, allowed to evaporate for one hour, and then 0.5 g of rearing food were spread in each flask. Controls were treated with the solvent alone. The side glass tube for each Erlenmeyer flask (treated and controlled) was joined to the other using a rubber tube with a hole in the middle (0.5 cm diameter). Once the flasks were joined by the rubber tube, ten unsexed adults of *Tribolium castaneum*, randomly selected, were released carefully in the hole. The hole was covered using a piece of sello tape to ensure a hermetic seal. Each treatment was replicated five times. Bioassays were conducted in complete darkness at  $25 \pm 1$ °C and 65 % RH. After 30,

60, 90, 150 and 210 minutes, a Response Index (RI) for beetles in the two-choice bioassays was calculated using  $RI = (T - C / Tot) \times 100$ , where T is the number of insects in the treated flasks; C is the number of insects distributed in the control flasks, and Tot is the total number of insects released. Positive RIs indicate attraction to the treatment, and negative RIs indicate repellency. Values could theoretically range from -100 for a complete repellency, to +100 for complete attraction (Phillips *et al.*, 1993). Data were analyzed using Two Way ANOVA Test at  $P \leq 0.05$ .

## RESULTS AND DISCUSSION

The aim of this work was to investigate toxic and repellent properties of *Eupatorium buniifolium*, *E. inulaefolium*, *E. arnottii* and *E. viscidum* essential oils against *Tribolium castaneum* adults. These essential oils contain a variety of terpenoids, although one of them (*E. viscidum*) presented a non-terpenoid compound (6-methyl-5-hepten-2-one). The identified constituents and their composition (percentage), listed in order of elution, are shown in Table I.

Several of the identified compounds were also present in other *Eupatorium* species which grow wild in the Amazon region (Maia *et al.*, 2002). Table I shows a different composition for each assayed essential oil. However, a set of four compounds was common in all oil samples, namely  $\beta$ -caryophyllene (range 1.11-27.72%),  $\alpha$ -caryophyllene (0.25-5.90 %), germacrene D (0.95-13.66 %), and (-)-spathulol (0.48-25.16 %).

When the essential oil of *Eupatorium buniifolium* was analyzed, 19 compounds were identified accounting for 92.98 % of the total oil. The most abundant component was  $\alpha$ -pinene (50.98 %) with significant amounts of D-limonene (9.63 %) and (+)-sabinene (7.45 %).  $\beta$ -caryophyllene (5.22%), (-)-spathulol (4.93%), and ocimene (4.78 %) were also present. A total of 21 compounds were identified representing 97.36% of the whole oil of *E. inulaefolium*.

$\beta$ -caryophyllene (27.72%), germacrene D (13.66%),  $\delta$ -elemene (10.57%), limonene (9.73%), patchoulene (9.24%) and viridiflorol (9.16%) were the most important components. The sesquiterpenes fraction accounted for 84.97 % of the total oil. The essential oils of *E. arnottii* showed notable compositions because the species was mainly constituted by sesquiterpene compounds. In these samples, 16 components representing 71.40% of the whole oil were identified. The principal constituents were (-)-spathulol (10.57%), germacrene D (9.83%), caryophyllene (7.92%),  $\gamma$ -elemene (5.92%) and (+)- $\delta$ -cadinene (5.83%). Finally, in the essential oil of *E. viscidum* the main identified constituents were (-)-spathulol (25.16%), (-)- $\delta$ -cadinol (2.67%),  $\square$ -santalene (2.53%),  $\beta$ -cubebene (1.64%), (+)-nerolidol (1.63%) and germacrene D (1.46%).

The biological effects of *Eupatorium buniifolium*, *E. inulaefolium*, *E. arnottii*, and *E. viscidum* essential oils on *T. castaneum* adults were evaluated through two kinds of bioassays. Results are shown in Tables II-III.

