

# DESARROLLO DE MICROECONOMÍAS REGIONALES EN LA PRODUCCIÓN DE ACEITES ESENCIALES COSECHADOS EN SUELOS MINEROS

**Producto 18: Informe del perfil químico de los aceites.**

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## Resumen producto 18

La caracterización fisicoquímica y biológica del aceite esencial de *Helianthus petiolaris* y su efecto repelente sobre *Tribolium castaneum*, comprometida como Producto 18 del proyecto, han sido publicadas en el formato de artículo científico en la revista *Semiárida*, revista indexada de mayor impacto en el contexto agronómico regional de La Pampa. A partir de estos estudios, profundizamos los conocimientos sobre la composición química y perfil de aceite esencial de *H. petiolaris* (identificando sus posibles principios activos) y sus efectos repelentes sobre el gorgojo de la harina (*T. castaneum*). Esto no solo constituye un avance en la identificación de nuevas moléculas de interés biotecnológico, sino la base científica para la formulación de un bioinsumo con enorme potencial de aplicación en la protección del grano ensilado contra esta plaga. Este producto de origen natural es descrito por primera vez por nuestro grupo de trabajo y representa una gran oportunidad para reemplazar productos químicos de alto riesgo toxicológico como los fosfuros metálicos.

A continuación, se presenta el artículo publicado, como entregable del Producto 18 comprometido para esta actividad.



## ***Actividad repelente de aceites esenciales de plantas nativas y su mezcla para controlar Tribolium castaneum en granos almacenados***

Los aceites esenciales (EO) de 5 plantas aromáticas nativas de La Pampa, Argentina, se obtuvieron mediante hidrodestilación y se analizaron para determinar su repelencia en adultos de *Tribolium castaneum* Herbst. Todos los EO probados mostraron una alta actividad de repelencia a la concentración más alta establecida (0.3 mg.cm<sup>2</sup>).

Los EO de *Baccharis spartioides* y *Helianthus petiolaris* fueron los más eficientes, mostrando una mayor repelencia en comparación con el control positivo estándar (DEET). A 3x10<sup>1</sup> mg.cm<sup>2</sup>, *B. spartioides* y *H. petiolaris* mostraron un 95% y un 92,2% de repelencia, respectivamente. La composición química de los EO se analizó luego por cromatografía de gases espectrometría de masas (GCMS), se presenta la primera determinación de la composición química del EO de *H. petiolaris*. Finalmente, para evaluar la actividad de repelencia en “condiciones de campo” simuladas, se evaluó el comportamiento de la plaga en harina tratada.

En presencia de EO *H. petiolaris*, el 90% de los insectos abandonaron el grano, mientras que *B. spartioides* indujo un 81% de emigración. Para maximizar los rendimientos en la producción de repelentes, se propuso una mezcla que utiliza EO limón comercial. Los resultados obtenidos muestran una diferencia significativa después de 24 h de tratamiento, donde la repelencia de la mezcla persistió en contraste con los EO puros. Estos hallazgos presentan las mezclas de EOs de *B. spartioides* y *H. petiolaris* como alternativas prometedoras para controlar *T. castaneum* en granos almacenados.

**Palabras Clave:** aceites esenciales, *Tribolium castaneum*, control de plagas, almacenamiento postcosecha

## REPELLENT ACTIVITY OF ESSENTIAL OILS FROM NATIVE PLANTS AND THEIR BLEND FOR *Tribolium castaneum* CONTROL IN STORE GRAINS

### ACTIVIDAD REPELENTE DE ACEITES ESENCIALES DE PLANTAS NATIVAS Y SU MEZCLA PARA CONTROLAR *Tribolium castaneum* EN GRANOS ALMACENADOS

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#### ABSTRACT

The essential oils (EOs) from 5 native aromatic plants from La Pampa, Argentina, were obtained by hydrodistillation and tested for repellency on *Tribolium castaneum* Herbst. adults. All tested EOs showed high repellency activity at the highest concentration established (0.3 mg.cm<sup>-2</sup>). The EOs from *Baccharis spartioides* and *Helianthus petiolaris* were the most efficient, showing higher repellency when compared to the standard positive control (DEET). At 3x10<sup>-1</sup> mg.cm<sup>-2</sup>, *B. spartioides* and *H. petiolaris* showed 95% and 92.2% of repellency respectively. The EOs chemical composition was then analyzed by Gas chromatography-mass spectrometry (GC-MS) and the first report of *H. petiolaris* EO composition is presented. Finally, in order to assess repellency activity in simulated "field conditions", pest behavior was evaluated in treated flour. In presence of *H. petiolaris* EO, 90% of the insects left the grain, while *B. spartioides* induced an 81% of emigration. To maximize the yields in repellent production, a blend using commercial lemon EO was proposed. The results obtained show a significant difference after 24 h of treatment, were the repellency of the blend persisted in contrast with pure EOs. These findings present the *B. spartioides* and *H. petiolaris* EOs blends as promising alternatives to control *T. castaneum* in stored grains.

