Bulletin of Insectology **67** *(1): 81-86, 2014*
ISSN 1721-8861

Bioactivity of microencapsulated essentials oils and perspectives of their use in the control of *Varroa destructor*

Sergio R. RUFFINENGO[1], Matías D. MAGGI[2,3], Sandra FUSELLI[2,4], Fiorella G. DE PIANO[1,4], Pedro NEGRI[2,3], Constanza BRASESCO[2], Alberto SATTA[5], Ignazio FLORIS[5], Martín J. EGUARAS[2,3]

[1]*Grupo Apicultura, Facultad de Ciencias Agrarias, U.N.M.d.P., Balcarce, Argentina*
[2]*Laboratorio de Artrópodos, Facultad de Ciencias Exactas y Naturales, U.N.M.d.P., Mar del Plata, Argentina*
[3]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina*
[4]*Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC), La Plata, Argentina*
[5]*Dipartimento di Agraria, Sezione di Patologia vegetale ed Entomologia, Università degli Studi di Sassari, Italy*

Abstract

Attractant/repellent and acaricidal effects of two microencapsulated essential oils, *Acantholippia seriphioides* (A. Gray) Mold. and *Schinus molle* L., were evaluated on the ectoparasitic mite *Varroa destructor* Anderson et Truemann using complete exposure and evaporation tests. Mites and honey bees (10 specimens of each per dish) were introduced in Petri dishes having different microencapsulated essential oil doses (0.25, 0.5 and 1 g). Mite and honey bee mortality were registered at 24, 48 and 72 hrs. An attractant/repellent test was performed using a device with two tubes of virgin wax closed on one side. Microencapsulated essential oils were placed at the end of one of the tubes. One mite was placed inside a tube with no oil and its position was observed after 90 min. Microencapsulated oils differed in the level of toxicity caused to *V. destructor*, *A. seriphioides* oil toxicity was higher than *S. molle* oil. Negative effects were registered on honey bees when they were completely exposed to microencapsulated oil of *A. seriphioides*. Microencapsulated oil of *S. molle* had attractant properties. None of the two microencapsulated oils tested had repellent effects on mites. This study might be a starting point for future researches of microencapsulated essential oils, as they offer a good alternative in the control of varroatosis.

Key words: Microencapsulated essential oils, *Acantholippia seriphioides*, *Schinus molle*, *Varroa destructor*, *Apis mellifera*.

Introduction

Varroa destructor Anderson et Truemann is the most serious parasitic mite species that infests *Apis mellifera* L. colonies (Anderson and Truemann, 2000). This parasite affects both adult bees and developing honey bee pupae. Therefore beekeepers must carry out different miticide treatments in order to control the development of the parasite population and prevent colony death. Mite control is mainly based on the use of synthetic acaricides especially pyrethroids and organophosphates. Consequently, the continuous use of these substances over time has caused an accumulation of the different products inside the beehive (Wallner, 1999) and the expansion of resistant mite populations (Milani, 1999; Elzen *et al.*, 2000; Mozes-Koch *et al.*, 2000; Maggi *et al.*, 2009; 2010a; 2011). Therefore it is important to conduct more researches for the control of *Varroa* mites, leading to best management practices as the integrated parasite management, to avoid stress conditions, parasitism side effects, and hive contamination from acaricide residues, which can weaken the honey bee immune system (Di Prisco *et al.*, 2013). In this context, it is also important the substitution of conventional synthetic acaricides with natural ones.

A range of essential oils, have been found to exhibit acaricide activity against *V. destructor*. These are distilled from aromatic plants, possess intense smell, exhibit low toxicity to mammals and bees and have less harmful effect over environment and a wide public acceptance (Isman, 2000). An important number of essential oils and their components have been proven to control the mite, with different results. Their miticide or attractant/repellent effects on *Varroa* and their influence on its reproduction have been evaluated. Essential oils are being locally administered, applied in pulverization or in a passive evaporation form (Imdorf *et al.*, 1999), and many of them have showed to be effective under laboratory conditions (Lindberg *et al.*, 2000; Melathopoulus *et al.*, 2000; Ardeshir *et al.*, 2002; Neira *et al.*, 2004; Ruffinengo, 2010; Ruffinengo *et al.*, 2005; Maggi *et al.*, 2010b). However, beside these positive results few commercial formulations exist based on essential oils or their components (Imdorf *et al.*, 1995; Mattila and Otis, 1999; Baggio *et al.*, 2004; Floris *et al.*, 2004).