The essential oils obtained from *Eupatorium buniifolium*, *E. inulaefolium*, and *E. arnottii* exhibited greater repellent and toxic effects against the *T. castaneum* adults. The essential oils of these species caused 98% of mortality after 24 hs of exposure oil at 0.212 mg/cm<sup>2</sup>. High mortality in contact toxicity test could also be due to the presence of constituents such as  $\alpha$ -pinene, limonene,  $\beta$ -caryophyllene and germacrene. The toxic effects of *E. buniifolium* essential oil might be attributed to its major components (mainly  $\alpha$ -pinene), as well as other major and/or trace compounds. A previous research using *Tribolium castaneum* adults, reported by the same working team, provides clear support for this assumption. In these experiments, limonene (present in the essential oil under analysis) showed a bioactivity similar to  $\alpha$ -pinene ( $LD_{50} = 1.16 \mu\text{M}/\text{cm}^2$  and  $LD_{50} = 1.12 \mu\text{M}/\text{cm}^2$  at 24 h of exposition, respectively) (García *et al.*, 2005). Ojmelukwe & Adler (1999) found  $\alpha$ -pinene was toxic to *Tribolium confusum* du Val., and exhibited negative chemotaxis against *Periplaneta americana* (Ngoh *et*

**Table I.** Chemical composition of essential oils of *Eupatorium* species.

Components <sup>*</sup>	Composition (%)			
	<i>E. buniifolium</i>	<i>E. inulaefolium</i>	<i>E.arnottii</i>	<i>E. viscidu</i>
$\alpha$ -Thujene		0.44		
$\alpha$ -Pinene	50.98			
(+) Sabinene	7.45	0.38		
$\beta$ -Pinene	1.91	0.17		
6-Metil-5-hepten-2-one				18.18
(+) 2-Carene	2.46			
<i>p</i> -Cymene	0.36			
Limonene**		9.73		
D-Limonene	9.63			
<i>cis</i> $\beta$ -Ocimene	0.18	0.47		
Ocimene**	4.78			
(+) Sabinene hydrate	0.21			
Alloocimene		0.41		
Terpinen-4-ol	0.53			
$\delta$ -Elemene		10.57	0.88	
( $\pm$ )Copaene		0.87	0.37	
$\beta$ -Bourbonene		0.20	0.81	
$\beta$ -Elemene		3.46		
$\beta$ -Cubebene	0.95		0.27	1.64
$\beta$ -Caryophyllene	5.22	27.72	7.92	1.11
$\gamma$ -Elemene			5.92	
$\alpha$ -Caryophyllene	0.25	5.90	1.23	0.90
Alloaromadrendrene		0.17		
Patchoulene		9.24		
Germacrene D	0.95	13.66	9.83	1.46
$\delta$ + $\epsilon$ adinene	0.33	2.49	5.83	
Germacrene B	0.43		4.30	0.69
(+) Nerolidol		0.13		1.63
Ledol		0.10		
(-)-Spathunelol	4.93	0.48	10.57	25.16
Caryophyllene oxide	1.17		0.89	
Viridiflorol		9.16	0.17	
$\tau$ -Cadinol			2.43	
$\delta$ - $\epsilon$ Cadinol	0.26	0.82		2.67
$\alpha$ -Cadinol			3.30	
Phytol			8.08	
(*) In order of elution on Ultra-2 colum      (**) correct isomer not identified				
Monoterpenes (%)	78.49	11.60	-	-
Sesquiterpenes (%)	14.49	84.97	54.72	35.26
Others	-	-	8.08	18.18
Total	92.98	96.57	62.80	53.44

**Table II.** Contact mortality data of *Eupatorium* species essential oils on *Tribolium castaneum* adults at different times (hours) in a contact toxicity bioassay.