**KEY WORDS:** essential oils, *Tribolium castaneum*, pest control, postharvest store

#### RESUMEN

Los aceites esenciales (EO) de 5 plantas aromáticas nativas de La Pampa, Argentina, se obtuvieron mediante hidrodestilación y se analizaron para determinar su repelencia en adultos de *Tribolium castaneum* Herbst. Todos los EO probados mostraron una alta actividad de repelencia a la concentración más alta establecida (0.3 mg.cm<sup>-2</sup>). Los EO de *Baccharis spartioides* y *Helianthus petiolaris* fueron los más eficientes, mostrando una mayor repelencia en comparación con el control positivo estándar (DEET). A 3x10<sup>-1</sup> mg.cm<sup>-2</sup>, *B. spartioides* y *H. petiolaris* mostraron un 95% y un 92,2% de repelencia, respectivamente. La composición química de los EO se analizó luego por cromatografía de gases-espectrometría de masas (GC-MS), se presenta la primera determinación de la composición química del EO de *H. petiolaris*. Finalmente, para evaluar la actividad de repelencia en "condiciones de campo" simuladas, se evaluó el comportamiento de la plaga en harina tratada. En presencia de EO *H. petiolaris*, el 90% de los insectos abandonaron el grano, mientras que *B. spartioides* indujo un 81% de emigración. Para maximizar los rendimientos en la producción de repelentes, se propuso una mezcla que utiliza EO limón comercial. Los resultados obtenidos muestran una diferencia significativa después de 24 h de tratamiento, donde la repelencia de la mezcla persistió en contraste con los EO puros. Estos hallazgos presentan las mezclas de EOs de *B. spartioides* y *H. petiolaris* como alternativas prometedoras para controlar *T. castaneum* en granos almacenados.

**PALABRAS CLAVE:** aceites esenciales, *Tribolium castaneum*, control de plagas, almacenamiento postcosecha

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#### INTRODUCTION

A large portion of tropical African, Asian and Latin American lands are under a unimodal and often highly variable rainfall pattern. Without

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irrigation systems, their local production is limited to one annual harvest and grains must be frequently stored for long periods in low tech deposits in small farms (Alonso Amelot & Avila Núñez, 2011). In this context, there is a continuous need of protecting the stored grains against deterioration, especially from loss in quality and weight during the post-harvest way from field to consumer (Padin *et al.*, 2002).

In Argentina, the post-harvest losses recorded for the main crops oscillating around 8%, which generates approximately an economic loss of 1,000 million dollars per crop (INTA, 2008).

*Tribolium castaneum* Herbst., is one of the most widespread destructive primary pest of stored grains and it has been found as one of the most prevalent in harbor areas of Buenos Aires, Argentina (Descamps *et al.*, 2008). Beetles and larvae feed on a wide variety of dry vegetable substances, such as milled cereal products, causing extensive loss in both quality and quantity of the stored product (Rees, 2004).

From decades, various types of fumigants and synthetic insecticides have been used for the control of insects in stored grain, but its frequently used has induced resistance in most of the pest strains (Subramanyam & Hagstrum, 1995). Furthermore, the levels of acceptable pesticide residues in grains and oilseed (FAO, 2013; SENASA, 2013) limit the doses and the use of these fumigants, putting the pest control in a crossroads.

Recently, secondary plant metabolites have been intensively explored as an attempts to develop new bio rational alternatives. Such alternatives are expected to be pest specific, with low mammalian toxicity, easy to use, readily biodegradable and reduced in health and environmental impacts, in order to minimize the excessive reliance on the chemical pesticides (Jindal *et al.*, 2013).

Volatile compounds produced by higher plants are responsible for plant to plant interaction, defense mechanism and for attracting pollinators (Batish *et al.*, 2008). These volatile compounds (EO) have also shown insecticidal and repellent activity against stored grain insect pests (Bakkali *et al.*, 2008). On the other hand, since botanicals pesticides have multiple mechanisms and sites of action, development of resistance is limited and needs long periods and large population for selection

to occur (Regnault Roger *et al.*, 2012). Although, its high volatility makes it necessary the formulation in order to increase its effectiveness, and that the concentrations in which they must be used are usually high, botanical insecticides have gained popularity in both, the integrated pest management and the agro-ecological production. As an example, Citrus EOs obtained as byproducts of the citrus processing have gained acceptance in the food industry since they have been recognized as safe, and many crops tolerate their presence (Fisher & Phillips, 2008).