Although many essential oils are effective in the control of varroatosis, they have, as well as other natural acaricides, a marked variability in their final acaricidal efficacy when these are applied in beehives (Mutinelli *et al.*, 1994; Satta *et al.*, 2005; 2008). Acaricide release is one of the most important factors that could modify its final efficacy and depends on the manner of administration of the product. Essential oils are broken down faster than synthetic compounds and despite their promising properties, problems related to their volatility, poor water solubility and ability to oxidize must be solved before being incorporated as alternative ways to control systems (Moretti *et al.*, 2002). Microencapsulation method is suitable for the entrapment of substances with physicochemical properties like those present in essential oils. Moreover, microencapsulation protects against different erosive factors, allowing a controlled release of the product. Encapsulation is a technique by which a membrane encloses small particles of solids, liquids or

gases, providing protection from adverse environmental conditions such as undesirable effects of light, moisture and oxygen and also, contributing to an increase in half-life of the active substance and promoting controlled release of the encapsulated (Shahidi and Han, 1993). Microcapsules solve the volatility and stability problems, allowing that liquid and volatile product became able to be efficiently manipulated as a solid substance (trapping the active compounds, masking the smell and releasing the drugs in a regulated and directed way) (Robinson, 1997). Substances that are used as roofing material can be selected from a wide variety of natural or synthetic polymers, depending on the material to be encapsulated and the characteristics desired for the final product. The composition of the cover is the most important issue in relation to the functional properties of encapsulation and also, the method to be used to improve the performance of a particular ingredient (Barbosa-Cánovas, 2005). The encapsulants most widely used include carbohydrates, cellulose and its derivatives, lipids, some proteins and gums (Shahidi and Han, 1993). Besides the good properties of the microencapsulation, there are no reports of use of microencapsulated essences against *V. destructor*.

Essential oils of *Acantholippia seriphioides* (A. Gray) Mold. and *Schinus molle* L. had shown acaricidal properties in previous works (Ruffinengo *et al*., 2005). Andean thyme (*A. seriphioides*) is an aromatic, perennial sub-bush belonging to the family Verbenaceae. This plant is native to Western Mediterranean and grows wild in Argentina, throughout San Juan, Mendoza, Neuquén, La Pampa, Río Negro, Chubut and Santa Cruz provinces, and is cultivated in San Luis, Córdoba and Buenos Aires provinces. Aguaribay (*S. molle*) is an aromatic, perennial tree belonging to the Anacardiaceae family and it is native to different countries of South America (Brazil, Uruguay and Argentina).

The aim of the present study was to evaluate under laboratory conditions, the bioactivity of two microencapsulated essential oils, *A. seriphioides* and *S. molle* on *V. destructor* and *A. mellifera*, and its attractant/repellent effects on mites.

Materials and methods

Plant material, oil isolation and chemical compounds identification

Essential oils of *A. seriphioides* and *S. molle* were used for the experiments. These oils had shown acaricidal properties in previous works (Ruffinengo *et al*., 2005).

Air-dried flowers and ground aerial parts of plants were collected from San Luis Province and Córdoba Province (Argentina). Plant material was submitted to water distillation for 4 hours using the technique reported by Aldicara (1976). The essential oil obtained was dried over anhydrous sodium sulphate and, after filtration, stored at 5 °C until tested. The composition of the headspace volatiles of the essential oils were determined by performing a solid phase microextraction (SPME) prior to the analysis by gas chromatography coupled to mass spectrometry (GC-MS) (Fuselli *et al*., 2007). A divinylbenzene-poly(dimethylsiloxane)-coated stable flex fiber (65 μm) and a manual SPME holder (Supelco Inc., Bellefonte, PA) were used after preconditioning according to the manufacturer's instruction manual. Before each headspace sampling, the fiber was exposed to the GC inlet for 5 min for thermal desorption at 250 °C in a blank run. Two millilitres of the oil sample were placed in 2 ml vials, and the vials were sealed by PTFE/silicon septa. The samples were then equilibrated for 15 min at 50 °C. The SPME fiber was exposed to each sample for 5 min by manually penetrating the septum, and finally, the fiber was inserted into the injection port of the GC for 5 min sample desorption.