Essential oil	(mg/cm <sup>2</sup> )	Percent mortality ( $\pm$ SD)		
		24 h	48 h	72 h
<i>E. buniifolium</i>				
Control	0.00		0.16 $\pm$ 0.40	0.16 $\pm$ 0.40
0.028	0.16 $\pm$ 0.40		0.16 $\pm$ 0.40	0.16 $\pm$ 0.40
0.056	29.75 $\pm$ 1.08		40.41 $\pm$ 1.49	40.41 $\pm$ 1.49
0.141	31.58 $\pm$ 2.76		52.41 $\pm$ 2.37*	52.41 $\pm$ 2.37*
0.169	94.58 $\pm$ 4.41*		94.58 $\pm$ 4.41*	94.58 $\pm$ 4.41*
0.212	98.16 $\pm$ 1.47*		98.16 $\pm$ 1.47*	98.16 $\pm$ 1.47*
ED <sub>50</sub>	0.15		0.10	0.10
(CL 95 %)	(0.1545-0.1856)		(0.044-0.1256)	(0.044-0.1256)
<i>E. inulaefolium</i>				
Control	0.00		0.16 $\pm$ 0.40	0.33 $\pm$ 0.81
0.028	0.16 $\pm$ 0.40		14.16 $\pm$ 2.01	21.66 $\pm$ 1.99
0.056	0.16 $\pm$ 0.40		41.16 $\pm$ 2.78	51.83 $\pm$ 2.22
0.141	19.16 $\pm$ 1.43		60.00 $\pm$ 1.41*	78.41 $\pm$ 2.10*
0.169	45.50 $\pm$ 0.89		98.75 $\pm$ 1.17*	98.75 $\pm$ 1.17*
0.212	98.25 $\pm$ 1.72*		98.25 $\pm$ 1.72*	98.25 $\pm$ 1.72*
ED <sub>50</sub>	0.15		0.087	0.053
(CL 95 %)	(0.086-0.55)		(0.076-0.099)	(0.076-0.098)
<i>E. arnottii</i>				
Control	0.00		0.16 $\pm$ 0.40	0.33 $\pm$ 0.81
0.028	12.33 $\pm$ 1.66		19.91 $\pm$ 1.90	22.33 $\pm$ 2.44
0.056	14.00 $\pm$ 1.38		15.58 $\pm$ 1.11	30.83 $\pm$ 1.96
0.141	49.25 $\pm$ 2.04		57.83 $\pm$ 1.94*	71.66 $\pm$ 1.63*
0.169	90.58 $\pm$ 1.49*		90.58 $\pm$ 1.49*	90.58 $\pm$ 1.49*
0.212	95.08 $\pm$ 3.38*		95.08 $\pm$ 3.38*	95.08 $\pm$ 3.38*
ED <sub>50</sub>	0.15		0.10	0.097
(CL 95 %)	0.0851-0.1569)		(0.0845-0.129)	(0.0758-0.160)
<i>E. viscidum</i>				
Control	0.00		0.16 $\pm$ 0.40	0.33 $\pm$ 0.81
0.028	0.16 $\pm$ 0.40		3.25 $\pm$ 1.47	9.75 $\pm$ 1.94
0.056	1.16 $\pm$ 1.16		4.75 $\pm$ 1.33	11.75 $\pm$ 1.72
0.141	0.16 $\pm$ 0.40		3.91 $\pm$ 1.20	24.91 $\pm$ 3.20
0.169	0.16 $\pm$ 0.40		0.16 $\pm$ 0.40	36.25 $\pm$ 4.28
0.212	7.16 $\pm$ 1.43		11.75 $\pm$ 1.66	69.66 $\pm$ 1.86*
ED <sub>50</sub>	-		-	0.19
(CL 95 %)				(0.1408-0.329)

Each value is expressed as mean  $\pm$  S.E.M from five replicates with 10 adults each (n=50). Means within a column followed with \* are significantly different from the control at  $P \leq 0.05$ .

*al.*, 1998). The antifeedant and growth inhibitory effects of this monoterpene toward *T. castaneum* were observed by Huang *et al.* (1998). Taking into account the bioactivity of  $\alpha$ -pinene toward other insect orders, i.e. *Lycoriella mali* Fitch (Choi *et al.*, 2006), this monoterpene seems to be a non selective allelochemical.