The main botanicals families from which EOs are extracted are Asteraceae, Myrtaceae, Lauraceae and Lamiaceae (Giuliano, 2001). Asteraceae (Asteraceae) family, also called “compound” (Compositae Giseke, cons. Name), gather more than 23.500 species, spread over some 1.600 genera, which is why they are the Angiosperm family with the greatest richness and biological diversity (Jeffrey, 2007). Asteraceae is the most numerous family in Argentina, with 227 genera (five are endemic) and 1400 species (92 are adventitious and 382 are endemic). There are Asteraceae in most of the biomes, which adapt to different soils, climates and reliefs. In the phytogeographic province of Puna, the Asteraceae have adapted to water deficit, low atmospheric humidity, high solar radiation and nocturnal temperatures below zero throughout the year. The most represented tribe in number of genera is Heliantheae and in number of species the tribe Senecioneae. In the province of La Pampa this family is the second most important after Poaseae (Rúgolo de Agrazar *et al.*, 2005). Based on the bioprospection carried out in this region (Troiani & Steibel, 1999) the most relevant species selected were: *Ambrosia tenuifolia*, *Baccharis articulata*, *Baccharis spartioides*, *Helianthus petiolaris* and *Senecio serratifolius*.

In this context the aim of this study was to assess the repellent properties of *A. tenuifolia*, *B. articulate*, *B. spartioides*, *H. petiolaris*, *S. serratifolius* EOs on *T. castaneum* and find a blend that allows its future application in store grains.

## MATERIAL AND METHODS

### Plant materials

The aerial parts of *A. tenuifolia*, *B. articulata*, *B. spartioides*, *H. petiolaris* and *S.*

*serratifolius* were collected from different locations of La Pampa (Argentina) from September 2013 to December 2013, during the flowering period. Plants were identified at the Botany Department, University of La Pampa. Plant material (biomass) was dried in the lab for 7 days at room temperature (25-28°C).

#### **Essential oil extraction**

The EOs were extracted by hydrodistillation from the dried plant biomass by using a bench top scale extractor (Figmay SRL, Argentina). The extraction conditions were: 1 Kg of powdered biomass, 5 l water volume, 4 h distillation. Anhydrous sodium sulphate was used to remove water traces in EOs after extraction. Yield was calculated and extracted oils were stored at 4°C in fulfilled, tightly closed amber flasks until analyzed and tested

#### **Insects**

*T. castaneum* specimens were obtained from the Center for Pest and Insecticide Research (CIPEIN-CITEFA), National University of General San Martín, Argentina. The strain was kept in plastic containers (3 l) covered with a nylon mesh. Insects were raised on wheat flour mixed with brewer's yeast (7:1 w/w) in a growth incubator at 25-27°C, relative humidity of 70-75% and 12:12 h light:dark photoperiod.

#### **Repellent bioassay**

The Area Preference Method (Zhe Zhang *et al.*, 2017) was followed for the evaluation of repellent activities of the essential oils extracted on *T. castaneum*. Filter papers (Whatman N° 1, diameter 9 cm) were divided into halves. One half was impregnated with 0.5 ml of acetonic solution of EOs and the other half was impregnated with 0.5 ml of acetone (control). The concentrations evaluated were  $3 \times 10^{-3}$ ,  $3 \times 10^{-2}$ ,  $3 \times 10^{-1}$ ,  $0.31 \text{ mg.cm}^{-2}$  for *B. articulata*, *B. spartioides*, *H. petiolaris*, *S. serratifolius* and  $1.5 \times 10^{-3}$ ,  $1.5 \times 10^{-2}$ ,  $1.5 \times 10^{-1}$ ,  $0.15 \text{ mg.cm}^{-2}$  for *A. tenuifolia*, different concentrations were used due to the amount of EO obtained from the distillation. Paper disks were air dried for 24 h until acetone evaporation, and then placed into a plastic petri

dishes. Ten adult of *T. castaneum* were released in the middle of each disk and covered with plastic tape. The test was carried out under the same environmental conditions of temperature, humidity and photoperiod described for the raising and the plates were rotated every day to avoid basal behavior of the insects. The number of insects on each half of the paper disks was recorded every hour for 5 h and at 24 h from the beginning of the test. DEET (N, N-diethyl-3-methylbenzamide from Sigma-Aldrich) was used as a positive control and a vehicle control with 0.5 ml acetone was also set up, with the other half of the filter paper left untreated. Five replicates were used for each treatment and the experiment was repeated twice.

Percentage of repellency (PR) was calculated as follows:  $PR = (C-T)/(C+T) \times 100$ , where C represent the number of insects on the untreated area, and T the number of insects on the treated area (Nerio *et al.*, 2009). According to the averages PR, EOs dilutions were then assigned to different classes from 0 to V: class 0 (0-0.1%), class I (0.1-20%), class II (20.1-40%), class III (40.1-60%), class IV (60.1-80%) and class V (80.1-100%) (Liu & Ho, 1999).