GC-MS analyses were carried out on an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA) coupled to an Agilent 5970 mass selective detector operating in electron impact mode (ionization voltage, 70 eV). A Chrompack CP- Wax 52 CB capillary column (50 m length, 0.32 mm, 1.2 μm df) was used (Chrompack, Middelburg, The Netherlands). The temperature program was 250 °C for 1 min. Injections were performed with a split ratio of 1:20 and He (1 ml/min) as the carrier gas. The compounds were identified by using the National Institute of Standards and Technology-United States Environmental Protection Agency-National Institute of Health and Wiley mass spectra libraries as well as literature MS data and, whenever possible, coinjections with authentic chemical compounds.

Essential oil microencapsulation

The microencapsulation was carried out atomizing an emulsion composed by ten parts of each essential oil, thirty parts of Arabic rubber and sixty parts of distilled water. The instantaneous evaporation was carried out in a Niro Atomizer Production Minor oven. The emulsion was homogenized between 10,000 and 20,000 rpm, obtaining 200 μm diameter spheres at temperatures between 85-190 °C. The size of the microparticles was checked by microscopy. The obtained powder was packed in hermetic flasks and stored in a dark and dry place until use.

Mite and honey bee toxicity

Microencapsulated essential oils were incorporated into unmodified Petri dishes (150 × 20 mm) in two different ways: a) dispensers that allowed the direct contact of the oil with mites and honey bees (complete exposure method - CE) (figure 1), and b) dispensers with a plastic mesh that avoided the direct contact of the microcapsule with mites and honey bees (evaporation method - Ev) (figure 2). Brand new Petri dishes were used in each experiment. Doses of 0.25, 0.5 and 1 g of microencapsulated oils were used for the experiments. Ten adult honey bees and 10 mites obtained from bee brood sampled from high infested *A. mellifera* colonies were placed inside each dish. The honey bees were fed with 3 g of candy (water + powdered sugar). Petri dishes were incubated at 30 °C and 70% RH during the test. Control treatments consisted of Petri dishes with microcapsules with distilled water in dispensers with and without the plastic mesh. Five replicates for each treatment were

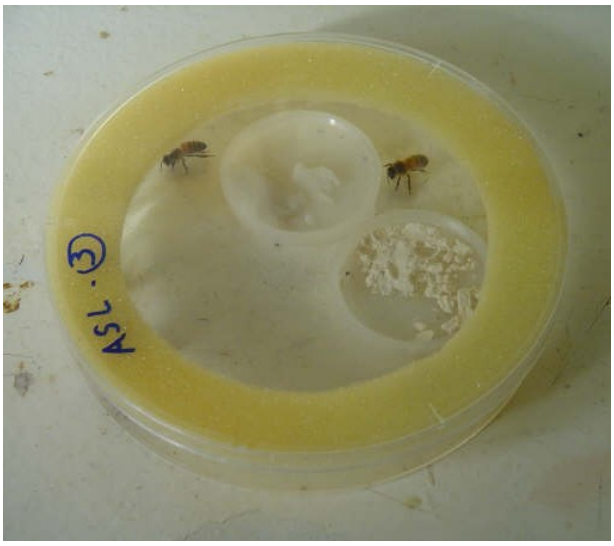

Figure 1. Complete exposure method.
(In colour at www.bulletinofinsectology.org)

Figure 2. Evaporation method.
(In colour at www.bulletinofinsectology.org)

used. The number of dead mites and honey bees was determined after visual inspection of the dish bottoms after 24, 48, and 72 hrs.

The proportion of mites and bees killed by both application methods was evaluated. The mortality rates of mites and bees were compared by means of statistical comparisons (χ^2 test for proportions). The model included the type of microencapsulated oil, species, time, and dose. The significance level was set at 5%.

Attractant and repellent effect

A device with two tubes of virgin wax (3 cm long by 1 cm wide) was developed (figure 3). Each tube was closed on one side. The microencapsulated essential oils were placed at the end of one of the tubes. One mite was placed inside a tube with no oil, which was then joined to a tube containing the microcapsules (figure 3). Mite position could be observed through the thin wax layer, and was recorded after 90 min. According to Kraus *et al*. (1994), *Varroa* orientation behaviour becomes evident in this period. Twenty replicates were carried out for each treatment and for the control tubes with no oil. Categorical data analysis was conducted using PROC CATMOD (SAS version 8) procedure. To compare oil effects with those obtained from the control groups, specific contrasts were used.