In the essential oil recovered from *Eupatorium inulaefolium* the main detected terpenes were  $\beta$ -caryophyllene (27.72 %), germacrene (13.66 %),  $\delta$ -elemene (10.57 %), and limonene (9.73 %). The essential oil of *E. betonicaeforme*, which includes in its composition the compound  $\beta$ -caryophyllene, has been reported as larvicidal toward *Aedes egyptii* (L) (Albuquerque *et al.*, 2004). In a previous search of biopesticides using *T. castaneum* adults, whereas the sesquiterpene germacrene was inactive in both bioassays, the monoterpene limonene showed both insecticidal and repellent bioactivities (García *et al.*, 2005).

In our experiments the exposure to *E. buniifolium* and *E. inulaefolium* essential oils led to liberation of defensive secretions used by *T. castaneum* as repellents and irritants (benzoquinones) (Unruh *et al.*, 1998). The presence of these quinones was recognizable because the food present in the treated flask acquired a pinkish color, caused by quinone binding in flour to form conjugates with amino groups (Hodge *et al.*, 1996; García *et al.*, 2005).

The essential oil recovered from *E. viscidum*, which showed the minor toxic bioactivity in the first 24 h, did not exhibit the terpenes  $\alpha$ -pinene, limonene, and  $\delta$ -elemene.

Some authors have suggested that the toxic activity of essential oils may be attributed to a reversible competitive inhibition of acetylcholinesterase by the occupation of hydrophobic site of the enzyme active center (Tapondjou *et al.*, 2005). In our experiments, aimed at determining the contact toxicity, it was possible to observe that the insects showed symptoms, including convulsion and tremors followed by paralysis (namely knock-down) (data not shown), similar to those produced by some essential oils isolated

from aromatic plants. This response might be due to an activation of octopaminergic receptors by several terpenes (Kostyukovsky *et al.*, 2002).

In conclusion, contact toxicity bioassays showed that *E. arnottii*, *E. inulaefolium*, and *E. buniifolium* essential oils caused the main deleterious effect after 24 hs of treatment. The dosage of ED<sub>50</sub> demonstrated that the insects were susceptible at 0.10-0.15 mg/cm<sup>2</sup>.

In the two-choice bioassays the essential oils exhibited repellent activity at the concentrations tested. However, the biggest activity was produced at 0.056 and 0.141 g/cm<sup>2</sup> from *E. buniifolium* and *E. inulaefolium* essential oils (Table III). There were no significant statistical differences between all concentrations for each essential oil after 150 minutes of exposure. Remarkably, the *E. buniifolium* essential oil showed the highest concentration of terpenes (78.49 %) with a lower concentration of sesquiterpenes (Table I), and it was the only one revealing the presence of  $\alpha$ -pinene. We have previously reported the noteworthy repellent and toxic activities of this compound toward the insect here assayed (García *et al.*, 2005). Besides, it has been observed that  $\alpha$ -pinene possesses important repellent effects toward *Tribolium confusum* du Val (Tapondjou *et al.*, 2005).

In the test for the two-choice bioassay, *E. viscidum* was significantly different in relation to the most active *E. buniifolium* ( $P \leq 0.05$ ). This observation could be ascribed with the fact that its essential oil showed the minor concentration of  $\beta$ -caryophyllene and, in addition,  $\alpha$ -pinene was absent. Essential oils obtained from *E. inulaefolium* and *E. arnottii* induce a notable mortality at high doses but with lower repellent activity than that described for *E. buniifolium*. These results could be explained keeping in mind some synergistic effects.

Since the structural characteristics of monoterpenoids can influence their insecticidal properties, the degree of penetration into the insect cuticle and the ability to move to and interact with an active site (Rice & Coats, 1994), the bioactivities here described cannot be accounted for the major components, and the existence of

**Table III.** Response Index data of *Eupatorium* species essential oils on *Tribolium castaneum* adults at different times (min) in a two-choice bioassay.