#### **GC-MS analysis of the EOs**

Once selected the most promising EOs, they were analyzed by GC-MS in order to determine their chemical composition, and eventually the main compound responsible for the repellence. So, *B. spartioides* and *H. petiolaris* EOs were analyzed in a HP 6890N Series Plus gas chromatograph (Agilent Technologies, Palo Alto, California, USA), equipped with a model 5973N mass selective detector (Agilent Technologies, Palo Alto, California, USA) and an HP 6890 Series autoinjector. The separation of the analytes was achieved using a HP-5 MS capillary column (30 m x 0.25 mm I.D., 0.25 m film thickness and 5% phenylmethylsiloxane), supplied by J & W Scientific (Folsom, CA, USA). Carrier gas (helium) was set at a constant flow rate. The volume of injection was 2 µl on splitless mode, the injection port and transfer line temperatures were set at 250°C and 280°C, respectively. The mass spectrometer was operated with a



filament current of 300 mA and electron energy 70 eV in the positive electron ionization mode. Based on the mass scan range of 50–550 atomic mass units (amu) with SCAN mode, retention times of the compounds were determined by comparing the MS fragmentation pattern of the standards and the National Institute of Standards and Technology (NIST) 2.0 GC–MS library. Chemstation Version D.01.00 was used for data acquisition, data processing, and instrument control.

#### Repellent activity in treated flour

According to Mohan and Fields (2002), movement of insects in the treated grains revealed the interaction between repellent and attractant abilities. In this way, a cup bioassay was conducted with minimal modifications. The best repellent concentrations previously obtain of *B. spartioides* and *H. petiolaris* EOs ( $3 \times 10^{-1}$  mg.cm<sup>-2</sup>) were used for this test. Briefly, 0.5 g of flour spiked with the essential oil dilution in acetone and 0.5 g of flour spiked with acetone (vehicle) were placed on each half of a petri dish. After air-drying the acetone, 15 larvae of *T. castaneum* were released in the middle of each container. The test was carried out under the same environmental conditions described for the rising. The number of the larvae which run away from the treatment flour was counted at two different intervals, 2 h and 24 h. Three replicates were used and the experiment was repeated twice. The repellency percentage was calculated with the same equation used in repellent activity assay.

#### Repellent activity of blends

In order to enhance the repellent effect of the *B. spartioides* and *H. petiolaris* EOs and maximize the production, the different EOs were blend 50/50 on a base of lemon EO (minimum limonene content 85%) purchased from Pico

Drugstore SA, La Pampa, Argentina. The final concentration obtained was 10 mg.ml<sup>-1</sup> of each EO in a lemon EO base. Once blended, the Area Preference Method (2.4) was followed for the evaluation of repellent activities.

#### Statistical analysis

Data were analyzed using InfoStat software. One-way analysis of variance (ANOVA) and Tukey test were performed on the data to determine significant differences ( $p < 0.05$ ) among concentrations for repellent activity test. The same analysis was made to find the differences in repellent activity between pure extracts and blends.

## RESULTS AND DISCUSSION

Sufficient biomass (from 100 to 500 Kg depending on EOs yield) of each plant species was steam-distilled to obtain 5 ml of each EO. The EO yields of the different species are presented in Table 1 with the coordinates and locations of the site of collection. In general, the yields of the 5 essential oils extracted range from 0.001% (*B. articulata* and *S. serratifolius*) to 0.1% (*B. spartioides* and *H. petiolaris*). Although it is necessary to harvest a considerable amount of biomass to obtain the oil, since these species are endemic and ruderal, the collection can be done without creating damage or selective pressure on the ecosystem (Elechosa, 2009).

Results of repellency assays for the tested EOs are presented in Table 2. Tested oils showed repellent activities against *T. castaneum* in a dose-dependent manner. Except for *A.*

Table 1. Plant species, collecting information and yield of essential oils.

Tabla 1. Especies vegetales, información de colecta y rendimiento de aceites esenciales.

Species	Origin	Latitude	Longitude	EO Yield (%w/w)
<i>A. tenuifolia</i>	Santa Rosa, LP	36°37'39.917"S	64°18'23.949"W	0.05
<i>B. articulata</i>	Toay, LP	36°42'55.011"S	64°20'27.788"W	0.001
<i>B. spartioides</i>	Puelches, LP	37°52'26.444"S	65°26'57.593"W	0.1
<i>H. petiolaris</i>	Santa Rosa, LP	36°37'8.348"S	64°19'11.76"W	0.1
<i>S. serratifolius</i>	Int. RN N°5-RN N°7, LP	36°32'12.383"S	63°56'40.017"W	0.001

LP: La Pampa province, Argentina. RN: Nacional Route.