Results

Microspheres of *A. seriphioides* and *S. molle* essential oils, obtained from the microencapsulation technique, presented a 10% (v/v) of bioactive principles, which were thymol (29.2%), p-cymene (23.3%), carvacrol (23.3%) and γ-terpinene (11.0%) for *A. seriphioides,* and β-phellandrene (28.3%) and α-phellandrene (11.5%) for *S. molle*.

Microencapsulated oils differed in the level of toxicity caused to *V. destructor*. *A. seriphioides* oil toxicity was higher than *S. molle* oil. Mite mortality increased positively with oil exposure-time and oil dosage. At 72 hrs, *A. seriphioides* essential oil yielded high values of acaricide effectiveness for the three doses tested in both methods of exposure (0.25 g, 88 ± 11.5% in CE and 83 ± 19.4% in Ev; 0.5 g, 96 ± 6.7% in CE and 78 ± 10.4% in Ev; 1 g, 99 ± 9.4% in CE and 97 ± 11.4% in Ev). However, *S. molle* presented a lower efficacy for the same time and dose (0.25 g, 48 ± 9.1% in CE and 30 ± 30% in Ev; 0.5 g, 36 ± 11.1% in CE and 52 ± 23.1% in Ev; 1 g, 54 ± 22.2% in CE and 58 ± 18.2% in Ev). Significant differences were observed between the two methods of exposure ($p < 0.05$). The complete exposure method produced more mite toxicity than the evaporation one (table 1).

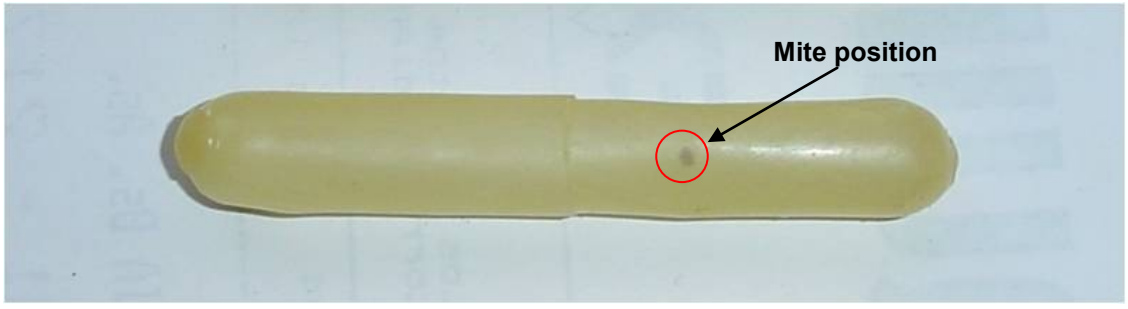

Figure 3. Device for the attractants/repellents effects evaluation of the microencapsulated essential oils on *V. destructor*.
(In colour at www.bulletinofinsectology.org)

Table 1. Percentage mortality (SD in brackets) of *V. destructor* exposed to different doses of microencapsulated oil, during complete exposure (CE) and evaporation (Ev) tests, at different exposure times. Five replicates for each treatment were used. Different letters represent significant differences ($P < 0.05$).

	A. seriphioides						*S. molle*					
Dose (g)	Complete exposure (CE)			Evaporation (Ev)			Complete exposure (CE)			Evaporation (Ev)		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
0.25	74 ^{a} (11.6)	79 ^{a} (22.1)	88 ^{b} (11.5)	38 ^{a} (47.2)	62 ^{b} (33.4)	83 ^{c} (19.4)	30 ^{a} (18.1)	40 ^{b} (9.4)	48 ^{b} (9.1)	20 ^{c} (38.2)	28 ^{a} (32.1)	30 ^{a} (30)
0.50	80 ^{a} (23.5)	87 ^{b} (14.8)	96 ^{b} (6.7)	45 ^{a} (12.3)	65 ^{b} (11.5)	78 ^{c} (10.4)	8 ^{c} (16.1)	30 ^{a} (18.7)	36 ^{a} (11.1)	20 ^{d} (42.1)	38 ^{a} (31.4)	52 ^{c} (23.1)
1.00	70 ^{a} (18.1)	91 ^{b} (22.6)	99 ^{b} (9.4)	78 ^{c} (21.1)	86 ^{c} (22)	97 ^{d} (11.4)	20 ^{c} (43.2)	48 ^{b} (25.3)	54 ^{d} (22.2)	30 ^{a} (38.4)	36 ^{a} (31.2)	58 ^{d} (18.2)
Control	6 ^{c} (12)	9 ^{c} (10.1)	13 ^{c} (8.4)	8 e (16.1)	8 ^{e} (16.1)	12 ^{e} (6.1)	7 ^{e} (12.1)	8 ^{e} (16.1)	10 ^{e} (5.6)	8 ^{e} (16.1)	8 ^{e} (16.1)	10 ^{e} (10.1)

