Essential oils from Uruguayan native plants against *Varroa destructor* (Acari: Varroidae). Chemical characterization and bioactivity .

Santos, E. [1], Umpiérrez, M. [1], González, A. [1], Mendoza, Y. [2], Ramallo, G. [2], Díaz-Cetti, S. [2], Rossini, C. [1]
esantos@fcien.edu.uy , mlumpierr@fq.edu.uy
[1]Laboratorio de Ecología Química, Facultad de Química - UdelaR - Montevideo, Uruguay
[2] Instituto Nacional de Investigación Agropecuaria - Colonia, Uruguay

INTRODUCTION

Uruguay: Beekeeping is in increasing development

Varroa destructor , *Apis mellifera's* ectoparasite

RESULT: depopulation of bee hives

CURRENT CONTROL STRATEGIES

Conventional pesticides and organic acids are the main control agents used. Resistance against some of these products was reported.

ALTERNATIVE

Use of essential oils (environmentally friendly and minor toxicity against bees, humans, beneficial insects, as well as less residues in honey)

ABSTRACT

A bioprospecting program of essential oils (EOs) from Uruguayan plants was carried out in search of alternative means for the control of *Varroa destructor* (Acari: Varroidae), ectoparasite of *Apis mellífera* (Hymenoptera: Apidae). An initial screening (by laboratory bioassays) of 46 EOs was performed obtaining four EOs with good safety margins (active against mites and innocuous on bees): *Aloysia* sp. leaves (Verbenaceae), *Schinus molle* leaves (Anacardiaceae) and *Eupatorium buniifolium* twigs and leaves (Asteraceae). The later was chemically characterized by gas chromatography (GC) and GC coupled to mass spectrometry (GC/MS) and the lethal dose (LD_{50}) was calculated. According to these results, the EO from leaves of *E. buniifolium* was chosen to be tested in hives in the field, comparing its activity with amitraz and oxalic acid (main control agents used against *V. destructor*).

MATERIALS AND METHODS

Collection and processing of plant material

Extraction of EOs with Clevenger

The chemical characterization of EOs was done by gas chromatography coupled to mass spectrometry (GC/MS). Identification of the individual compounds was done by comparing fragmentations patterns with databases (NIST05 and SHIM2205) and retention index with those in Adams (2007) and the Pherobase (2009).

Laboratory bioassays:

N=3

5 bees + 5 mites in the upper chamber

Filter paper with 0.5 mL of EO solution in ethanol (treatment), 0.5 mL of ethanol (solvent control) or no treatment (negative control).

The number of dead or dying bees and mites, as well as the number of mites that fell off of the bees was recorded at 24 and 48 hrs .

Field assays:
EO from leaves of *E. buniifolium* was chosen to be tested in hives in the field, comparing its activity with amitraz and oxalic acid.
Oxalic acid: Alpplied in sucrose syrup to 6.2% (5 cm^3), drip dispenser between the frames, 3 applications every 7 days (N =10)
Amitraz: 2 strips per hive of Amivar ® (N = 10)
EO: 2 applications (1st application: 4.3 g, 2nd application: 8.6 g) at intervals of 12 days on oasis ® (N = 10)
Control: untreated hives (N = 10)

RESULTS AND DISCUSSION

- Out of the 46 EOs tested in their acaricidal activity, four showed selective activity against *V. destructor: Aloysia* sp. (leaves), *S. molle* (leaves) and *Eupatorium buniifolium* (leaves and twigs).
- EO from leaves of *E. buniifolium* was chemically characterized (Figure 1, Table 1), and its dose variation response was analyzed (Figure 2).
- This EO was also tested in hives in the field. At the doses tested neither activity against *V. destructor,* nor mortality of bees were observed (Figure 3).

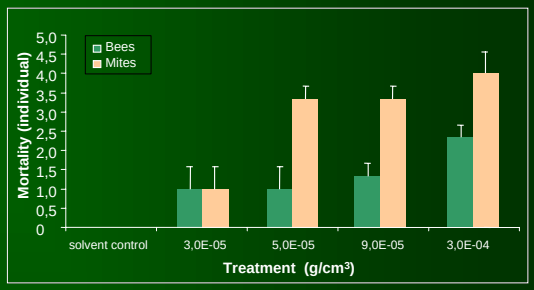

Figure 2. Bees and mites mortality vs dose of EO from leaves of *E. buniifolium* (48 hrs).

Perspectives:

- Given the margin of safety obtained in laboratory assays, the dosage of EO should be increased for future field tests.

Compound	%A	IA calc.	IA ref.
(1) α-pineno	3,45	930	932
(2) β-pineno	3,39	972	974
(3) limoneno	2,31	1028	1024
(4) E-β-ocimeno	2,87	1045	1044
(5) β-elemeno	6,09	1395	1389
(6) germacreno D	7,02	1489	1484
(7) biciclogermacreno	2,05	1504	1500
sesquiterpeno	5,38	1514	
(8) δ-cadineno	2,39	1528	1522
(9) germacreno B	3,16	1565	1559
sesquiterpeno oxigenado	2,19	1617	
sesquiterpeno oxigenado	3,3	1660	
sesquiterpeno oxigenado	2,08	1681	
(10) 8-cedren-13-ol	5,18	1691	1688
sesquiterpeno oxigenado	2,42	1703	
Only compounds with a %Area > 2 % are shown			

Table 1. Major compounds of EO from leaves of *E. buniifolium*

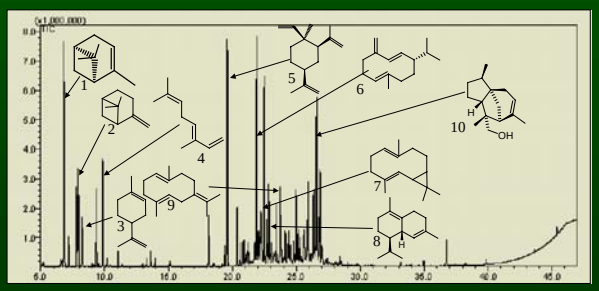

Figure 1. EO of *E. buniifolium* leaves chromatogram, and structural assignment of major components. GC/MS Column OPTIMA-5-MS (30m x 0.25mm, 0.25µm); injection in split mode en CH_2Cl_2; 40ºC (2min) - 240 (1min) to 5ºC/min - 320 to 10ºC/min; injector temperature and interphase 250ºC.

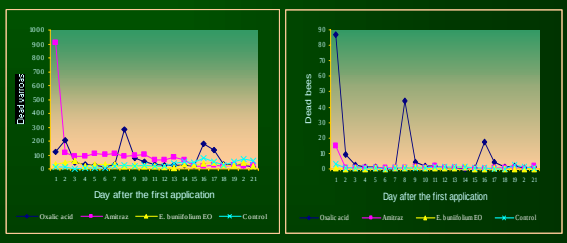

Figure 3. Field assays results

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