%w/w: gr of EO/ gr of plant material distilled



Repellent activity of essential oils from native plants and their blend for *Tribolium castaneum* control in store grains

Table 2. Repellency of EOs.  
Tabla 2. Repelencia de los EOs

Essential oil	Repellency Percentage (PR)							RC <sup>2</sup>
	Hours after treatment							
	C <sup>1</sup>	1	2	3	4	5	24	
<i>A. tenuifolia</i>	0.15	91.1 <sup>b</sup>	84.4 <sup>b</sup>	86.7 <sup>b</sup>	82.2 <sup>b</sup>	75.6 <sup>bc</sup>	68.9 <sup>b</sup>	V
	1.5x10 <sup>-1</sup>	71.1 <sup>b</sup>	64 <sup>b</sup>	60 <sup>b</sup>	75.6 <sup>b</sup>	48.9 <sup>c</sup>	-11.1 <sup>a</sup>	V
	1.5x10 <sup>-2</sup>	71.1 <sup>b</sup>	64 <sup>b</sup>	60 <sup>b</sup>	75.6 <sup>b</sup>	48.9 <sup>b</sup>	-11.1 <sup>a</sup>	III
	1.5x10 <sup>-3</sup>	24.4 <sup>a</sup>	6.7 <sup>a</sup>	-8.9 <sup>a</sup>	17.8 <sup>a</sup>	-13.3 <sup>a</sup>	-15.6 <sup>a</sup>	I
<i>B. articulata</i>	0.3	91.1 <sup>b</sup>	86.7 <sup>b</sup>	86.7 <sup>b</sup>	86.7 <sup>b</sup>	66.7 <sup>b</sup>	9.9 <sup>ab</sup>	IV
	3x10 <sup>-1</sup>	93.3 <sup>b</sup>	91.1 <sup>b</sup>	91.1 <sup>b</sup>	80.2 <sup>b</sup>	73.7 <sup>b</sup>	73.3 <sup>b</sup>	V
	3x10 <sup>-2</sup>	75.6 <sup>b</sup>	60 <sup>b</sup>	-8.9 <sup>a</sup>	62.2 <sup>b</sup>	39 <sup>b</sup>	31.1 <sup>ab</sup>	III
	3x10 <sup>-3</sup>	-35.6 <sup>a</sup>	-22.2 <sup>a</sup>	-28.9 <sup>a</sup>	-51.1 <sup>a</sup>	-44.4 <sup>a</sup>	-20 <sup>a</sup>	-
<i>B. spartioides</i>	0.3	31.1 <sup>a</sup>	58.6 <sup>bc</sup>	47.1 <sup>a</sup>	11.1 <sup>a</sup>	28.9 <sup>a</sup>	11.1 <sup>a</sup>	II
	3x10 <sup>-1</sup>	100 <sup>c</sup>	95.6 <sup>c</sup>	97.8 <sup>b</sup>	97.8 <sup>b</sup>	100 <sup>b</sup>	80 <sup>b</sup>	V
	3x10 <sup>-2</sup>	73.3 <sup>b</sup>	33.3 <sup>b</sup>	36.8 <sup>a</sup>	13.3 <sup>a</sup>	40 <sup>b</sup>	-4.4 <sup>a</sup>	II
	3x10 <sup>-3</sup>	4.4 <sup>a</sup>	-53.3 <sup>a</sup>	6.3 <sup>a</sup>	11.1 <sup>a</sup>	4.4 <sup>a</sup>	-17.8 <sup>a</sup>	-
<i>H. petiolaris</i>	0.3	100 <sup>c</sup>	100 <sup>b</sup>	100 <sup>b</sup>	97.8 <sup>cb</sup>	95.6 <sup>a</sup>	0 <sup>b</sup>	V
	3x10 <sup>-1</sup>	97.8 <sup>c</sup>	93.3 <sup>b</sup>	97.8 <sup>b</sup>	97.8 <sup>c</sup>	95.6 <sup>a</sup>	71.1 <sup>c</sup>	V
	3x10 <sup>-2</sup>	66.7 <sup>b</sup>	84.4 <sup>b</sup>	73.3 <sup>a</sup>	66.7 <sup>a</sup>	71.1 <sup>a</sup>	33.3 <sup>b</sup>	IV
	3x10 <sup>-3</sup>	8.9 <sup>a</sup>	35.6 <sup>a</sup>	66.7 <sup>a</sup>	75.6 <sup>ab</sup>	73.3 <sup>a</sup>	-55.6 <sup>a</sup>	II
<i>S. serratifolius</i>	0.3	91.1 <sup>ab</sup>	77.8 <sup>a</sup>	86.7 <sup>a</sup>	91.1 <sup>a</sup>	91.1 <sup>a</sup>	20 <sup>ab</sup>	IV
	3x10 <sup>-1</sup>	97.8 <sup>a</sup>	91.1 <sup>a</sup>	91.1 <sup>a</sup>	88.9 <sup>a</sup>	75.6 <sup>a</sup>	33.3 <sup>a</sup>	IV
	3x10 <sup>-2</sup>	51.1 <sup>bc</sup>	60 <sup>a</sup>	51.1 <sup>ab</sup>	55.6 <sup>a</sup>	-51.1 <sup>b</sup>	-42.2 <sup>c</sup>	II
	3x10 <sup>-3</sup>	17.8 <sup>c</sup>	6.7 <sup>b</sup>	22.2 <sup>b</sup>	-8.9 <sup>b</sup>	-20 <sup>b</sup>	-24.4 <sup>bc</sup>	-
DEET	0.3	80 <sup>b</sup>	93.3 <sup>b</sup>	97.8 <sup>b</sup>	97.8 <sup>b</sup>	97.8 <sup>b</sup>	68.9 <sup>b</sup>	V
	3x10 <sup>-1</sup>	44.4 <sup>b</sup>	55.6 <sup>ab</sup>	8.9 <sup>a</sup>	51.6 <sup>ab</sup>	20 <sup>a</sup>	-2.2 <sup>a</sup>	II
	3x10 <sup>-2</sup>	4.4 <sup>a</sup>	6.7 <sup>a</sup>	11.1 <sup>a</sup>	6.7 <sup>a</sup>	20 <sup>a</sup>	11.1 <sup>a</sup>	I
	3x10 <sup>-3</sup>	66.7 <sup>b</sup>	66.7 <sup>b</sup>	55.6 <sup>ab</sup>	15.6 <sup>a</sup>	55.6 <sup>ab</sup>	-11.1 <sup>a</sup>	II
Lemon	0.3	97.8 <sup>b</sup>	95.6 <sup>b</sup>	91.1 <sup>b</sup>	100 <sup>b</sup>	97.8 <sup>b</sup>	42.2 <sup>b</sup>	V
	0.15	97.8 <sup>b</sup>	100 <sup>b</sup>	100 <sup>b</sup>	93.3 <sup>b</sup>	100 <sup>b</sup>	51.1 <sup>b</sup>	V
	9x10 <sup>-1</sup>	97.8 <sup>b</sup>	97.8 <sup>b</sup>	80 <sup>b</sup>	95.6 <sup>b</sup>	97.8 <sup>b</sup>	52.6 <sup>ab</sup>	V
	3x10 <sup>-1</sup>	60 <sup>a</sup>	13.3 <sup>a</sup>	-17.8 <sup>a</sup>	-8.9 <sup>a</sup>	-33.3 <sup>a</sup>	-15.6 <sup>a</sup>	-