Table 2. Percentage mortality (SD in brackets) of *A. mellifera* exposed to different doses of microencapsulated oil, during complete exposure (CE) and evaporation (Ev) tests, at different exposure times. Five replicates for each treatment were used. Different letters represent significant differences ($P < 0.05$).

	A. seriphioides						*S. molle*					
Dose (g)	Complete exposure CE			Evaporation Ev			Complete exposure CE			Evaporation Ev		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
0.25	47 ^{a} (33.2)	55 ^{a} (34.2)	79 ^{b} (11.4)	9 ^{d} (25.5)	14 ^{d} (18.5)	38 ^{c} (12.2)	6 ^{a} (18.2)	6 ^{a} (18.2)	12 ^{a} (12.6)	2 ^{a} (4.2)	2 ^{a} (4.2)	6 ^{a} (10.4)
0.50	16 ^{c} (41.1)	62 ^{a} (32)	82 ^{b} (12.2)	5 ^{d} (20.1)	19 ^{e} (10.2)	39 ^{c} (10)	8 ^{a} (5.6)	8 ^{a} (5.6)	8 ^{a} (5.6)	6 ^{a} (22.4)	6 ^{a} (22.4)	8 ^{a} (16.1)
1.00	29 ^{c} (33.2)	62 ^{a} (10.5)	87 ^{b} (6.5)	6 ^{d} (22.4)	12 ^{d} (18.2)	16 ^{d} (15.1)	8 ^{a} (16.1)	18 ^{a} (8.1)	42 ^{c} (10)	8 ^{a} (16.1)	8 ^{a} (16.1)	8 ^{a} (16.1)
Control	5 ^{d} (20.1)	13 ^{d} (11)	14 ^{d} (8.5)	5 ^{d} (20.1)	8 ^{d} (8.2)	17 ^{d} (8.6)	6 ^{a} (10.4)	7 ^{a} (10.5)	12 ^{a} (12.4)	3 ^{a} (6.2)	6 ^{a} (6.2)	8 ^{a} (5.6)

With regard to bee toxicity, the microencapsulated oil *A. seriphioides* provides high values of honey bee mortality for the doses tested, also at 72 hrs (0.25 g, 79 ± 11.4% in CE and 38 ± 12.2% in EV; 0.5 g, 82 ± 12.2% in CE and 39 ± 10% in EV; 1 g, 87 ± 6.5% in CE and 16 ± 15.1% in EV). Nevertheless, *S. molle* oil presented a moderate bee mortality rate when it was exposed to 1g at 72hs (42 ± 10% in CE and 8 ± 16.1% in Ev). Significant differences were observed between the two methods of exposure ($p < 0.05$). The complete exposure method produced more bee toxicity than the evaporation one (table 2).

The number of times that mites were found in the tube area with pure wax and in the tube area with both wax and oils was listed in table 3. Mite numbers in tubes containing wax and the microencapsulated oil of *S. molle* were significantly higher than those in control tubes ($p < 0.001$). Therefore, the essential oil of *S. molle* presented attractant effects.

Table 3. Mite number related to position inside the test tube for each treatment. Twenty replicates for each treatment were used.

Oil	Wax + oil	Pure wax	χ^2	Pr > χ^2
S. molle	19 (95%)	1 (5%)	6.86	0.0088
A. seriphioides	7 (35%)	13 (65%)	0.42	0.5164
Control	9 (45%)	11 (55%)		

Discussion

Essential oils can interfere with basic behavioural functions of certain arthropods (Imdorf *et al*., 1999). Some exhibit acute toxicity while others may act as repellent substances (Watanabe *et al*., 1993), antifeedand (Hough-Goldstein, 1990), or may stop the growth, development or reproduction, or interfere with physiological and biochemical processes (Gershenzon and Croteau, 1991). However, many essential oils have not been included yet in commercial formulations due to the lack of adequate substrates for their incorporation in beehives (Eguaras *et al*., 2005). In this way, essential oil microencapsulation could be an option for *Varroa* treatments. It involves the use of a polymer to protect the oils and it has been tested for adhesive, cosmetics, insecticides, pharmaceutical and medical applications (Park *et al*., 2001).