Essential oil	(g/cm <sup>2</sup> )	repellency (± SD)				
		30 min	60 min	90 min	150 min	210 min
<i>E. buniifolium</i>						
Control		0.00	0.00	0.00	0.00	0.00
0.028		-56.66 ± 0.11*	-56.66 ± 2.50*	-60.00 ± 1.10*	-70.00 ± 2.30*	-60.00 ± 1.20*
0.056		-76.66 ± 1.87*	-70.00 ± 6.10*	-70.00 ± 1.30*	-71.30 ± 6.50*	-71.00 ± 2.80*
0.141		-53.30 ± 2.61*	-53.30 ± 3.60*	-56.66 ± 2.50*	-66.60 ± 3.30*	-66.60 ± 3.30*
0.169		-53.30 ± 1.22*	-60.00 ± 1.82*	-63.30 ± 0.40*	-63.30 ± 3.80*	-70.00 ± 3.50*
0.212		-76.66 ± 3.4*	-60.00 ± 1.09*	-56.00 ± 1.98*	-63.00 ± 2.35 *	-70.00 ± 3.50*
<i>E. inulaefolium</i>						
Control		0.00	0.00	0.00	0.00	0.00
0.028		-26.30 ± 0.80*	-30.00 ± 4.50*	-30.00 ± 2.70*	-30.00 ± 4.70	-30.33 ± 0.80
0.056		-45.00 ± 2.90*	-40.00 ± 5.10*	-55.00 ± 2.50*	-55.00 ± 2.60*	-55.00 ± 2.60*
0.141		-40.00 ± 2.60*	-47.00 ± 3.10*	-56.00 ± 2.80*	-54.00 ± 3.40*	-54.60 ± 3.20*
0.169		-36.00 ± 1.88*	-36.00 ± 3.70*	-47.08 ± 1.50*	-47.00 ± 4.10*	-47.00 ± 0.66*
0.212		-36.60 ± 2.01*	-36.00 ± 3.50*	-43.08 ± 1.50*	-43.00 ± 0.33*	-43.00 ± 0.66*
<i>E. arnottii</i>						
Control		0.00	0.00	0.00	0.00	0.00
0.028		2.50 ± 1.50	3.30 ± 0.20	-2.40 ± 1.80	-3.50 ± 1.10	-3.10 ± 1.50
0.056		-22.20 ± 1.33	-22.00 ± 3.40	-26.66 ± 1.80	-30.00 ± 0.70*	-30.00 ± 1.60*
0.141		-23.33 ± 2.60	-13.30 ± 1.80	-30.00 ± 1.40*	-30.00 ± 1.40*	-30.00 ± 1.40*
0.169		-26.40 ± 2.10*	-27.60 ± 2.30	-37.80 ± 2.40*	-33.30 ± 0.90	-33.50 ± 3.00*
0.212		-47.70 ± 1.50*	-50.00 ± 2.50*	-47.00 ± 3.40*	-47.07 ± 1.60*	-47.10 ± 2.40*
<i>E. viscidum</i>						
Control		0.00	0.00	0.00	0.00	0.00
0.028		16.13 ± 1.34	30.00 ± 1.80	30.00 ± 1.14	-22.00 ± 3.60	-26.00 ± 1.64*
0.056		30.00 ± 1.87	-18.00 ± 4.40	-42.00 ± 0.20*	-42.00 ± 2.80*	-52.00 ± 0.66*
0.141		-26.00 ± 1.30*	-24.00 ± 0.70	-24.00 ± 3.90	-30.00 ± 4.10*	-32.00 ± 3.70*
0.169		-20.22 ± 3.10	-34.00 ± 4.80*	-36.00 ± 4.50*	-35.00 ± 3.30*	-35.00 ± 5.60*
0.212		-20.22 ± 2.90	-20.00 ± 2.90	-26.00 ± 5.00	-22.04 ± 2.90	-22.00 ± 1.40

Each data point represents the mean of five replicates with 10 adults each (n=50). Means within a column followed with \* are significantly different from the control at P ≤ 0.05

synergistic effects is possible.

## CONCLUSIONS

In conclusion, this study suggests that *Eupatorium buniifolium*, *E. inulaefolium*, *E. arnottii*, and *E. viscidum* essential oils may act as potential grain protectant due to their combined contact toxicity and repellency against *Tribolium castaneum*. However, further investigations for the insecticidal action mode of these essential oils, as well as the evaluation of the major components presented in each complex sample, and field evaluation studies are needed.

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