C<sup>1</sup>: concentration (mg.cm<sup>-2</sup>); RC<sup>2</sup>: repellency class. Means within the same column followed by the same letter are not significantly different. ANOVA, Tukey (P > 0.05).

*tenuifolia*, all plant species show higher repellency at the 3x10<sup>-1</sup> mg.cm<sup>-2</sup>. In this way, Zhe Zhang *et al.* (2017) reported that the activity of Artemisia ordosica EOs didn't increase with the increase in the concentration on *T. castaneum*. In addition, it was observed that the repellency activity of all species have a fluctuating behavior in the first 5 h, decreasing before 24 h of exposure. *B. spartioides* EO presented the strongest repellent activity against *T. castaneum* at 3x10<sup>-1</sup> mg.cm<sup>-2</sup>, class V of

repellent. *H. petiolaris* EO was the second most effective, showing its maximum repellent activity at the same concentration, also class V of repellent. Both oils exhibited higher repellent activity than the positive control DEET (class II) at this concentration. At the lowest assayed dose (3x10<sup>-3</sup> mg.cm<sup>-2</sup>), only *H. petiolaris* showed moderate repellency (class II). However, all EOs were under DEET repellency (class II). Comparing the percentage of repellency obtained at different concentrations, significant differences were observed in the third dilution (3x10<sup>-2</sup> mg.cm<sup>-2</sup>) in 50% of the samples.

In the best of our knowledge, this is the first report of repellent activity on *T. castaneum* for the EOs of *A. tenuifolia*, *B. articulata*, *H. petiolaris* and *Senecio serratifolius*. For *B. spartioides*, Jofré Barud *et al.* (2014) reported repellent activity of the pure essential oil against the fruit fly pest, *Ceratitis capitata* and it was reported too its antimicrobial (Oliva *et al.*, 2007) and nematocidal (Sosa *et al.*, 2012) activity. There were also some reports about EO from other *Baccharis* species, such as *B. salicifolia* and *B. darwinii*, possessing great repellent activity against insects and plant-parasites nematodes (Sosa *et al.*, 2012; Kurdelas *et al.*, 2012).

The results of GC-MS analysis of *B. spartioides* and *H. petiolaris* EOs are shown in Table 3. In *B. spartioides* EO, 5 major compounds were identified, which represented



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Table 3. Identification of constituents present in *B. spartioides* and *H. petiolaris* EOs.

Tabla 3. Identificación de los constituyentes presentes en los EOs de *B. spartioides* y *H. petiolaris*.