The essential oils of *A. seriphioides* and *S. molle* were previously successfully tested to control the mite *V. destructor* by means of bioassays (Ruffinengo, 2010; Ruffinengo *et al*., 2005). On the bases of the present results, the microencapsulated essential oil of *A. seriphioides* showed higher level of toxicity respect to *S. molle* in laboratory conditions against *V. destructor*. However, both oils, when applied in dispensers, produce harmful effects on honey bees, probably due to the constant movement of the bees within the Petri dishes that pro-

duces a cloud of microencapsulated powder in the atmosphere, facilitating the inhalation of the same into the honey bee respiratory system.

V. destructor comes in contact with a large number of host honey bees during its life cycle and therefore the orientation is very important to differentiate and recognize them. In the mite, olfactory stimuli play an important role in the recognition of volatile compounds present in preimaginal developmental stages and in adult honey bees of different ages. It is therefore expected that an influence in the olfactory orientation to volatiles produces disturbances that affect development and reproduction of the mite.

The literature reports few essential oils attractant to mites (Imdorf *et al*., 1999). Ruffinengo *et al*. (2005) evaluated the attractant and repellent effects of different essentials liquid oils. They observed that the liquid oil of *A. seriphioides* and *S. molle* did not show any effect. However, the results obtained in this study showed that the microencapsulated oil of *S. molle* exhibited a marked attractant effect on mites, while *A. seriphioides* did not show effects in its microencapsulated state. That said, none of the tested oils had microencapsulated repellent effects on mites. Studies in the encapsulation process should be conducted, given the possibility to detect some chemical change in any of the constituents of the aguaribay oil.

Essential oils have beneficial aspects; their physicochemical properties make them much less risky for the environment than synthetic pesticides, and resistance will develop more slowly due to the complex mixture of constituents that comprise (Koul *et al*., 2008). Through microencapsulation can improve the performance of the oils also reduce the effects related to its volatility, poor water solubility and ability to oxidize. In this context, this study might be a starting point for researches of microencapsulated essential oils, as they offer a good alternative in future integrated pest management programmes in honey bee colonies.

References

ALDICARA J. R., 1976.- Essential oil, p. 262. In: *Encyclopedic of Chemical Processing and Desing* (MACKETTA J. J., CUNNINGHAM W. A., Eds).- Marcel Dekker, New York, USA.

ANDERSON D. L., TRUEMANN J. W. H., 2000.- *Varroa jacobsoni* (Acari: Varroidae) is more than one species.- *Experimental and Applied Acarology*, 24: 165-189.

ARDESHIR A., RAHIM E., GHOLAMHOSIEN T., 2002.- Laboratory evaluation of some plant essences to control *Varroa destructor* (Acari: Varroidae).- *Experimental and Applied Acarology*, 27: 319-327.

BAGGIO A., ARCULEO P., NANETTI A., MARINELLI E., MUTINELLI F., 2004.- Field trials with different thymol-based products for the control of varroosis.- *American Bee Journal*, 144: 395-400.

BARBOSA-CÁNOVAS G., JULIANO P., KOUTCHMA T., BALASUBRAMANIAM V., MATHEWS J., DUNNE C., 2005.- High pressure thermal sterilization of precooked egg patties: factors affecting preheating efficiency, p. 572.- In: *High pressure processing, American institute of chemical engineers annual meeting*, Cincinnati, USA.

DI PRISCO G., CAVALIERE V., ANNOSCIA D., VARRICCHIO P., CAPRIO E., NAZZI F., GARGIULO G., PENNACCHIO F., 2013.- Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees.- *Proceedings of the National Academy of Sciences of the United States of America*, 110 (46): 18466-18471.

EGUARAS M., FUSELLI S., GENDE L., FRITZ R., RUFFINENGO S., CLEMENTE G., GONZALEZ A., BAILAC P., PONZI M., 2005.- An in vitro evaluation of *Tagetes minuta* essential oil for the control of the honeybee pathogens *Paenibacillus larvae* and *Ascosphaera apis*, and the parasitic mite *Varroa destructor*.- *Journal of Essential Oil Research*, 17: 336-340.

ELZEN P. J., BAXTER J. R., SPIVAK M., WILSON W. T., 2000.- Control of *Varroa jacobsoni* Oud. resistant to fluvalinate and amitraz using coumaphos.- *Apidologie*, 31: 437-441.

FLORIS I., SATTA A., CABRAS P., GARAU V., ANGIONI A., 2004.- Comparison between two thymol formulations in the control of *Varroa destructor*: effectiveness, persistence, and residues.- *Journal of Economic Entomology*, 97: 187-191.

FUSELLI S., GARCÍA DE LA ROSA S., EGUARAS M., FRITZ R., NDAGIJIMANA M., VANNINI L., GUERZONI M., 2007.- Efficacy of indigenous plant essential oil Andean thyme (*Acantholippia seriphioides* A. Gray) to control American foulbrood (AFB) in honey bee (*Apis mellifera* L.) hives.- *Journal of Essential Oil Research*, 19: 514-519.

GERSHENZON J., CROTEAU R., 1991.- Terpenoids, pp. 165-219. In: *Herbivores: their interactions with secondary plant metabolites*, Vol. 1 (ROSENTHAL G. A., BERENBAUM M. R., Eds).- Academic Press, San Diego, California, USA.

HOUGH-GOLDSTEIN J. A., 1990.- Antifeedant effects of common herbs on the Colorado potato beetle (Coleoptera: Chrysomelidae).- *Environmental Entomology*, 19: 234-238.

IMDORF A., BOGDANOV S., KILCHENMANN V., MAQUELIN C., 1995.- Apilife Var: a new miticide with thymol as the main ingredient.- *Bee World*, 76: 77-83.

IMDORF A., BOGDANOV S., IBAÑEZ OCHOA R., CALDERONE N. W., 1999.- Use of the essential oils for the control of *Varroa jacobsoni* in honey bee colonies.- *Apidologie*, 30: 209-229.

ISMAN M. B., 2000.- Plant essential oils for pest and disease management.- *Crop protection*, 19: 603-608.

KOUL O., WAGLIA S., DHALIWAL G., 2008.- Essential oils as green pesticides: potential and constraints.- *Biopesticide International*, 4: 63-84.

KRAUS B., KOENIGER N., FUCHS S., 1994.- Screening of substances for their effect on *Varroa jacobsoni* - attractiveness, repellency, toxicity and masking effects of ethereal oils.- *Journal of Apicultural Research*, 33: 34-43.

LINDBERG C., MELATHOPOULUS A., WINSTON M., 2000.- Laboratory evaluation of miticides to control *Varroa jacobsoni*, a honey bee parasite.- *Journal of Economic Entomology*, 93: 189-198.

MAGGI M., RUFFINENGO S., DAMIANI N., SARDELLA N., EGUARAS M., 2009.- First detection of *Varroa destructor* resistance to coumaphos in Argentina.- *Experimental and Applied Acarology*, 47: 317-320.

MAGGI M., RUFFINENGO S., NEGRI P., EGUARAS M., 2010a.- Resistance phenomena to amitraz from populations of the ectoparasitic mite *Varroa destructor* of Argentina.- *Parasitology Research*, 107: 1189-1192.

MAGGI M., RUFFINENGO S., GENDE L., SARLO G., EGUARAS M., 2010b.- Laboratory evaluations of *Sicygium aromaticum* (L.) Merr. et Perry essential oil against *Varroa destructor*.- *Journal of Essential Oil Research*, 22: 119-122.

MAGGI M., RUFFINENGO S., MENDOZA Y., OJEDA P., RAMALLO G., FLORIS I., EGUARAS M., 2011.- Susceptibility of *Varroa destructor* (Acari: Varroidae) to synthetic acaricides in Uruguay: Varroa mites' potential to develop acaricide resistance.- *Parasitology Research*, 108: 815-821.

MATTILA H., OTIS G., 1999.- Trials of Apiguard, a thymol-based miticide. Part 1. Efficacy for control of parasitic mites and residues in honey.- *American Bee Journal*, 139: 947-952.