Rt (min)	Compounds	<i>B. spartioides</i>	<i>H. petiolaris</i>
7.134	(S) cisVerbenol	-	12.54
8.143	Verbrnone	-	6.08
7.301	Verbenol	-	10.79
8.950	Bornyl acetate	-	18.97
13.187	$\alpha$ -Bisabolol	1.32	-
14.135	Ledene oxide (I)	-	5.53
14.068	Spathulenol	35.15	-
14.135	Ledene oxide (I)	-	5.53
14.719	Caryophyllene oxide	31.1	27
16.438	Isoaromadendrene	-	3.53
16.863	1,6(1-(Hydroxymethyl)vinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-2(1H)-naphthalenone	6.42	-
19.132	$\alpha$ -Pipene oxide	20.74	3.56
24.538	Manoyl oxide	-	1
Total		94.73	89
Ni		5.27	11

Values are expressed as relative percentages and compounds are listed by elution order according to GC-MS analyses

Ni: Not identified

about 95% of the total composition. The main compounds identified (concentrations higher than 30%) were Spathulenol (35.15%) and Caryophyllene oxide (31.10%). Jofré Barud *et al.*, (2014) reported that the composition of *B. spartioides* EO from San Juan province was dominated by the monoterpene hydrocarbons,  $\alpha$ -phellandrene (44.5%), sabinene (20.7%) and  $\alpha$ -pinene (15.9%). In contrast, Oliva *et al.*, (2007) reported the composition of *B. spartioides* from three populations of Argentina (Northwest, Central and Patagonia areas) were the main constituents of the oils were camphor (26.5–50%), limonene (4.3–35.8%), citronellal (12%), carvone (10%) and spathulenol (2.1–11.8%), finding a relatively high variation in their composition and antimicrobial activity depending on the origin. Such divergence in results reveals the intrinsic variability of the EOs composition depending on the complex interaction between plant phenotype and environment. In addition, other sources of compositional variability can also include the part of the plant extracted, the phenological state

of the plant and time of year (Isman & Machial, 2006). In this context, EOs composition predictability is one of the main biotechnological challenges in bio-formulated production and identifying the responsible for repellence as well as toxic compounds is critical (Canter *et al.*, 2005).

In *H. petiolaris* EO, 9 major compounds were identified, which represent about 90% of the total composition. All components that represented more than 1% in composition are listed, being the major compounds (concentrations higher than 12%) the (S) cis-Verbenol (12.54%) and Caryophyllene oxide (27.00%). As far as we know, this report constitutes the first description of the *H. petiolaris* essential oil composition by GC-MS analysis.

The results of the repellent activity assay performed in treated flour are presented in Table 4. In the case of *H. petiolaris* EO, 90% of the insects rejected the treated flour while *B. spartioides* induced 67.6% of emigration. In both cases, the repellency decreased after 24 hours of treatment in about 20 and 25%, respectively.

Sadeghi *et al.* (2013) reported the repellency of Palizin® (Coconut Soap 65%) on *T. castaneum* and others stored product pest using the cup bioassay as a method that simulates complexity of real condition in repellent activity, crop and pest behavior. Silva *et al.* (2002) reported that the effect of most plants used in pest control, more than being insecticidal are insectistatic (growth regulators) because they inhibit normal development of insects. In this way, the application of sub-lethal doses of natural products, or even chemical pesticides as a new pest control approach has become more common nowadays. This is particularly relevant

Table 4. Repellent activity of treated flour with *B. spartioides* and *H. petiolaris* EOs.

Tabla 4. Actividad repelente de harina tratada con los EOs de *B. spartioides* y *H. petiolaris*.

Essential oil	concentration (mg/cm <sup>2</sup> )	PR <sup>2</sup> 2 h	PR <sup>2</sup> 24 h
<i>B. spartioides</i>	3x10 <sup>-1</sup>	95.5	67.6
<i>H. petiolaris</i>	3x10 <sup>-1</sup>	100	80

in a context where the use of broadly toxic chemical pesticides is now under serious restrictions in an increasingly regulated world. This pest control strategy has become crucial in the case of insects in stored grain, since the accumulation of toxic residues in food products is strictly scrutinized and matter of public concern.

Fumigant activity was also assayed but the concentration of EOs tested ( $3 \times 10^{-1} \text{ mg.cm}^{-2}$ ) result not lethal against *T. castaneum* (data not shown). More studies must be undertaken to elucidate the differences found between the repellent and fumigant activity but a possible

explanation could be based on the respiration rates of *T. castaneum* (Emekci *et al.*, 2002). Benzi *et al.*, (2014) reported fumigant activities of two South American plants against the stored grain pests *T. castaneum* and *T. confusum* and found differences between the susceptibility of both beetles, not reaching lethal effects on *T. castaneum*.

The results of repellency activity for the blends proposed are presented in Figure 1 and Figure 2. Repellent activity of *H. petiolaris*-Lemon blend (Figure 2) persisted after 24 h of treatment while the pure EO of *H. petiolaris* decreased by 24% in the same period. In the case of *B. spartioides*-Lemon blend (Figure 1), both the blend and the pure EO of *B. spartioides* decreased in a 20%. When the pure lemon EO was used at the concentration of  $3 \times 10^{-1} \text{ mg.cm}^{-2}$ , it did not show any repellency activity, however when it was used in combination with *H. petiolaris* EO, the blend increased by 20% its repellency at 24 h of treatment. Although the concentration to generate the repellent effect is high compared to the yield of the oil of this species, the proposed blend reduces by half the concentration of oil needed and in turn increases the repellent effect over time.

Lee *et al.*, (2002) reported the fumigant toxicity of lemon oil against *T. castaneum* obtaining the second most potent activity ( $\text{LD}_{50} = 16.2 \mu\text{l.l}^{-1} \text{ air}$ ). Besides Karamaouna *et al.*, 2013 informed that in range from 2.7 to 8.1  $\text{mg.mL}^{-1}$  of concentration *Citrus limon* L. peel EOs (mainly limonene) were the most toxic of all the tested EOs, against the vine mealybug. We found that in a range from 30 to 100  $\text{mg.ml}^{-1}$  of concentration Lemon EO is classified as class V repellency against adults of *T. castaneum* (Table 2). Also limonene was

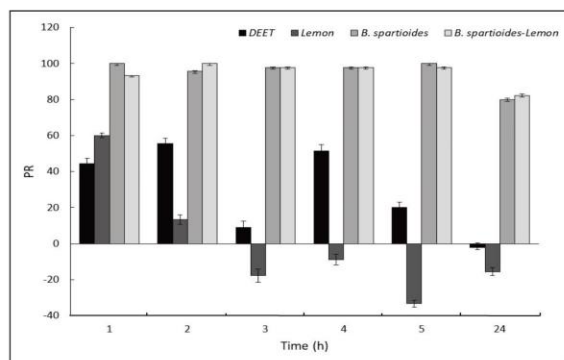


Figure 1. Repellency activity for the blend proposed *B. spartioides*-Lemon, compared with pure EOs and DEET at  $3 \times 10^{-1} \text{ mg.cm}^{-2}$  of concentration.

Figura 1. Actividad repelente de la mezcla propuesta *B. spartioides*-Lemon, comparada con los EOs puros y DEET a  $3 \times 10^{-1} \text{ mg.cm}^{-2}$  de concentración.

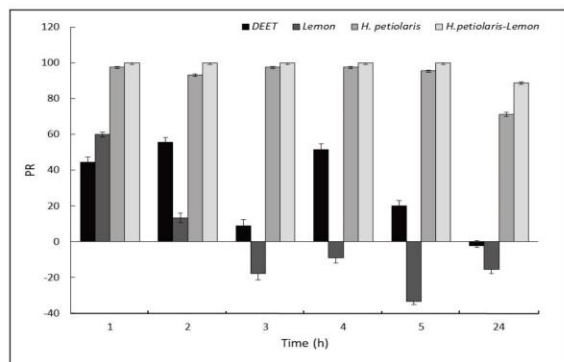


Figure 2. Repellency activity for the blend proposed *H. petiolaris*-Lemon, compared with pure EOs and DEET at  $3 \times 10^{-1} \text{ mg.cm}^{-2}$  of concentration.

Figura 2. Actividad repelente de la mezcla propuesta *H. petiolaris*-Lemon, comparada con los EOs puros y DEET a  $3 \times 10^{-1} \text{ mg.cm}^{-2}$  de concentración.

registered as a pesticide active ingredient in 15 products: for use against ticks and fleas, as an insecticide spray, as an outdoor dog and cat repellent, as a by repellent on tablecloths, as an insect repellent for use on humans, and as a mosquito larvicide (EPA, 1994). At least one U.S. patent (N° 5.653.991 published in 1997 by Robert L. Rod; USPTO 1997) refers to the use of various oil-based formulations of d-limonene, with or without a water carrier, against plant pests such as whiteflies.

Essential oils are complex mixtures of numerous molecules and their bioactivity could be affected by interactions among their structural components due to additive action and/or synergism between chemical classes (Hummelbrunner & Isman, 2001). The activity of the main components could also be modulated by other minor molecules (Franzios *et al.*, 1997).

These findings present the *B. spartioides* and *H. petiolaris* EOs blend as promising alternatives to control *T. castaneum* in stored grains, as safer and more suitable substitutes of the synthetic insecticides currently used

## CONCLUSIONS

Bioprospecting for less toxic and environmental friendly bio-products as substitutes in stored grain protection is raising research area, and native species are a promising reservoir.

In this context, the use of the EOs extracted from these five plant species could be proposed as a new botanical insecticide, since they are able to control the pest *T. castaneum* at doses 10 times lower than standard synthetic products used nowadays (DEET). However, based on the persistence of repellency and its low production cost, we recommend the blend of the *B. spartioides* and *H. petiolaris* EOs, with a base of lemon EO, as the best bio-product. Additional trials are needed to comprehensively assess toxicity, evaluate the effect of this blend and validate their efficacy under conditions of large scale grain storage

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