MELATHOPOULUS A., WINSTON M., WHITTINGTON R., SMITH T., LINDBERG C., MUKAI A., MOORE M., 2000.- Comparative laboratory toxicity of Neem pesticide to honey bees, their mite parasites *Varroa jacobsoni* and *Acarapis woodi*, and brood pathogens *Paenibacillus larvae* and *Ascosphaera apis*.- *Journal of Economic Entomology*, 93: 199-209.

MILANI N., 1999.- The resistance of *Varroa jacobsoni* Oud. to acaricides.- *Apidologie*, 30: 229-234.

MORETTI M., SANNA-PASSINO G., DEMONTIS S., BAZZONI E., 2002.- Essential oil formulations useful as a new tool for insect pest control.- *AAPS PharmSciTech*, 3 (2): 64-74.

MOZES-KOCH R., SLABEZKI Y., EFRAT H., KALEV H., KAMER Y., YAKOBSON B. A., DAG A., 2000.- First detection in Israel of fluvalinate resistance in the varroa mite using bioassay and biochemical methods.- *Experimental and Applied Acarology*, 24: 35-43.

MUTINELLI F., CREMASCO S., IRSARA A., 1994.- Formic acid in the control of varroatosis: a practical approach.- *Journal of Veterinary Medicine B*, 41: 433-440.

NEIRA M., HEINSOHN P., CARRILLO R., BÁEZ A., FUENTEALBA J., 2004.- Efecto de aceites esenciales de lavanda y laurel sobre el ácaro *Varroa destructor* Anderson y Trueman (Acari: Varroidae).- *Agricultura Tecnica*, 64: 238-244.

PARK S. Y., LEE B. I., JUNG S. T., PARK J. H., 2001.- Biopolymer composite films based on carrageenan and chitosan.- *Materials Research Bulletin*, 36: 511-519.

ROBINSON J. R., 1997.- Controlled drug delivery. Past, present, and future, pp: 1-7. In: *Controlled drug delivery: challenges and strategies* (PARK K., Ed.).- American Chemical Society, Washington, USA.

RUFFINENGO S., EGUARAS M., FLORIS I., FAVERIN C., BAILAC P., PONZI M., 2005.- LD50 and repellent effect to *Varroa destructor* mite of different essential oil from Argentina wild plants species.- *Journal of Economic Entomology*, 98: 651-655.

RUFFINENGO S., 2010.- Actividad biológica de aceites esenciales sobre el ácaro parásito *Varroa destructor* (Acari: Varroidae). 138 pp. *Tesis Doctoral* en Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Argentina.

SATTA A., FLORIS I., CABRAS P., GARAU V. L., MELIS M., 2005.- Formic acid-based treatments for control of *Varroa destructor* in a Mediterranean area.- *Journal of Economic Entomology*, 98: 267-273.

SATTA A., FLORIS I., CABONI P., EGUARAS M., VELIS G., 2008.- New experimental data on use of rotenone as an acaricide for control of *Varroa destructor* in honey bee colonies.- *Journal of Economic Entomology*, 101: 1075-1080.

SHAHIDI F., HAN X., 1993.- Encapsulation of food ingredients.- *Critical Reviews in Food Science and Nutrition*, 33: 501-547.

WALLNER K., 1999.- Varroacides and their residues in bee products.- *Apidologie*, 30: 235-248.

WATANABE A., SHONO Y., KAKIMIZU A., MATSUO N., SATON A., NISHIMURA H., 1993.- New mosquito repellent from *Eucalyptus camaldulensis*.- *Journal of Agricultural and Food Chemistry*, 41: 2164-2166.

Authors' addresses: Alberto SATTA (corresponding author, e-mail: albsatta@uniss.it), Ignazio FLORIS, Dipartimento di Agraria, Sezione di Patologia vegetale ed Entomologia, Università degli Studi di Sassari, viale Italia 39, 07100 Sassari, Italy; Sergio R. RUFFINENGO, Fiorella G. DE PIANO, Grupo Apicultura, Facultad de Ciencias Agrarias, U.N.M.d.P., Ruta Nacional 226, Km 73.5, CC 276 (7620) Balcarce, Argentina; Matías D. MAGGI, Sandra FUSELLI, Pedro NEGRI, Constanza BRASESCO, Martín J. EGUARAS, Laboratorio de Artrópodos, Facultad de Ciencias Exactas y Naturales, U.N.M.d.P., Funes 3350 (7600) Mar del Plata, Argentina.

Received July 16, 2013. Accepted February 11, 2014.